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Optimizing the productivity of eggplant grown in polybags with the application of buffalo manure tea

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ARTICLE INFO	ABSTRACT
Research Paper Received: December 17, 2024 Revised: February 12, 2025 Accepted: February 24, 2025 Keywords Buffalo manure tea Container gardening Eggplant Organic farming Polybag-growing *Corresponding author Francisco Olermo Esgrina Email: foesgrina@cfcst.edu.ph	By optimizing container gardening practices for solanaceous vegetables like eggplant, urban agriculture and food security initiatives can be significantly advanced. This study examined the productivity of polybag-grown eggplants, focusing on polybag sizes and buffalo manure tea levels. A randomized complete block design (RCBD) was utilized, featuring a 3 × 8 factorial arrangement with 24 treatments and 3 replicates. The experimental results showed that eggplants grown in 40.64 x 40.64 cm polybags demonstrated significantly higher growth and yield. Specifically, these polybags achieved average plant heights of 46.73 cm, a leaf count of 28.39, and a total yield of 2.01 kg per plant. The polybags required at least 6.5 kg of growing media, composed of a 2:2:1 ratio of garden soil, aged rice hull, and vermicompost. These findings offer valuable insights for urban gardeners and agricultural practitioners seeking to enhance eggplant productivity sustainably.

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1. Introduction

Eggplant (*Solanum melongena* Linn) a versatile and nutritious vegetable, thrives in container gardens, making it an ideal choice for urban or small-space gardening (Roll & Wilson, 2020; Kiefer, 2023). Container gardening offers better control over soil quality and moisture levels (Relf, 2020; Harrison, 2021), which are essential for eggplant growth. Container-grown eggplants

benefit from organic nutrients like compost tea or organic fertilizers, which enhance soil quality, promote root health, and improve nutrient uptake (Montgomery & Bikle, 2021; Ye et al., 2023; Hu et al., 2024). Furthermore, eggplants grown without synthetic fertilizers tend to have superior postharvest qualities, including firmer texture, better flavor, richer nutritional content, longer shelf life, and reduced environmental impact (Ebrahimi et al., 2021; Mahamad et al., 2022).

Manure tea application to vegetables enhances growth and yield by supplying essential nutrients such as NPK (Hamaïel et al., 2015; Tuan, 2019; Gad et al., 2023). Its organic matter content improves soil structure, aiding water retention and nutrient availability to plant roots. Beneficial microorganisms in manure tea also enhance soil health and plant resilience against pests and diseases (Schonbeck et al., 2019; Silva & Canellas, 2022). The use of manure tea optimizes crop productivity, reduces reliance on synthetic fertilizers, and supports sustainable agricultural practices (Golabi et al., 2004; Gamage et al., 2023). Specifically, buffalo manure tea (BMT), a liquid organic fertilizer derived from composted buffalo manure, is particularly effective in improving soil fertility and plant health (DAATI, 2011; Leauthaud et al., 2022; Ramírez-Gottfried et al., 2023).

Moreover, despite the benefits, limited studies address the interaction of BMT application levels with container size, particularly for eggplant cultivation. Thus, determining the optimal container size for growing fruit vegetables remains challenging due to gaps in understanding. Fruit vegetables, including eggplant, have specific root structure and growth habit requirements (GCE, 2016; JICA, 2016). Additionally, container size can impact moisture retention, nutrient availability, and microclimate conditions around the plant (NeSmith & Duval, 1998; Llewellyn et al., 2016; Dumroese & Haase, 2018).

Although several studies have explored the effects of manure tea on vegetable growth (Radovich & Arancon, 2011; González-Hernández et al., 2023; Sunaryo et al., 2023), there is still limited standardized guidance on the appropriate concentration and application frequency of manure tea for solanaceous crops like eggplants. Addressing these knowledge gaps through empirical research will provide clearer

guidelines for selecting the best polybag size and manure tea application rate for eggplants. Further, it may provide practical recommendations for urban agricultural practices.

This study aimed to fill these gaps by examining the two-factor effects of polybag size and buffalo manure tea (BMT) application levels on the vegetative, reproductive, and postharvest quality of polybag-grown eggplants in the local environmental conditions of Arakan, Cotabato. It specifically investigated how polybag size influences eggplant growth and yield, the effects of varying BMT levels on eggplant performance, and the interaction between polybag size and manure tea application.

2. Materials and Methods

2.1. Duration and location of the study

This open-field experiment was conducted from January to April 2024 at the agricultural experimental area of Cotabato Foundation College of Science and Technology, Arakan, Cotabato, Philippines.

2.2. Experimental design, treatments, and field layout

This open-field study employed a factorial arrangement in Randomized Complete Block Design (RCBD), with three polybag sizes (30.48 x 35.56 cm, 35.56 x 35.56 cm, 40.64 x 40.64 cm) as factor A and eight buffalo manure tea levels (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, and 1.75 liters per liter of water) as factor B. The design included 24 treatment combinations, three replicates, and 10 plants per treatment (Table 1). A total of 72 plots (3.0 x 1.25 m) were established, with 0.5 m spacing between plots, covering an area of 625 m² (62.5 x 10 m). Each plot had two rows, with six hills per row, and a planting distance of 20 inches (50 cm) between hills and 30 inches (75 cm) between rows.

Table 1. Summary of this two-factor experimental design

Factor B: Buffalo manure tea levels (liters of BMT per liter of H ₂ O)	Factor A: Polybag sizes (cm)		
	A1 (30.48 x 35.56 cm polybag)	A2 (35.56 x 35.56 cm polybag)	A3 (40.64 x 40.64 cm polybag)
B1 (0 liter)	A1B1 (30.48 x 35.56 cm polybag; 0 L BMT/L H ₂ O)	A2B1 (35.56 x 35.56 cm polybag; 0 L BMT/L H ₂ O)	A3B1 (40.64 x 40.64 cm polybag; 0 liter of BMT/L H ₂ O)
B2 (0.25 liter)	A1B2 (30.48 x 35.56 cm polybag; 0.25 L BMT/L H ₂ O)	A2B2 (35.56 x 35.56 cm polybag; 0.25 L BMT/L H ₂ O)	A3B2 (40.64 x 40.64 cm polybag; 0.25 L BMT/L H ₂ O)
B3 (0.50 liter)	A1B3 (30.48 x 35.56 cm polybag; 0.50 L BMT/L H ₂ O)	A2B3 (35.56 x 35.56 cm polybag; 0.50 L BMT/L H ₂ O)	A3B3 (40.64 x 40.64 cm polybag; 0.50 L BMT/L H ₂ O)
B4 (0.75 liter)	A1B4 (30.48 x 35.56 cm polybag; 0.75 L BMT/L H ₂ O)	A2B4 (35.56 x 35.56 cm polybag; 0.75 L BMT/L H ₂ O)	A3B4 (40.64 x 40.64 cm polybag; 0.75 L BMT/L H ₂ O)
B5 (1.00 liter)	A1B5 (30.48 x 35.56 cm polybag; 1.00 L BMT/L H ₂ O)	A2B5 (35.56 x 35.56 cm polybag; 1.00 L BMT/L H ₂ O)	A3B5 (40.64 x 40.64 cm polybag; 1.00 L BMT/L H ₂ O)
B6 (1.25 liters)	A1B6 (30.48 x 35.56 cm polybag; 1.25 L BMT/L H ₂ O)	A2B6 (35.56 x 35.56 cm polybag; 1.25 L BMT/L H ₂ O)	A3B6 (40.64 x 40.64 cm polybag; 1.25 L BMT/L H ₂ O)
B7 (1.50 liters)	A1B7 (30.48 x 35.56 cm polybag; 1.50 L BMT/L H ₂ O)	A2B7 (35.56 x 35.56 cm polybag; 1.50 L BMT/L H ₂ O)	A3B7 (40.64 x 40.64 cm polybag; 1.50 L BMT/L H ₂ O)
B8 (1.75 liters)	A1B8 (30.48 x 35.56 cm polybag; 1.75 L BMT/L H ₂ O)	A2B8 (35.56 x 35.56 cm polybag; 1.75 L BMT/L H ₂ O)	A3B8 (40.64 x 40.64 cm polybag; 1.75 L BMT/L H ₂ O)
Field layout			
a. Parameter	Specification		
b. Plot dimensions	3.0 x 1.25 m		
c. Total plots	72		
d. Total area	625 m ²		
e. Spacing between plots	0.5 m		
f. Planting distance	50 cm (hills) 75 cm (rows)		
Number of replicates	3		
Number of plants per treatment	10 plants per treatment		
Treatment combinations (factor A x factor B)	3 x 8 = 24 treatment combinations		
Total number of plant population (treatment combination x replication x number of plants per treatment)	24 x 3 x 10 = 720 plants		

2.3. Sowing and transplanting

Eggplant seeds (var. ‘Dumaguete Long’) from East West Seed Company were sown in trays and transplanted after four weeks when they have

already produced four (4) leaves. The growing media consisted of a 2:2:1 mixture of garden soil, aged rice hull, and vermicompost, with each polybag containing 6.5 kg.

2.4. Preparation of growing media and buffalo manure tea

The planting media in the polybags was modified using a 2:2:1 ratio of ordinary garden soil, aged rice hull, and vermicompost, with each polybag containing 6.5 kg of the mixture. Buffalo manure tea was prepared following the DAATI (2011) guidelines by placing partially dried

buffalo manure in a jute sack, creating a "tea bag." This was submerged in a 200 L plastic drum, weighed down with stones, and covered with a net. Water was added, leaving a 20% air gap, and the mixture was soaked for one week to produce a rich, dark brown manure tea. The chemical test report for the growing media and buffalo manure tea is presented in Table 2.

Table 2. Chemical test results on growing media and buffalo manure tea (analysed at Department of Agriculture, Caraga Region, Regional Soils Laboratory)

Constituents (content)	Oven-dried basis		Methodology
	Growing media	Buffalo manure tea	
Total Nitrogen (N), %	0.54	BDL	<i>Kjeldahl Method</i>
Total Phosphorus (P_2O_5), %	0.38	7.00	<i>Vanadomolybdate Method</i>
Total Potassium (K_2O), %	0.28	495.00	<i>Aqua-regia digestion</i>
Moisture Content, %	18.51	-	<i>Gravimetric Method</i>
pH (1:10, sample:water (water by volume))	7.41	7.60	<i>Potentiometric Method</i>
Sodium (Na)*, ppm	368.00	77.00	<i>Aqua-regia digestion</i>
Calcium (Ca)*, ppm	5047.00	68.00	<i>Aqua-regia digestion</i>
Magnesium (Mg)*, ppm	2592.00	44.00	<i>Aqua-regia digestion</i>
Zinc (Zn)*, ppm	12.00	2.00	<i>Aqua-regia digestion</i>
Iron (Fe)*, ppm	57967.00	11.00	<i>Aqua-regia digestion</i>
Copper (Cu)*, ppm	58.00	3.00	<i>Aqua-regia digestion</i>
Manganese (Mn)*, ppm	13268.00	10.00	<i>Aqua-regia digestion</i>
Molybdenum (Mo)*, ppm	72495.00	355.00	<i>Aqua-regia digestion</i>
Lead (Pb)*, ppm	BDL	BDL	<i>Aqua-regia digestion</i>
Chromium (Cr)*, ppm	1297.00	BDL	<i>Aqua-regia digestion</i>
Nickel (Ni)*, ppm	1.00	1.00	<i>Aqua-regia digestion</i>
Cadmium (Cd)*, ppm	BDL	BDL	<i>Aqua-regia digestion</i>

*Remarks: To convert ppm to %: Starting from the decimal point, count four (4) places to the left and affix the decimal point (Example: 10.50 ppm = 0.00105%. BDL = below detection limit. *Microwave-assisted acid digestion and determination by Microwave Plasma-Atomic Emission Spectrometer.*

2.5. Rate and frequency of application of manure tea

This research followed DAATI (2011) guidelines, applying 250 mL of undiluted buffalo

manure tea (BMT) to the growing media one day before transplanting to provide micronutrients and boost microbial activity. After plant establishment, BMT was applied as a foliar spray every 14 days, ensuring full coverage of the eggplant leaves

throughout their growth stages. BMT, in fact, is a liquid organic fertilizer that is effective in improving soil and plant health (Leauthaud et al., 2022; Ramírez-Gottfried et al., 2023).

2.6. Care and maintenance of the experimental plants

Irrigation was conducted twice a day, with plants receiving 500 mL of water per day before flowering, increasing to 1,000 mL per day once flowering began. Weeds and insect pests were controlled manually through handpicking.

2.7. Data collection and monitoring

Growth parameters such as plant height, number of leaves, stem circumference, and leaf area were measured weekly. Yield indicators included fruit diameter, weight, and total yield per plant were gathered during harvest time. Plant height (cm) was measured weekly from the base of the stem to the tip using a ruler; number of leaves was counted weekly for all plants in a treatment group; stem diameter (cm) was measured at the middle part of the plant using a caliper; leaf area (cm²) was determined using the Easy Leaf® digital application; days to fruit setting was recorded from transplanting until the first visible fruit formation; fruit diameter (cm) was measured using a caliper; number of marketable fruits per plant was counted at each harvest; weight (g) of marketable fruits was measured using a digital scale; and the total yield per plant (kg) was calculated by summing the total weight of harvested fruits per plant.

2.8. Harvesting and storing

Sample fruits were collected weekly at harvestable size or commercial maturity, starting at nine weeks (63rd day) after transplanting. Five fruits per treatment, ideally uniform in size and weight, were placed on paper plates and kept at ambient room temperature for less than a day to collect the different data with minimal

statistical errors. Please add how to measure/weight the fruits.

2.9. Statistical data analysis

Data were analyzed using the statistical tool for agricultural research (STAR-2.0.1) through analysis of variance (ANOVA). Significant results from the quantitative treatments were further analyzed using Pairwise Mean Comparison, specifically the least significant difference (LSD).

3. Results and Discussion

The summary of analysis of variance (Table 3), on the one hand, highlighted the significant impact of polybag sizes (factor A) on various growth and reproduction parameters of polybag-grown eggplants. However, the buffalo manure tea levels (factor B) and the interaction between polybag sizes and BMT did not show significant effects on these parameters. Table 4, on the other, presents the significant and highly significant effects of polybag sizes on the plant height, number of leaves, and stem diameter of polybag-grown eggplants across various weeks after transplanting.

Similar studies have demonstrated that larger containers allow for better root expansion, improved water retention, and increased nutrient availability, resulting in enhanced plant growth (NeSmith & Duval, 1998; Bouzo & Favaro, 2015). However, the lack of significant effects from BMT application could be attributed to the nutrient sufficiency of the growing media used (2:2:1 garden soil, aged rice hull, vermicompost), which may have already provided adequate macro- and micronutrients for plant development.

Also, studies on manure tea application in other crops (Gad et al., 2023; Ramírez-Gottfried et al., 2023) suggest that its effects are more pronounced when applied to nutrient-deficient

soils. The neutral pH and relatively low nitrogen content of the BMT used in this study might have further contributed to its limited influence on growth parameters. Future research should explore varying concentrations of BMT or combinations with other organic amendments to assess their cumulative effects on polybag-grown vegetables.

Plant height (cm): Measured weekly for 13 weeks, plant height (Table 4) showed significant variation starting from the third week after transplanting, with steady growth from weeks 2 to 7, followed by slower growth as flowering and fruiting began. BMT application and the interaction between polybag sizes and BMT levels had no significant effect on plant height (5 and 1%, LSD). Only polybag size significantly impacted height (Table 3), with larger polybags (40.64 x 40.64 cm) producing the highest/tallest eggplants. These results align with recommendations from various studies on the advantages of larger container sizes for plant growth (Cotner, 2008; Lerner, 2009; Roll & Wilson, 2020).

Number of leaves: The weekly observations in Table 4 showed significant differences in leaf

number due to polybag size (5 and 1%, LSD), especially from weeks 4 to 13. Eggplants grown in 40.64 x 40.64 cm polybags had the highest leaf counts. Neither BMT levels nor the interaction between BMT and polybag sizes significantly affected leaf production (Table 3). Larger polybags promote better vegetative growth, such as more leaves, likely due to better root growth and moisture retention, even though all polybags contained nearly the same number of growing media (6.5 kg) (NeSmith & Duval, 1998; Bouzo & Favaro, 2015).

Stem diameter (cm): Polybag size significantly affected stem diameter (Table 4), with highly significant differences observed in all weeks except week 9 (5 and 1%, LSD). The interaction between BMT levels and polybag sizes, as well as the individual effect of BMT, had no impact on stem diameter.

The r coefficient (Table 3): Eggplants grown in 35.56 x 35.56 cm or 40.64 x 40.64 cm polybags showed similar stem diameters, suggesting that both sizes supported comparable stem growth. Smaller polybags, however, may limit vegetative growth and root development (Bouzo & Favaro, 2015; UT, 2015).

Table 3. Summary of analysis of variance of the polybag sizes (factor A), buffalo manure tea (BMT) levels (factor B) and their interactions on growth and reproductive parameters of polybag-grown eggplant in Arakan, Cotabato, Philippines

Parameters	Polybag sizes (A)	Levels of BMT (B)	Interaction (A x B)
Average plant height (cm)	** LSD	ns	ns
Average number of leaves	** LSD	ns	ns
Average stem diameter (cm)	** LSD	ns	ns
Leaf area (cm ²)	** LSD	ns	ns
Days to fruit setting	* LSD	ns	ns
Fruit diameter (cm)	* LSD	ns	ns
Number of marketable fruits	** LSD	ns	ns
Weight of marketable fruits (g)	** LSD	ns	ns
Total yield / plant (kg)	** LSD	ns	ns

** : highly significant @ 1%; * : significant @ 5%; ns : not significant; LSD : least significant difference test.

Table 4. Significant and highly significant effects of polybag sizes on the plant height (cm); number of leaves; and stem diameter (cm) of polybag-grown eggplants in Arakan, Cotabato, Philippines

Weeks after transplanting												
Plant height (cm)												
Polybag sizes _{1/}	1 ^{ns}	2 ^{**}	3 ^{**}	4 ^{**}	5 ^{**}	6 ^{**}	7 ^{**}	8 ^{**}	9 [*]	10 ^{**}	11 ^{**}	12 ^{**} 13 ^{**}
A ₁ (30.48 x 35.56 cm)	1.99	3.72 ^b	6.29 ^b	12.10 ^b	19.45 ^b	24.13 ^b	32.67 ^b	33.88 ^b	36.22 ^b	38.77 ^b	40.39 ^b	42.63 ^b 44.49 ^b
A ₂ (35.56 x 35.56 cm)	1.96	4.19 ^a	7.56 ^a	14.10 ^a	22.11 ^a	26.51 ^a	35.29 ^a	36.48 ^a	38.72 ^a	41.34 ^a	42.89 ^a	44.85 ^a 46.73 ^a
A ₃ (40.64 x 40.64 cm)	1.88	4.04 ^{ab}	7.40 ^a	14.09 ^a	21.04 ^a	25.67 ^a	34.24 ^a	36.08 ^a	38.31 ^a	40.88 ^a	42.87 ^a	45.01 ^a 46.64 ^a
CV (%)	20.31	14.74	14.81	13.01	9.13	7.49	6.71	6.22	6.34	6.06	6.00	5.97 5.64
Number of leaves (Leaves)												
Polybag sizes _{1/}	1 ^{**}	2 ^{ns}	3 ^{ns}	4 ^{**}	5 ^{**}	6 ^{**}	7 ^{**}	8 ^{**}	9 [*]	10 ^{**}	11 ^{**}	12 ^{**} 13 ^{**}
A ₁ (30.48 x 35.56 cm)	5.38 ^b	6.70	6.23	9.18 ^b	10.00 ^b	9.51 ^b	14.99 ^b	19.51 ^b	20.85 ^c	24.22 ^b	27.27 ^b	24.77 ^c 23.10 ^b
A ₂ (35.56 x 35.56 cm)	5.85 ^a	6.92	6.58	9.37 ^{ab}	10.50 ^a	9.92 ^a	15.42 ^a	20.13 ^a	22.15 ^b	24.78 ^b	28.57 ^b	26.98 ^b 23.51 ^b
A ₃ (40.64 x 40.64 cm)	5.65 ^{ab}	6.65	6.54	9.46 ^a	10.57 ^a	9.85 ^a	15.33 ^a	20.46 ^a	25.33 ^a	29.93 ^a	34.43 ^a	32.54 ^a 28.39 ^a
CV (%)	8.34	8.28	8.08	4.15	4.32	6.03	3.09	3.03	9.29	12.44	10.46	13.37 13.39
Stem diameter (cm)												
Polybag sizes _{1/}	1 ^{**}	2 ^{**}	3 ^{**}	4 ^{**}	5 ^{**}	6 ^{**}	7 ^{**}	8 ^{**}	9 [*]	10 ^{**}	11 ^{**}	12 ^{**} 13 ^{**}
A ₁ (30.48 x 35.56 cm)	0.23 ^b	0.37 ^b	0.57 ^b	0.82 ^b	0.91 ^b	0.93 ^b	0.95 ^b	0.99 ^b	1.02 ^b	1.04 ^b	1.06 ^b	1.10 ^b 1.13 ^b
A ₂ (35.56 x 35.56 cm)	0.30 ^a	0.48 ^a	0.68 ^a	0.87 ^a	0.93 ^b	0.95 ^b	0.97 ^b	1.02 ^a	1.06 ^a	1.09 ^a	1.11 ^a	1.14 ^a 1.16 ^a
A ₃ (40.64 x 40.64 cm)	0.29 ^a	0.46 ^a	0.65 ^a	0.89 ^a	0.95 ^a	0.97 ^a	0.99 ^a	1.04 ^a	1.07 ^a	1.10 ^a	1.13 ^a	1.16 ^a 1.18 ^a
CV (%)	14.29	13.54	12.17	4.52	3.35	3.46	3.97	5.34	6.00	6.06	5.54	4.58 4.54

*: means in the same column with common letter manuscript do not significantly differ at 5%, LSD; **: means in the same column with common letter manuscript do not significantly differ at 1%, LSD; n.s.: not significant.

Leaf area (cm²): Significant differences (Table 5) in the leaf area of the crops were caused by the varying polybag sizes used in the study (1% level, LSD Test), but not by the individual effect of manure tea nor by the interaction of polybag sizes and levels of manure tea. The results in Table 5 agree with what authors (Bouzo & Favaro, 2015; Oagile et al., 2016) have posited about the enhanced growth and development of solanaceous crops like eggplant.

Number of days to fruit setting: The interaction between polybag sizes and BMT levels, as well as the individual effect of BMT, did not significantly affect (Table 5) the number of days to fruit setting. The interaction effect ranged from 43.67 to 47.33 days, while the individual effect of BMT ranged from 44.78 to 46.89 days. Only polybag size had a significant impact (Table 3) on fruit setting (5% level, LSD Test), with eggplants in larger polybags being the first to produce buds that developed into fruits. Plants with more leaves, branches, and a sturdier, taller structure are more likely to bear flowers and fruits earlier. Fruit setting is influenced by the availability of macro- and micronutrients, and trace elements (Tisdale et al., 1993), which are present in the manure tea and growing media, albeit in smaller quantities (DARSL, 2020a; 2020b).

Fruit diameter (cm): Polybag size significantly affected (Table 5) fruit diameter (5% level, LSD Test), while the application of BMT and the interaction between polybag sizes and BMT had no significant impact (Table 3). The interaction effect ranged from 2.70 to 3.05 cm, and the individual effect of BMT ranged from 2.77 to 2.95 cm. The fruits in this experiment were relatively smaller compared to field-grown eggplants (Daunay, 2016; Zaro et al., 2016). Photosynthesis, a key plant process where food

is produced and stored in fruits, shoots, stems, and leaves (Johnson, 2016; Simkin et al., 2019), was likely limited by restricted root growth, shoot expansion, and nutrient and moisture availability in the polybags, affecting fruit size and other related parameters.

Number of marketable fruits: The use of different polybag sizes significantly affected (Table 5) the number of marketable fruits (1% level, LSD Test), while the individual effect of BMT application and the interaction between polybag sizes and BMT did not have a significant impact (Table 3). The number of marketable fruits ranged from 22.89 to 28.22 for polybag sizes, and from 20 to 32.67 for the interaction effect. These results suggest that larger polybags support the expansion of vegetative parts, leading to more marketable fruits. However, the low nitrogen content in organic substances, as reflected in the manure tea and growing media (DA, 2020a; 2020b), limits the ability of eggplants to produce fruits that match the appearance of those grown with synthetic fertilizers in conventional field farming.

Weight (g) of marketable fruits: Polybag size significantly affected (Table 5) the weight of marketable fruits (1% level, LSD Test), but neither the interaction between polybag sizes and BMT levels nor the individual effect of BMT significantly influenced (Table 3) fruit weight. The interaction effect ranged from 13.62 to 28.10 g, while the individual BMT effect ranged from 17.78 to 22.29 g. These results suggest that larger polybags enable the plants to produce more and heavier fruits. Fruit weight is closely linked to moisture content, with eggplant fruits typically containing 92% water (Siemonsma & Piluek, 1994). However, the limited root growth due to polybag size restricted the plants' ability to access

moisture and nutrients, and despite frequent watering, the hot and dry conditions (January to April) led to the drying of the growing media and increased evapotranspiration (NeSmith & Duval, 1998; Bouzo & Favaro, 2015).

Total yield/plant (kg): The only significant factor (Table 5) affecting the total yield of polybag-grown eggplants was polybag size (1% level, LSD Test). The interaction between polybag sizes and BMT levels, as well as the individual effect of BMT, did not show a significant difference (Table 3), with yield ranging from 1.17 to 2.28 kg for polybag sizes and 1.50 to

1.87 kg for BMT levels. These results align with studies by Bouzo & Favaro (2015), which showed that larger containers resulted in higher total yields. However, the yields in this study were much lower compared to conventionally field-planted crops, likely due to limited root growth, restricted nutrient absorption, and exposure to drying conditions caused by wind and heat (NeSmith & Duval, 1998). Additionally, the BMT and growing media lacked essential nutrients, affecting the plants' vegetative and reproductive functions, further reducing yield of eggplant (DA, 2020a; 2020b).

Table 5. Highly significant and significant effects of polybag sizes on the growth parameters of polybag-grown eggplant applied with different levels of buffalo manure tea in Arakan, Cotabato, Philippines

Polybag sizes	Leaf area (cm ²)**	Days to fruit setting (days)*	Fruit diameter (cm)*	No. of marketable fruits (fruits)**	Weight of marketable fruits (g)**	Total yield/plant (kg)**
A1 (30.48 x 35.56 cm)	68.21 ^b	46.42 ^a	2.77 ^b	21.46 ^c	15.94 ^c	1.34 ^c
A2 (35.56 x 35.56 cm)	70.88 ^b	45.62 ^{ab}	2.87 ^{ab}	25.08 ^b	19.67 ^b	1.64 ^b
A3 (40.64 x 40.64 cm)	76.25 ^a	45.29 ^b	2.92 ^a	30.79 ^a	24.04 ^a	2.01 ^a
CV (%)	8.21	3.31	6.27	18.53	22.63	19.02

***: means with the same letter do not significantly differ, 1% LSD; **: means with the same letter do not significantly differ, 5% LSD.**

4. Conclusions

The results of this study underscore the substantial effect of polybag sizes on the growth and yield parameters of polybag-grown eggplants. Especially, the results suggest that a minimum size of 40.36 x 40.36 cm polybags, with 6.5 kg of growing media that is composed of 2:2:1 garden soil, aged rice hull, and vermicompost,

is optimal for promoting the vegetative growth and reproductive development of eggplants, particularly in urban or backyard gardening settings. However, BMT application did not have a significant effect on plant performance. This suggests that nutrient availability from the growing media was sufficient, rendering additional organic fertilization unnecessary

under the experimental conditions. These findings contribute to best practices in container gardening by emphasizing the importance of selecting appropriate polybag sizes. Future studies may investigate the effects of higher BMT concentrations, alternative organic inputs, or nutrient-deficient growing media conditions to determine more specific recommendations for organic fertilization in polybag cultivation.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Authors contribution

The corresponding author, FOEsgrina, conceptualized, conducted the experiments, analyzed the results, wrote the manuscript, and prepared the article for publication. The co-author, AABaladjay, provided the technical and intellectual support for the study.

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Effects of gamma radiation (^{60}Co) on the *in vitro* mutation of the Son La ancient chrysanthemum variety (*Chrysanthemum* sp. “Cuc Son La”)

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ABSTRACT

The Son La ancient chrysanthemum, a woody-stemmed variety, is prized for pot cultivation and bonsai, reflecting the cultural significance of chrysanthemums as symbols of longevity. This study, conducted from February to November 2024 at Nong Lam University, Ho Chi Minh City, aimed to evaluate the impact of gamma radiation (^{60}Co) on the *in vitro* propagation and mutation induction of Son La ancient chrysanthemum plants, thereby identifying the optimal radiation dose for mutation breeding. The experiment was carried out using a completely randomized design with a two-factor setup: tissue type (shoot clusters, callus) and radiation dose (0, 20, 30 and 40 Gy). Results showed significant impacts on survival, growth, and mutation frequency. The LD_{50} (lethal dose, 50%) values at 50 days after irradiation (DAI) were 36.8 Gy for shoot clusters and 16.0 Gy for callus, indicating higher callus sensitivity. In shoot clusters, 20 Gy significantly stimulated growth (6.1 shoots, 6.2 cm height, 43.4 leaves), while 40 Gy severely inhibited it (2 shoots, 1.1 cm height, 8.9 leaves); callus survival sharply declined at 20 Gy (31.1%) and reached 0% at 30 and 40 Gy. A dose of 20 Gy induced diverse morphological mutations in shoot clusters (leaf color, shape, stem), while 40 Gy caused 100% yellowing and stunting; callus primarily showed chlorophyll loss (6.9%) and shoot regeneration failure (15.6%). Statistical analysis revealed a significant tissue type-dose interaction ($P < 0.01$). A dose of 20 Gy was optimal for mutation induction in shoot clusters, whereas callus being too sensitive. This study provides new LD_{50} data, tissue-specific radiation sensitivity, and mutation potential, recommending 20 Gy for future breeding studies focusing on shoot clusters, and suggesting further investigation into shoot cluster radiation tolerance.

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1. Introduction

In the agricultural industry, alongside staple crops, the flower-growing sector has gained attention for economic potential. Chrysanthemums, particularly the Son La ancient variety, are highly favored, ranking second in the global cut flower industry. The woody-stemmed Son La ancient chrysanthemum is ideal for pot cultivation and bonsai, aligning with current ornamental trends. However, the variety requires new strains with diverse flower colors, blooming periods, and adaptability to different climates to meet consumer demands. Therefore, development of the Son La ancient chrysanthemum should focus on rapid propagation and creating mutant lines with varied flower colors, stem shapes, and leaf characteristics to meet market needs and adapt to ecological conditions, especially in southern regions.

Among the methods of inducing mutations, radiation treatment is an effective approach that allows for changes in the genetic structure and promotes a high mutation frequency through irradiation. Specifically, the use of gamma rays from a ^{60}Co source has proven effective in generating plant lines with new traits (Beyaz et al., 2017). However, factors such as the type of specimen, dosage, and irradiation duration still need further research to optimize the mutation induction process. The combination of tissue culture techniques and gamma radiation mutation treatment offers promising prospects for improving chrysanthemum varieties and targeting new mutation traits.

2. Materials and Methods

2.1. Time and place of the study

The study was conducted from February to November 2024 at the tissue culture laboratory of the Department of Physiology and Biochemistry, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam.

Gamma ray irradiation with a ^{60}Co source was performed at Ho Chi Minh City Biotechnology Center. The plant materials used in this study included shoot clusters with a diameter of approximately 1 cm and square-shaped callus samples with a side length of 0.5 cm.

2.2. Experimental design

The experiment had 2 two factors: tissue type factor (T): shoot clusters, callus and irradiation dose factor (I): 0, 20, 30 and 40 Gy. The experiment consisted of 8 treatments, with 3 replicates, arranged in a completely randomized design. Each treatment in a replicate had 2 petri dishes. Each petri dish had 10 samples.

2.3. Experimental method

The callus formation medium was MS (Murashige & Skoog, 1962) with 7 g/L agar, 30 g/L sucrose, 1.0 mg/L BA, and 0.1 mg/L NAA. The basic shoot multiplication medium was prepared using the same MS formulation, supplemented with 7 g/L agar, 30 g/L sucrose, and 1.5 mg/L BA, as identified in the previous experiments.

For *in vitro* shoot cluster samples: shoot clusters with a diameter of approximately 1 cm are cultured in petri dishes (10 cm in diameter; 2 cm in height), with each petri dish containing 20 mL of culture medium and 10 shoot clusters per

dish. The irradiation procedure was conducted at the Ho Chi Minh City Biotechnology Center. The samples were irradiated with gamma rays from a ^{60}Co gamma radiation source at a dose rate of 2.5 kGy/h. The non-irradiated treatment serves as the control. After 10 days of irradiation, the shoot clusters were transferred to the shoot multiplication medium.

For in vitro callus samples: leaves from 5-week-old in vitro plants cultured on MS medium are used, specifically the third and fourth leaves. These leaves are cut into square pieces (0.5 cm per side) and cultured in the optimal medium determined in previous experiment to induce callus formation. After 30 days, callus samples that are uniform in color are selected and cut into squares (0.5 cm per side). The samples were treated with irradiation as shoot cluster samples. After 10 days of irradiation, the calluses were transferred to the shoot multiplication medium.

2.4. Parameters

2.4.1. Determination of LD_{50} (lethal dose, 50%) value at 50 DAI (days after irradiation)

The damage rate of callus samples (%) was evaluated based on visual changes in color before and after irradiation. Observations were recorded every 10 days over a 50-day period. The survival rate of shoots and callus (%) was calculated as $(\text{number of surviving samples} / \text{total initial cultured samples}) \times 100$, recorded at 10 and 50 DAI.

2.4.2. Growth parameters at 50 DAI

For shoot clusters, the number of shoots (shoot) was counted when they reached 0.5 cm in height. Shoot height (cm) was measured from the medium surface to the highest point of the

cluster, and the total number of leaves per plant (leaf) was calculated as $(\text{total number of leaves per shoot} / \text{total number of shoots surveyed})$. For callus tissue samples, the shoot regeneration rate (%) was determined as $(\text{number of samples regenerating shoots} / \text{total initial samples}) \times 100$, and the number of shoots per sample (shoot) was calculated as $(\text{total number of shoots} / \text{the total number of samples with shoots})$.

2.4.3. Mutation parameters at 50 DAI

For shoot clusters, mutations in leaves and stems were observed, including leaf arrangement, shape, and color, as well as stem morphology and color. For callus tissue samples, mutations such as chlorophyll loss, failure to regenerate shoots, or death were recorded. The mutation frequency for both groups was calculated as $(\text{number of mutated individuals} / \text{total number of individuals}) \times 100$.

2.5. Data processing and analyses

Data were processed with Excel 2019 (Microsoft, USA). R (version 4.4.2) was used to perform ANOVA and rank the treatments with Duncan's multiple range test at $\alpha = 0.01$ or $\alpha = 0.05$. The damage rate at 10 and 50 DAI, the survival rates of shoot clusters and calluses at 10 DAI, the shoot regeneration rate, and the number of shoots at 50 DAI were transformed using the formula $\sqrt{x+0.5}$ before statistical analysis. The survival rates of shoot clusters and calluses at 50 DAI were transformed using the formula $\arcsin\sqrt{x}$ before statistical analysis, the value 0 replaced with $1/4n$ and 100 replaced with $100 - 1/4n$ (with $n = 15$ samples) before the formula $\arcsin\sqrt{x}$ was applied (Gomez & Gomez, 1984).

3. Results and Discussion

Gamma rays, a type of high-energy electromagnetic radiation, have unique characteristics that allow them to penetrate plant tissues and induce genetic mutations. The biological effects are influenced by various factors, including the source, type of radiation, dose rate, absorbed dose, and the sensitivity of tissues and organs. Gamma rays have been successfully applied to various plant species, creating superior new mutations for cultivation. Gamma radiation has diverse effects on plants, and its impact depends on factors such as dosage, exposure time, and plant species. Gamma radiation can induce beneficial mutations in plants, leading to changes in growth, development, and physiological characteristics (Bharath et al., 2024).

3.1. Survey of LD₅₀ Value

3.1.1. Effects of ⁶⁰Co gamma radiation on the damage rate of callus samples

The results in Table 1 indicate that the damage rate in the calluses is directly proportional to the radiation dose. The higher the dose, the greater the damage rate, and the damage in the callus samples increased over time.

At 10 DAI, the 40 Gy dose resulted in the highest damage rate of 51.9%, which was statistically significant compared to the control treatment (0%) and the other treatments (Figure 1).

At 20 DAI, the 40 Gy dose still showed the highest damage rate at 58.8%, with no statistically significant difference compared to the 30 Gy dose (40.1%). However, the difference was statistically significant compared to the control treatment (0.5%) and the 20 Gy treatment (34.1%).

At 30 DAI, the 40 Gy radiation dose resulted in the highest damage rate of 83.3%, with no statistically significant difference compared to the 30 Gy radiation dose (68.5%), but a highly significant difference compared to the control (2.3%) and the 20 Gy dose (56.9%).

Table 1. Effects of ⁶⁰Co gamma radiation on the damage rate (%) of callus sample

Irradiation dose (Gy)	Observation time (DAI)				
	10	20	30	40	50
0 (Control)	0 ^c	0.5 ^c	2.3 ^c	2.3 ^c	2.6 ^c
20	15.3 ^c	34.1 ^b	56.9 ^b	73.8 ^b	95.7 ^b
30	34.2 ^b	40.1 ^{ab}	68.5 ^{ab}	90.7 ^{ab}	100 ^a
40	51.9 ^a	58.8 ^a	83.3 ^a	95 ^a	100 ^a
CV (%)	22.4	24.7	12.9	10.4	7.2
F value	40.4 ^{**}	26.0 ^{**}	80.9 ^{**}	119.3 ^{**}	249.3 ^{**}

Within the same group, mean values with the same letter following indicate no significant statistical difference.
**: significant difference with $P \leq 0.01$. DAI: days after irradiation.

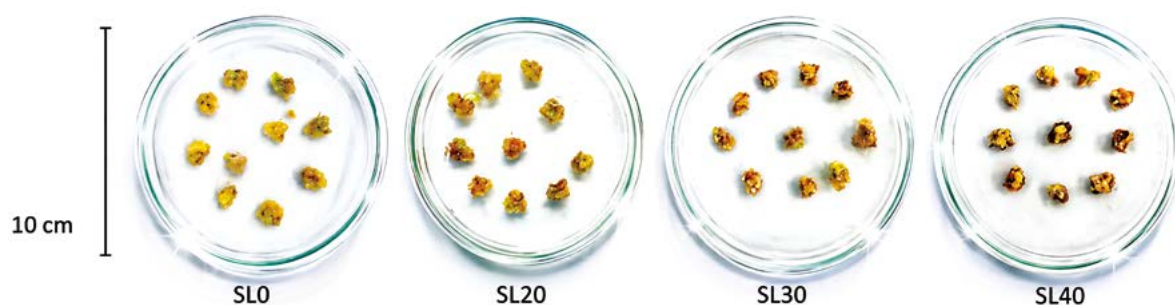


Figure 1. Callus samples at 10 days after irradiation following gamma radiation exposure.

SL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for the callus samples.

At 40 DAI, the 40 Gy radiation dose resulted in the highest damage rate of 95%, with no statistically significant difference compared to the 30 Gy dose (90.7%), but a highly significant difference compared to the control (2.3%) and the 20 Gy dose (73.8%).

At 50 DAI, both the 40 Gy and 30 Gy doses resulted in the highest damage rate of 100%, with

a highly significant difference compared to the control (2.6%) and the 20 Gy dose (95.7%).

As the radiation dose increases, the damage rate of the samples increases. At 50 DAI, the damage rate reached 100% for the 40 and 30 Gy doses, with the samples completely devoid of life.

3.1.2. Effects of ^{60}Co gamma radiation on the survival rate of Son La ancient chrysanthemum variety

Table 2. Effects of ^{60}Co gamma radiation on the survival rate (%) of Son La ancient chrysanthemum variety

Observation time	Tissue type	Irradiation dose (Gy)				Average (T)
		0 (Control)	20	30	40	
10 DAI	Shoot cluster	100 ^a	100 ^a	100 ^a	100 ^a	100 ^A
	Callus	100 ^a	95.6 ^a	86.7 ^b	75.6 ^c	89.5 ^B
	Average (I)	100 ^A	97.8 ^A	93.4 ^{AB}	87.8 ^B	
CV (%) = 3.6%; $F_I = 4.1^*$; $F_T = 15.3^{**}$; $F_{I \times T} = 4.1^*$						
50 DAI	Shoot cluster	100 ^a	100 ^a	57.8 ^b	46.7 ^c	76.1 ^A
	Callus	100 ^a	31.1 ^d	0 ^e	0 ^e	32.8 ^B
	Average (I)	100 ^A	65.6 ^B	28.9 ^C	23.4 ^C	
CV (%) = 4.9%; $F_I = 1048.6^{**}$; $F_T = 1370.4^{**}$; $F_{I \times T} = 159.5^{**}$						

*Within the same group, the mean values with the same letter following indicate no statistically significant difference. *: significant difference at $P \leq 0.05$; **: significant difference at $P \leq 0.01$. DAI: days after irradiation.*

The results in Table 2 at 10 DAI indicate that, considering the tissue type factor, the survival rate of the Son La ancient chrysanthemum variety in shoot cluster samples was higher at 100%, with a statistically significant difference at $P < 0.05$ compared to callus samples (89.5%). Regarding the irradiation dose factor, the survival rate of the non-irradiated samples were the highest at 100%, showing a statistically significant difference at $P < 0.01$ compared to the survival rate at 40 Gy (87.8%). In terms of the interaction between tissue type and irradiation dose, the survival rate of the non-irradiated samples (control) and the shoot cluster samples irradiated at 20, 30, and 40 Gy remained at 100%, whereas the callus samples irradiated at 40 Gy had a significantly lower survival rate of only 75.6%, with a statistically significant difference at $P < 0.05$ (Figure 2).

At 50 DAI, regarding the tissue type factor, the survival rate of the Son La ancient chrysanthemum variety in shoot cluster samples was higher at 76.1%, showing a statistically significant difference at $P < 0.01$ compared to callus samples (32.8%). In terms of the irradiation dose factor, the non-irradiated samples showed the highest survival rate at 100%, with a statistically significant difference at $P < 0.01$ compared to the survival rate at 40 Gy (23.4%). Regarding the interaction between tissue type and irradiation dose, the survival rate of the non-irradiated samples (control) and the shoot cluster samples irradiated at 20 Gy remained at 100%, whereas the callus samples irradiated at 40 Gy had a significantly lower survival rate of only 23.4%, with a statistically significant difference at $P < 0.01$.

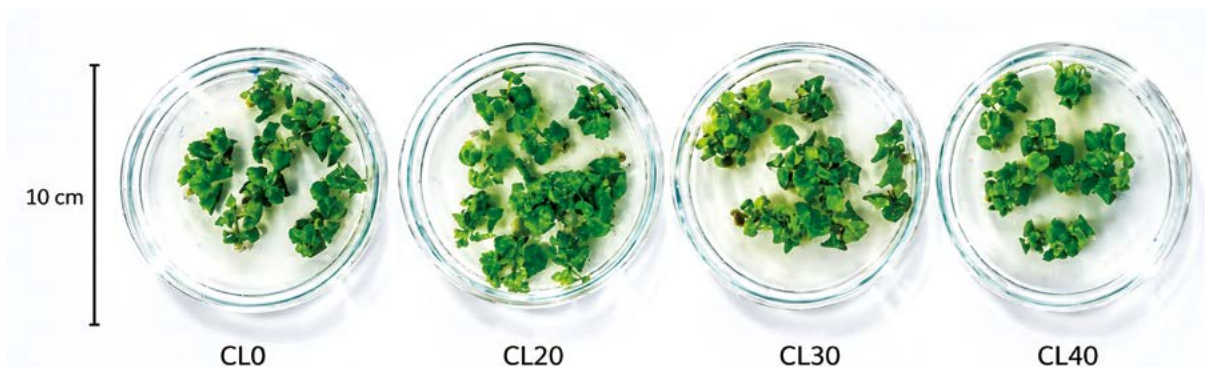


Figure 2. Shoot cluster samples at 10 days after irradiation following gamma radiation exposure.

CL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for shoot clusters samples.

These findings highlight the dose-dependent sensitivity of callus tissue. At 10 DAI, survival gradually decreased with increasing radiation doses, whereas by 50 DAI, callus samples exposed to 30 and 40 Gy were completely nonviable, turning brown-black due to pigmentation loss. This confirms that the lethal irradiation dose for callus tissue is 30 Gy, whereas shoot clusters exhibit greater resistance to radiation stress.

Physiologically, high irradiation doses affect the cell wall and membrane, disrupting the exchange of water, minerals, and growth regulators (Le & Vu, 2021). When gamma irradiation doses increase, the mortality rate in chrysanthemum varieties can rise significantly.

Vucic et al. (2006) indicated that irradiation doses ranging from 20 to 50 Gy can cause high

mortality in plant individuals under *in vitro* conditions. The mortality rate can reach up to 100% at excessively high doses, rendering the plants unable to develop.

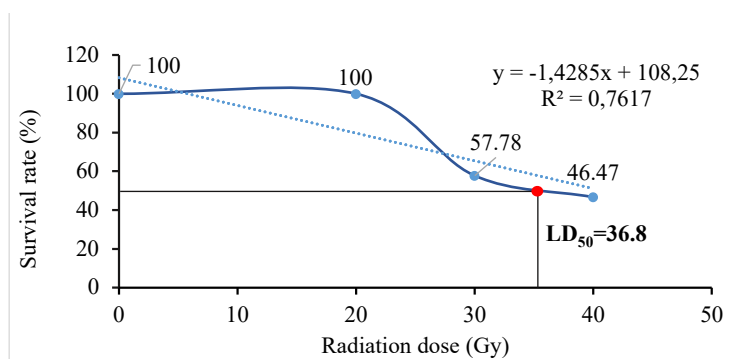


Figure 3. The correlation between survival rate and irradiation dose of shoot cluster samples at 50 days after irradiation.

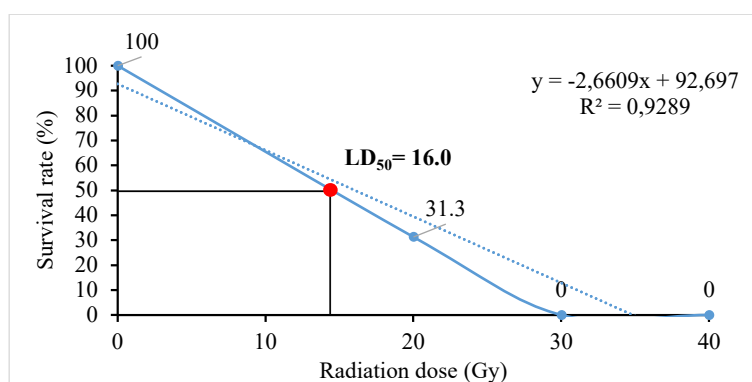


Figure 4. The correlation between survival rate and radiation dose of callus samples at 50 days after irradiation.

The results from Figures 3 and 4 allow for the determination of the LD_{50} values for Son La ancient chrysanthemum samples at 50 DAI. For the shoot cluster sample, the LD_{50} is 36.8 Gy, while for the callus sample, it is 16.0 Gy.

These experimental results confirm that the effects of gamma radiation vary depending on the plant species and the type of tissue. Determining

the LD_{50} value provides a basis for selecting the most effective radiation dose for inducing mutations, achieving a high mutation frequency while ensuring the survival and viability of the mutated plants meets the required standards.

3.2. The effect of gamma radiation from ^{60}Co source on the growth of Son La ancient chrysanthemum *in vitro*

Table 3. The effect of gamma radiation from ^{60}Co source on the growth of Son La chrysanthemum shoot clusters *in vitro* at 50 days after irradiation

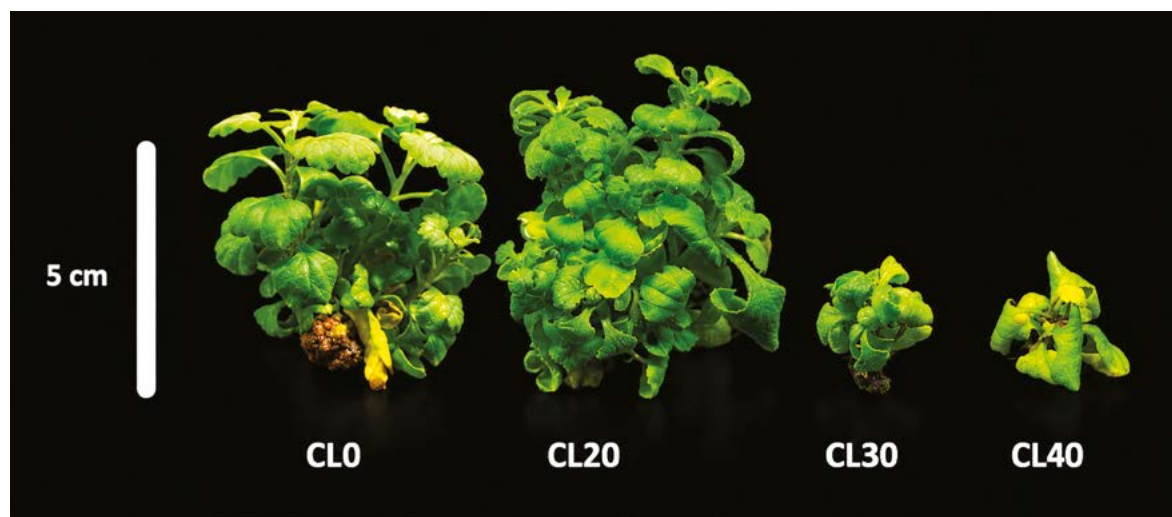
Irradiation dose (Gy)	Observed indicators		
	Number of shoots (shoots)	Shoot height (cm)	Total number of leaves (leaves)
0 (Control)	3.3 ^b	5.7 ^a	21.5 ^b
20	6.1 ^a	6.2 ^a	43.4 ^a
30	3.0 ^{bc}	1.7 ^c	11.7 ^c
40	2.0 ^c	1.1 ^c	8.9 ^c
CV (%)	10.6	17.6	13.7
F value	62.9 ^{**}	50.5 ^{**}	88.0 ^{**}

Within the same group, mean values followed by the same letter indicate no statistically significant difference.

^{**}: significant difference at $P \leq 0.01$.

From the results in Table 3, there was a highly significant difference in the number of shoots, shoot height, and number of leaves among the irradiation doses. The highest number of shoots was observed at the 20 Gy dose, reaching 6.1 shoots, which was statistically significantly different from the control (3.3 shoots) and the other treatments (Figure 5). The formation of new shoots is typically

influenced by growth regulators such as cytokinin and auxin in the culture medium. All treatments were affected by these substances to induce shoot proliferation. However, different irradiation doses led to variations in shoot numbers. In other words, irradiation doses caused damage to some lateral buds, affecting shoot formation (Nguyen et al., 2014).

**Figure 5.** Shoot cluster samples at 50 days after irradiation.

CL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for shoot cluster samples.

Lal et al. (2024) indicated that low-dose irradiation (approximately 10 to 20 Gy) can significantly stimulate the growth of chrysanthemum varieties. Chrysanthemum plants irradiated at low doses generally exhibited better growth compared to the non-irradiated control group.

Regarding shoot height, an increase in irradiation dose resulted in a decrease in shoot height. The lowest shoot height was recorded at 40 Gy (1.1 cm) compared to the control (5.7 cm) and other treatments. The highest shoot height was 6.2 cm at the 20 Gy dose, which was not significantly different from the control (5.7 cm) but was highly significantly different from the 30 (1.7 cm) and 40 Gy (1.1 cm) doses. The variations in shoot height at different irradiation doses after 50 days indicate that irradiation caused cellular damage, affecting cell division and expansion, leading to reduced shoot height at higher irradiation doses.

The increase in the number of leaves per shoot also varied among the treatments. However, strong responses to high-dose irradiation resulted in morphological variations such as

smaller leaves and shorter internodes. The highest number of leaves was observed in the control (0 Gy), reaching 43.4 leaves at the 20 Gy dose, which was significantly different from the control (21.5 leaves) and other irradiation doses. Research by Wang et al. (2020) showed that as the irradiation dose increased (above 30 Gy), negative effects started to appear. Irradiation doses higher than 35 Gy significantly reduced shoot height and leaf number.

During *in vitro* culture after irradiation, shoot cluster samples exposed to 30 and 40 Gy doses suffered severe damage to the apical meristem due to radiation effects, preventing further development. At the 40 Gy dose, samples ceased growing and gradually died over time, showing almost no growth or new leaf formation. This confirms that irradiation at 30 and 40 Gy negatively affected the growth and development of Son La ancient chrysanthemum shoot clusters. However, the highest shoot growth was observed at the 20 Gy dose. Gamma irradiation at 20 Gy was found to be the optimal condition for promoting the development of Son La ancient chrysanthemum shoots, leading to stronger growth compared to higher irradiation doses.

Table 4. The effect of gamma radiation from ^{60}Co source on the shoot regeneration rate and number of shoots of callus samples at 50 days after irradiation

Irradiation dose (Gy)	Observed indicators	
	Shoot regeneration rate (%)	Number of shoots (shoots/sample)
0 (Control)	100 ^a	5.5 ^a
20	46.7 ^b	3.12 ^b
30	0 ^c	0 ^c
40	0 ^c	0 ^c
CV (%)	13.7	11.2
F value	163.5**	87.7**

Within the same group, mean values followed by the same letter indicate no statistically significant difference.

** : Significant difference at $P \leq 0.01$.



Figure 6. Callus samples at 50 days after irradiation.

SL: the corresponding radiation doses of 0, 20, 30, or 40 Gy for callus samples.

In plant cell culture, especially in callus culture, mutations often occur at a certain frequency. However, the mutation frequency in somatic cells during plant cell culture is typically very low (Le et al., 2012). Therefore, some scientists have used artificial mutation induction through gamma rays on callus material to increase the mutation frequency, yielding some promising results. This experiment also used callus as the starting material.

The results in Table 4 indicate that at 50 DAI, the highest shoot regeneration rate and number of shoots were observed in the control group, with statistically significant differences compared to the treatments with mutation induction. The 20 Gy irradiation dose resulted in a shoot regeneration rate of 46.7% and an average of 3.12 shoots, showing a highly significant difference compared to the 30 Gy and 40 Gy doses. Notably, no shoot regeneration was observed at 30 Gy and 40 Gy (Figure 6).

3.3. Effect of gamma radiation on the *in vitro* mutation potential of Son La ancient chrysanthemum *in vitro* at 50 DAI

Gamma radiation can cause significant changes in morphological characteristics such

as plant height, leaf area, and leaf number. These changes not only slow down plant growth but can also lead to defects in the structure of leaves and stems, affecting photosynthetic capacity and overall survival (Riviello-Flores et al., 2022).

During the observation, Table 5 indicated that at the 20 Gy irradiation dose, many morphological mutations were clearly expressed through color variations (dark green, light green), with less leaf lobing and tight internodes.

At 50 DAI, Son La chrysanthemum shoot clusters exhibited leaf and stem mutations at doses ranging from 20 to 40 Gy. Observations revealed differences in shape between the irradiated plants and the control plants, such as darker leaves, yellowing leaves, fewer lobes on the leaf edges, tight internodes, and lack of height growth. The frequency of leaf morphological mutations due to *in vitro* irradiation was relatively high, with leaf color being particularly sensitive to gamma rays, leading to various chlorophyll mutations within the 20 - 40 Gy dose range.

At the 20 Gy dose, several mutations were observed, particularly small leaves and tight internodes, with high frequencies (35.4 and 24.5%), along with other forms such as darker

leaves, lighter leaves, and stems with grooves at lower frequencies. The 30 Gy dose resulted in dark leaves and tight internodes with high frequencies (27.8 and 34.2%), but caused slower growth in terms of height, shoot number, and leaf number. The 40 Gy dose had a negative impact on growth potential, damaging the apical meristem and preventing the development of stems, leaves, and new shoots, while leaves turned yellow and no height growth occurred at 50 DAI.

Table 5. *In vitro* mutation patterns of shoot cluster samples at 50 days after irradiation

Irradiation dose (Gy)	Total number of shoots monitored	Mutation type	Number of mutated plants	Mutation frequency (%)
20	274	Dark green leaves	12	4.4
	274	Light green leaves	5	1.8
	274	Slightly lobed leaves	4	1.5
	274	Small leaves	97	35.4
	274	Compact internodes	67	24.5
	274	Grooved stem	2	0.7
30	79	Dark green leaves	3	3.8
	79	Light green leaves	0	0
	79	Slightly lobed leaves	2	2.5
	79	Small leaves	22	27.8
	79	Compact internodes	27	34.2
	79	Grooved stem	0	0
40	60	Yellow leaves	60	100
	60	Stunted height	60	100

For shoot cluster samples, gamma radiation with a ⁶⁰Co source at doses of 20, 30, and 40 Gy was suitable for creating a wide and diverse spectrum of mutations, with significant effects on the growth characteristics, morphological mutations, and color changes in various plant parts such as stems and leaves. However, at the 30 and 40 Gy doses, there was a relatively high mortality rate and a high rate of damage to the samples (Figures 7 and 8).

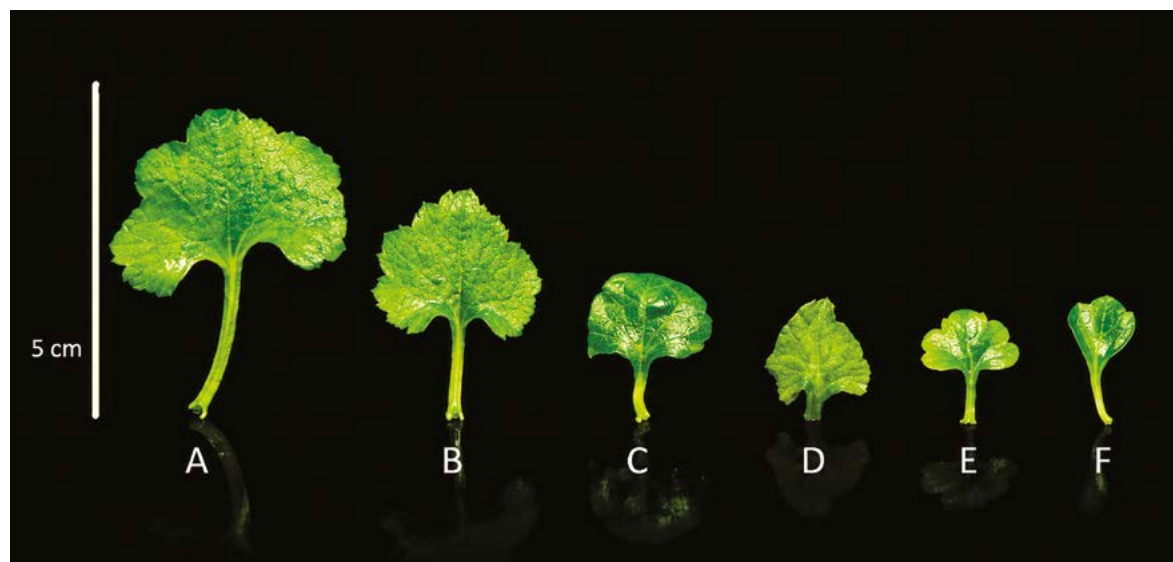


Figure 7. Some morphological leaf mutations observed in shoot cluster samples.

A) Control sample, B) Light-colored leaves, C) Yellow leaves, D) Dark-colored leaves, E) Small leaves, F) Leaves with few lobes.

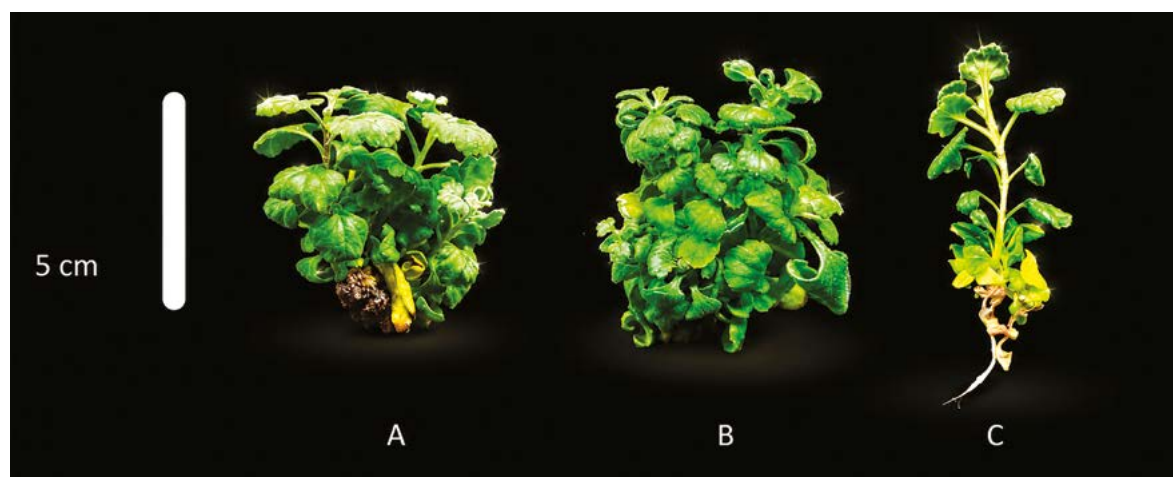


Figure 8. Some morphological stem mutations observed in shoot cluster samples.

A) Control sample, B) Tight internodes, C) Stem with grooves.

3.3.2. Effect of gamma radiation on the *in vitro* mutation potential of callus samples at 50 DAI

In plant cell culture, especially in callus culture, mutations often occur at a certain frequency. However, the mutation frequency in

somatic cells during plant cell culture is generally very low. Therefore, some scientists have used artificial mutation induction through gamma rays on callus material to increase the mutation frequency, yielding some promising results (Le & Vu, 2021).

Table 6. *In vitro* mutation potential of callus samples at 50 days after irradiation by a dose of 20 Gy

Total number of samples monitored	Mutation type	Number of mutated samples	Mutation frequency (%)
45	Callus regenerating shoots without chlorophyll	2	6.9
45	Callus failing to regenerate shoots	7	15.6
45	Callus regenerating normal shoots	5	11.1
45	Dead callus	31	68.9

In this experiment, as the irradiation dose increased from 20 to 40 Gy, the survival rate of the callus decreased over time. After 50 days of irradiation treatment, at doses ranging from 20 to 40 Gy, the callus lost its pigmentation, turning brown-black, and the survival rate of the callus was lower than that of the shoot samples at the same irradiation dose and cultivation time. This indicates that callus is more sensitive to irradiation intensity than shoot material. At the 20 Gy dose, color mutations in the shoots (loss of chlorophyll) appeared with a low frequency (6.9%). As shown in Table 6, the mortality rate of the callus was 68.9% of the total samples, with a higher percentage of non-regenerating callus (15.6%) compared to the normal shoot regenerating callus (11.1%), and the survival rate of the samples was low (31.1%). At the 30 and 40 Gy doses, the calluses died completely.

Many previous studies have also demonstrated the effects of gamma radiation from a ^{60}Co source on survival and growth in other plant species. According to Le et al. (2016), when high gamma doses were used, the survival rate of the callus decreased significantly. The negative effects of radiation can cause cellular damage and inhibit

cell division, leading to reduced survival ability of the tissue.

Therefore, it can be seen that for Son La ancient chrysanthemum, irradiation doses ranging from 20 to 40 Gy caused significant damage to the cells. While the 20 Gy dose induced chlorophyll mutations, the survival rate of the samples decreased significantly.

4. Conclusions

Gamma radiation is a valuable tool for inducing mutations in Son La ancient chrysanthemum, but its effectiveness is highly dependent on both the irradiation dose and the tissue type. The 20 Gy dose proved optimal for inducing a wide spectrum of mutations in shoot clusters while maintaining viable growth. Callus tissue exhibited extreme sensitivity to radiation, limiting its utility for mutation breeding in this variety. The significant interaction between tissue type and irradiation dose highlights the importance of considering these factors in future studies. The newly identified LD_{50} values, the documentation of specific mutation types, and the clear differences in radiation tolerance between

tissue types, provide essential information for developing new chrysanthemum varieties with enhanced ornamental characteristics and adaptability.

Conflict of interest

We certify that this is our own research work. The data and results presented in the study are genuine and have never been published in any other journal.

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Effects of copper sulphate levels on growth, yield, physiological and biochemical characteristics of mugwort (*Artemisia vulgaris* L.) on grey fertile soil in Ho Chi Minh City

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ABSTRACT

This study was conducted to investigate the influence of copper sulphate (CuSO_4) levels and spraying frequency on growth, yield and some physiological and biochemical characteristics in mugwort (*Artemisia vulgaris* L.) on grey fertile soil in Ho Chi Minh City. The two-factor experiment was arranged in a split-plot design, including nine treatments with three replicates. The main plot factor was three concentrations of CuSO_4 with 0, 400 and 800 mg/L. The sub-plot factor was the number of sprays including 1 spray (10 days after cutting: DAC), 2 sprays (10 and 20 DAC), 3 sprays (10, 20 and 30 DAC), 4 sprays (10, 20, 30 and 40 DAC). The results indicated that mugwort was treated with copper sulphate at a concentration of 800 mg/L and sprayed three times, the physiological indices, including chlorophyll a and chlorophyll b contents in the leaves, reached the highest values with 9.34 and 4.74 mg/g, respectively. In addition, the highest total flavonoid, polyphenol and essential oil content (6.32 mg RE/g, 8.86 mg GAE/g and 0.18%, respectively) were attained when compared with other copper sulphate concentration. Finally, among the graded levels of CuSO_4 application, the yield of essential oil increased progressively with increasing levels of CuSO_4 , obtaining the highest yield of essential oil at the treatment of 800 mg/L CuSO_4 , reaching 31.7 L/ha.

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1. Introduction

Mugwort (*Artemisia vulgaris* L.) is a medicinal herb which is widely used around the world. In addition to medicine, it is used as an ingredient for everyday dishes too. In Traditional Medicine, sun-dried wormwood has a bitter taste, aroma, warm properties. Besides, it also has the effect regulating blood circulation, regulating menstruation, calming pregnancy, and treating rheumatism as claimed by Vo (2012). Moreover, the stems and leaves of wormwood are rich chemical composition, which especially includes flavonoids, polyphenols, sesquiterpene lactones, phenolic acids, essential oils, coumarins, and other groups of metabolites. Recently, this species has been taken under consideration to be active toward the virus SARS-CoV-2 and disease COVID-19 (Efferth et al., 2015; Haq et al., 2020).

Plants require not only macronutrients but also vital micronutrients to grow and complete the life cycle. According to Yruela (2005), copper is a major metal for normal plant growth and development. What's more, it participates in numerous physiological processes and is an essential cofactor for many metalloproteins.

It plays a crucial role in photosynthetic and respiratory electron transport chains, in ethylene sensing, cell wall metabolism, oxidative stress protection and biogenesis of molybdenum cofactor into the bargain. Thus, deficiency in the copper supply can alter essential functions in plant metabolism (Aftab & Hakeem, 2020). Furthermore, Droux (2004) approved sulfur appears in two primary amino-acids, cysteine (Cys) and methionine (Met), essential for the primary and secondary metabolism of the plant.

Foliar nutrition promotes the efficiency of fertilizer use which help plants to absorb nutrients

quickly and reduce the lost of nutrients. The foliar application of nutrients provides a swift and efficient means of facilitating the rapid uptake of essential elements during periods of fast plant. Foliar fertilizer application is a routine measure to improve nutrients status of plants (Mengel, 2001).

The foliar application of CuSO_4 may be sprayed with the suitable concentrate and number of the sprayed liquid depending on the type of crop planted, weather conditions and growth stage of plant. If the concentration of foliar spray is high, it could cause physiological damage to cell membranes. On the contrary, if the foliar spraying concentration is too low, the plant will not absorb enough nutrients. In addition, foliar sprays many times increases production costs too.

From the above practical needs, research was conducted to determine the appropriate concentration and number of copper sulphate sprays to help mugwort achieve good productivity, quality and high economic efficiency.

2. Materials and Methods

2.1. Materials

Mugwort plants which used in the experiment were regenerated from the original crop at the Experimental Farm of the Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam. Before spraying copper sulphate to treatments in the experiment, the regenerated plants in all treatments were cut at the same height approximately 4 - 5 cm with 3 - 4 leaves.

Fertilizers were used in the experiment included copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (Cu: 25.5%, S: 12.8%); Sfarm Pb01 (SF) vermicompost of Dang Gia Trang company (0.98% N, 1.05% P_2O_5 , 0.29% K_2O); Phu My urea fertilizer (46.3% N); Lam Thao superphosphate fertilizer (16% P_2O_5 , 10% S, 2 mg Cd/kg); Vietnam General

Corporation of Agricultural Materials potassium sulphate fertilizer (50% K_2O).

Soils sample was taken and sent to the laboratory at the Research Institute for Biotechnology and Environment (RIBE) to analyze the soil physical and chemical properties.

Table 1. Physical and chemical properties of soil sample used in the experiment

Texture (%)			pH_{KCl}	Organic matter (%)	Total N (%)	Total P_2O_5 (%)	Total K_2O (%)	Cu (mg/kg)	S (%)
Sand	Loam	Clay	4.6	1.2	0.056	0.109	0.082	7.83	0.12
58.3	31.7	10.0							

Data were analyzed by the Research Institute for Biotechnology and Environment in 2004.

The main characteristics of soil properties were presented in Table 1. The data showed that the soil was classified as a sandy loam texture, highly acidic, low in soil organic matter, total nitrogen, phosphorus and potassium. Therefore, the use of lime would raise the soil pH and improve soil quality by increasing nutrient bioavailability.

2.2. Experimental design

The experiment was carried out in the Experimental Research Station at Nong Lam University, Ho Chi Minh City, Vietnam from August to November 2024.

The two-factor experiment was laid out in a split-plot design with three replicates. The main plot was the concentration of copper sulphate, with three levels of 0, 400 and 800 mg/L/spray. The sub-plot was the number of sprays including 1 spray (10 DAC), 2 sprays (10, 20 DAC), 3 sprays (10, 20 and 30 DAC), and 4 sprays (10, 20,

30 and 40 DAC). All treatments were applied the same amount fertilizer with 500 kg lime, 1000 kg vermicompost, 90 kg N, 60 kg P_2O_5 and 30 K_2O for one ha.

The plot size was 5.4 m² (3.0 m in length and 1.8 m in width). The regenerated plants of mugwort were planted at the distance of 30 x 25 cm. The distance between blocks and plots were 1.0 and 0.5 m, respectively.

2.3. Data collection and analysis

Growth parameters, yield components, yield and essential oil content were collected randomly from ten plants in the middle three rows of each base plot. In details, the plant height and stem diameter observed at 55 days after cutting (DAC), the number of primary branches before harvesting. Plant height (cm) was measured starting from the base of the stem to the tip of the highest leaf by using ruler; stem diameter (mm) was measured with a diameter tape placed around

the circumference of the stem and at 2 cm away from the base. The average fresh weight of stems and leaves (g/plant) calculated in harvest stage: Measured the average weight fresh of stems and leaves of 10 target plants, cut with a sickle at 3 cm from the ground (P1). Theoretical fresh yield (ton/ha) = $P1 \text{ (g/plant)} \times 10^{-6} \times \text{planting density (plant/ha)}$. Actual harvested yield (ton/ha) = $[\text{weight of fresh stems and leaves of each base plot (kg)} \times 10^{-3} \times 10,000] / \text{area of the base plot (m}^2\text{)}$.

Determination of polyphenol and flavonoid content were adapted to the method of Atanassova et al. (2011): 500 mg of dried leaf was dissolved in 2.5 mL methanol, shaken for 5 h, and centrifuged. For phenolic content, Folin-Ciocalteu reagent was added, incubated, and measured at 750 nm. For flavonoid content, NaNO_2 , AlCl_3 , and NaOH were added to the extract, incubated, and measured at 510 nm.

Chlorophyll a and b were evaluated according to the recommendations of Lichtenthaler (1987) which are based on absorbance measurements at different wavelengths 648 nm and 664 nm. Then, chlorophyll content was calculated using Lichtenthaler's formula: Chlorophyll a (mg/g) = $13.36A_{664} - 5.19A_{648}$; chlorophyll b (mg/g) = $27.43A_{648} - 8.12A_{664}$.

For analysis of the essential oil content of the mugwort, 100 g/pot of branches and leaves of 10 target plants, collected at the time point when the plants had buds and begun to bloom, was used. Essential oil content was calculated by the percentage of essential oil obtained from the mass of the starting material after being extracted by steam distillation (Nguyen & Pham, 2001). The calculation was based on the formula at the harvesting stage (65 DAC): Content of essential oil in leaves, stems (%) = $(\text{volume of essential}$

oil)/(weight of sample) $\times 100$. Yield of essential oil (L/ha): Calculated as essential oil content of leaves and stems (mL/100 g) $\times$ actual fresh yield (ton/ha) $\times 10^3$.

For the analysis of the economic efficiency, total cost (VND/ha per crop) = seedlings + fertilizers + pesticides + labor + electricity and water; total revenue (VND/ha per harvest) = essential oil yield (L/ha) $\times$ retail price (VND/kg); total profit (VND/ha per harvest) = total revenue - total cost, and benefit - cost ratio = total profit/Total cost, were measured.

All variables were subjected to analysis of variance (SPD) using mixed models of R.4.2.2. Differences between treatments were tested using the least significant difference (LSD) test at the probability of 0.05.

3. Results and Discussion

3.1. Effect of copper sulphate levels and sprayed frequency on growth of mugwort

The data presented in Table 2 clearly showed that copper sulphate significantly affected plant height (cm) and stem diameter (mm). The highest vegetative growth characters were recorded in 55 DAC with amount of 800 mg/L since the differences between 0 and 400 mg/L were insignificant. The highest mean value of plant height (97.9 cm) was recorded with the treatment of 800 mg/L of CuSO_4 , while the greatest stem diameter (9.0 mm) was recorded in 400 or 800 mg/L of CuSO_4 . The lowest mean values of vegetative growth characters were obtained as a result of the control. On the other hand, copper sulphate at 1, 2, 3 or 4 sprays as between the number of copper sulphate sprays and the concentration sprays had not effects on the height and stem diameter of plant.

Table 2. Effect of copper sulphate levels and spraying frequency on growth of mugwort at 65 days after cutting

Parameters	Treatments					Average A
	(A)	1	2	3	4	
Plant height (cm)	Control	88.1	90.5	90.9	90.8	90.1 ^B
	400 mg/L	91.9	92.9	93.0	92.2	92.5 ^B
	800 mg/L	96.7	97.1	99.0	98.7	97.9 ^A
	Average B	92.2	93.5	94.3	93.9	
CV (%) = 4.0; $F_A = 8.7^*$; $F_B = 0.5^{ns}$; $F_{A*B} = 0.1^{ns}$						
Stem diameter (mm)	Control	7.5	7.8	7.9	7.8	7.7 ^B
	400 mg/L	8.8	8.7	9.1	9.1	9.0 ^A
	800 mg/L	8.9	9.0	9.2	9.1	9.0 ^A
	Average B	8.4	8.5	8.7	8.7	
CV (%) = 9.7; $F_A = 17.5^*$; $F_B = 0.3^{ns}$; $F_{A*B} = 0.03^{ns}$						

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant.

In fact, the application of copper sulphate to mugwort with different concentrations and frequencies did not significantly affect plant height, stem diameter. However, the application of copper sulphate tends to increase some growth parameters compared to the control. Studying on tobacco plant, the treatment of the substrate with 100 μM CuSO_4 led to significant increase in the plant height by 16% (Tugbaeva et al., 2023). Özyazıcı & Bektaş (2021) also showed that the use of CuSO_4 had a significant impact on growth characteristics, especially plant height and stem diameter of coriander (*Coriandrum sativum* L.).

3.2. Effect of copper sulphate levels on physiological and biochemical indicators

The data in Table 3 indicates that spraying 800 mg/L copper sulphate recorded the highest chlorophyll a (8.53 mg/g FW) and chlorophyll

b content (4.33 mg/g FW), which significantly outperformed the control (0 mg/L) and 400 mg/L. The interaction among concentration and spraying as well as spray frequency with copper sulphate was not significant in both chlorophyll a and chlorophyll b content. Notwithstanding, chlorophyll content tended to reduce when plants were sprayed CuSO_4 for 4 times.

Actually, copper participates in numerous physiological processes and is an crucial cofactor for metalloproteins (Yruela, 2005). Similarly, Saghirzadeh et al. (2019) reported similar results (an 2-fold increase in the total chlorophyll content) where 5, 10 and 25 μM of copper sulphate added to *Artemisia annua* L. Also, this study demonstrates that copper sulphate is also necessary for the formation of chlorophyll in plants (Al-Doori, 2024).

Table 3. Effect of copper sulphate levels and spraying frequency on physiological and biochemical indicators

Parameters	Treatments					Average A
	(A)	1	2	3	4	
Chlorophyll a content (mg/g)	Control	5.24	5.55	6.5	6.06	5.84 ^B
	400 mg/L	6.58	6.83	7.33	7.20	6.98 ^{AB}
	800 mg/L	7.92	7.92	9.34	8.95	8.53 ^A
	Average B	6.58 ^B	6.77 ^B	7.72 ^A	7.41 ^{AB}	
	CV (%) = 12.28; $F_A = 11.54^*$; $F_B = 3.38^*$; $F_{A*B} = 0.18^{ns}$					
Chlorophyll b content (mg/g)	Control	2.66	2.82	3.21	3.26	2.99 ^B
	400 mg/L	3.34	3.47	3.72	3.66	3.55 ^B
	800 mg/L	4.02	4.02	4.74	4.54	4.33 ^A
	Average B	3.34 ^C	3.44 ^{BC}	3.89 ^A	3.82 ^{AB}	
	CV (%) = 12.51; $F_A = 12.25^*$; $F_B = 3.32^*$; $F_{A*B} = 0.20^{ns}$					

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant.

On top of that, sulfur is a constituent of the proteinaceous amino acids such as methionine and cysteine, glutathione, vitamins (biotin and thiamine), phytochelatins, chlorophyll, coenzyme A, and S-adenosyl-methionine (Scherer et al., 2008; Li et al., 2020; Nakai & Maruyama-Nakashita, 2020). Consequently, optimal copper sulphate levels enhanced chlorophyll a and chlorophyll b in leaves.

3.3. Effect of copper sulphate levels and sprayed frequency on yield of mugwort

Foliar application of CuSO_4 has many beneficial effects. Our finding indicated the positive effect of low concentration of copper sulphate on dry matter yield.

The results of analysis of variance for yield are presented in Table 4 showed that frequency application as well as its and doses of CuSO_4

showed non-significant difference in yield measured at 65 days after cutting. Nevertheless, the maximum theoretical and actual fresh yield were observed to be 16.2 and 4.9 tons/ha, respectively with application of 800 mg/L CuSO_4 which significantly increased to 13.1; 3.1 tons/ha with 0 mg/L CuSO_4 and 14.7; 4.0 tons/ha, respectively with 400 mg/L CuSO_4 . Likewise, copper has enhanced the yield of the plants due to providing nutritional requirements (Kovacik et al., 2009).

Additionally, the result can be explained by the deficiency of sulfur could lead to stunted growth of plants and ultimately loss of yield (Narayan et al., 2023). Yet, at higher concentrations (800 mg/L) and spraying for 4 times, fresh yield tended to decrease in dry matter weight of 65-day-old mugwort (Table 4).

Table 4. Effect of copper sulphate levels and spraying frequency on theoretical and actual fresh yield of mugwort (65 days after cutting)

Parameters	Treatments					Average A
	(A)	1	2	3	4	
Theoretical fresh yield (Ton/ha)	Control	12.7	13.2	12.7	13.7	13.1 ^C
	400 mg/L	14.5	13.8	15.5	15.0	14.7 ^B
	800 mg/L	15.7	16.5	16.7	16.3	16.2 ^A
	Average B	14.3	14.4	15.0	15.0	
CV (%) = 7.6; $F_A = 62.0^{**}$; $F_B = 1.0^{ns}$; $F_{A \times B} = 0.6^{ns}$						
Actual fresh yield (Ton/ha)	Control	3.1	3.2	3.1	3.1	3.1 ^C
	400 mg/L	4.0	3.3	4.6	4.1	4.0 ^B
	800 mg/L	4.8	5.1	5.0	4.8	4.9 ^A
	Average B	3.9	3.9	4.2	4.0	
CV (%) = 16.4; $F_A = 56.8^{**}$; $F_B = 0.5^{ns}$; $F_{A \times B} = 0.7^{ns}$						

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; **: significant at 1% level; ns: non significant.

3.4. Effect of copper sulphate on essential oil of mugwort

Table 5. Effect of copper sulphate levels and spraying frequency on essential oil content and yield of mugwort

Parameters	Treatments					Average A
	(A)	1	2	3	4	
Essential oil content (%)	Control	0.13	0.14	0.14	0.13	0.14 ^B
	400 mg/L	0.15	0.16	0.19	0.17	0.16 ^A
	800 mg/L	0.17	0.18	0.19	0.17	0.18 ^A
	Average B	0.15	0.16	0.17	0.16	
CV (%) = 14.0; $F_A = 13.6^*$; $F_B = 0.7^{ns}$; $F_{A \times B} = 0.1^{ns}$						
Essential oil yield (L/ha)	Control	16.9	18.5	18.3	18.4	18.0 ^C
	400 mg/L	22.4	21.7	25.8	24.5	23.6 ^B
	800 mg/L	26.7	29.6	31.7	28.7	29.2 ^A
	Average B	22.0	23.3	25.3	23.9	
CV (%) = 16.8; $F_A = 29.1^{**}$; $F_B = 1.0^{ns}$; $F_{A \times B} = 0.3^{ns}$						

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant.

Copper sulphate (800 mg/L) significantly increased the essential oil yield to 29.2 L/ha, compared with 18.0 L/ha in the control (improved 62.2% compared to the control). Nonetheless, an excess of this micronutrient may reduce essential oil yield when spraying four times although the interaction between the concentration and spray times of CuSO₄ were not significant difference. Considering the fact that in this study, although the influence of copper sulphate was positive on essential oil content, but essential oil content and essential oil yield were highest at a copper sulphate concentration of 800 mg/L (Table 5).

This result can be explained by the role of sulfur in increasing of herb, oil, and menthol yield. It plays important roles in protein structure and function and serves as a component of various enzyme cofactors, as well as in various metabolic pathways in the body (Ajay et al., 2010). Hence, this may explain the increase in essential oil content and yield in mugwort.

By the same token, Lafmejani et al. (2018) reported that the application of copper significantly increased essential oil content of peppermint (*Mentha piperita* L.). In the same way, copper sulfate increased the essential oil content of *Pelargonium* sp. by 41.9% as compared to the control (Prasad et al., 2008). However, essential oil content of mugwort enhanced 0.04% as compared to 0 mg/L CuSO₄.

3.5. Effect of copper sulphate levels and sprayed frequency on total flavonoids (TFC) and polyphenol content (TPC) of mugwort

Copper sulphate concentrations of 0, 400, and 800 mg/L induced TFC accumulation. The highest TFC of 5.04 mg RE/g dry extract was observed with the 800 mg/L CuSO₄ treatment. While the control and 400 mg/L CuSO₄ treatments resulted in lower total phenolic contents of 3.32 and 4.44 mg RE/g dry extract, respectively. Nonetheless,

the data in Table 6 showed no significant difference in TFC among spraying frequencies as well as CuSO₄ concentration and spraying frequency.

The data presented in Table 6 showed that changes in TPC under foliar CuSO₄ application followed a trend similar to that of TFC. Foliar application of copper sulfate at various concentrations affected TPC in mugwort. The maximum TPC of 8.55 mg GAE/g dry extract was observed with the 800 mg/L CuSO₄ treatment, which significantly increased 1.3 times over control (0 mg/L CuSO₄) and 1.1 times over the concentration of 400 mg/L CuSO₄. TPC values ranging from 6.30 to 8.86 mg GAE/g dry extract were obtained in the combination of CuSO₄ concentration and spraying frequency, which were not significantly different from the control. In the same way, no significant differences in TPC were observed among CuSO₄ spraying-frequency treatments.

These results were consistent with a previous study reporting improved TFC and TPC following CuO application to the leaves of lavender plants (*Lavandula stoechas* L.) (Asadollahi et al., 2023). This result can be explained by the role of copper in catalyzing the synthesis of phenolic compounds (Aftab & Hakeem, 2020).

Likewise, sulfur has already been shown to have a strong influence on the formation of S-containing secondary metabolites in vegetables and agricultural crops (Schnug et al., 1995; Hanelauks, 1999). In addition, copper plays an essential role in the biosynthesis of phenols in the plant and a deficiency of copper can lead to a reduction in phenolic compounds (Dicko et al., 2006). On the other hand, copper is an element that plays an essential role in the mitochondrial electron transport system and may influence the production of secondary metabolites in plants (Morimoto et al., 1988).

Table 6. Effect of copper sulphate levels and spraying frequency on TFC and TPC of mugwort

Parameters	Treatments					Average A
	(A)	1	2	3	4	
TFC (mg RE/g)	Control	3.53	3.33	2.94	3.46	3.32 ^C
	400 mg/L	3.59	4.41	5.13	4.65	4.44 ^B
	800 mg/L	4.17	4.09	6.32	5.57	5.04 ^A
	Average B	3.76 ^C	3.95 ^{BC}	4.80 ^A	4.56 ^{AB}	
	CV (%) = 18.85; $F_A = 42.86^{**}$; $F_B = 3.37^*$; $F_{A \times B} = 2.20^{ns}$					
TPC (mg GAE/g)	Control	6.30	6.58	6.90	6.78	6.64 ^C
	400 mg/L	7.44	7.44	8.14	7.56	7.65 ^B
	800 mg/L	8.36	8.39	8.86	8.60	8.55 ^A
	Average B	7.37	7.47	7.96	7.65	
	CV (%) = 7.55; $F_A = 18.99^{**}$; $F_B = 1.87^{ns}$; $F_{A \times B} = 0.12^{ns}$					

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant. TFC: Total flavonoid content; TPC: Total polyphenol content.

3.5. Economic efficiency of spraying copper sulphate on mugwort (*Artemisia vulgaris* L.)

Table 7. Economic efficiency of different copper sulfate levels and spraying frequencies in mugwort (*Artemisia vulgaris* L.)

Copper sulfate levels and number of sprays	Essential oil yield	Total costs	Total	Profit	P/C ratio
	(L/ha per harvest)	(million/ha per harvest)			
Spray once time with 0 mg/L	16.86	88.36	118.02	29.66	0.34
Spray twice times with 0 mg/L	18.50	88.36	129.5	41.14	0.47
Spray three times with 0 mg/L	18.26	88.36	127.82	39.46	0.44
Spray four times with 0 mg/L	18.37	88.36	128.59	40.23	0.46
Spray once time with 400 mg/L	22.44	91.00	157.08	66.08	0.73
Spray twice times with 400 mg/L	21.67	93.63	151.69	58.06	0.62
Spray three times with 400 mg/L	25.77	96.26	180.39	84.13	0.87
Spray four times with 400 mg/L	24.47	98.9	171.29	72.40	0.73
Spray once time with 800 mg/L	26.68	91.03	186.76	95.73	1.05
Spray twice times with 800 mg/L	29.58	93.69	207.06	113.37	1.21
Spray three times with 800 mg/L	31.71	96.36	221.97	125.61	1.30
Spray four times with 800 mg/L	28.75	99.02	201.25	102.22	1.03

Price: 7 million VND per liter of mugwort essential oil; CuSO_4 price: 134,000 VND/kg; labour cost: 250,000 VND/day; P/C ratio: profit-to-cost ratio.

The data in Table 7 showed that spraying CuSO_4 at a concentration of 800 mg/L four times, this treatment had the highest total cost (99.02 million VND/ha per harvest). In contrast, the control treatment had the lowest total cost (88.36 million VND/ha per harvest).

Considering the economic efficiency of copper sulfate concentration and spraying frequency based on profit and BCR, copper sulphate gave the highest P/C ratio followed by 1.3 of 800 mg/L concentration with three spraying times (125.61 million VND/ha per harvest). The control treatments were profitable, with profits ranging from 29.66 to 40.23 million VND/ha per harvest and achieved the lowest BCR (0.34 to 0.46).

4. Conclusions

The results of the experiment showed that spraying copper sulfate three times at a concentration of 800 mg/L resulted in the best growth, quality, and yield of mugwort. The growth parameters, including plant height (99.0 cm) and stem diameter (9.2 mm), reached the highest values. Physiological and biochemical indicators, including chlorophyll a, chlorophyll b, flavonoid, polyphenol, and essential oil contents, also reached the highest values comparing to the other copper sulphate concentrations. The potential fresh yield, actual fresh yield and essential oil yield were 23.3 ton/ha; 16.7 ton/ha, and 31.7 L/ha, respectively.

Conflict of interest

The authors declare no conflict of interest.

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Prevalence and hematological parameters of Feline Panleukopenia virus (FPV) infection in cats in Ho Chi Minh City

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ARTICLE INFO	ABSTRACT
Research Paper Received: November 13, 2024 Revised: January 09, 2025 Accepted: February 10, 2025 Keywords Cat Feline panleukopenia virus Hematological parameters Ho Chi Minh City Leukopenia *Corresponding author Vo Phong Vu Anh Tuan Email: vpvatuan@ntt.edu.vn	Feline panleukopenia, caused by the Feline panleukopenia virus (FPV), a small DNA virus in the Parvoviridae family, is a highly contagious and often fatal disease affecting all feline species. This study was conducted from April to September 2024 at the K9 Veterinary Clinic system in Ho Chi Minh City. During this period, 47 FPV-positive cats were identified from 132 suspected cases, representing a prevalence rate of 35.61%. Age and the number of cats per household were significantly associated with FPV infection rates ($P < 0.05$). The FPV-positive cats exhibited alterations in specific hematological parameters, with a reduction in both white blood cell (WBC) count ($2.79 \times 10^9/L$) and neutrophil count ($1.77 \times 10^9/L$). The most commonly observed clinical signs in FPV-positive cats included vomiting, non-bloody diarrhea, poor skin turgor, lethargy, and anorexia. The results of the treatment indicated that 29 out of the 47 infected cats recovered, representing a success rate of 61.70%. In conclusion, feline panleukopenia remains a significant risk for cats, particularly in young or unvaccinated individuals. Early treatment of cats with leukopenia, combined with strict adherence to the treatment regimen, is essential to improve the recovery rate.

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1. Introduction

Feline leukopenia is caused by the Feline Panleukopenia virus (FPV), a small DNA virus belonging to the Parvoviridae family (Tuzio, 2021). The FPV has been demonstrated to infect all species within the Felidae family, causing

disease in both household and stray cats (Brussel, 2019). The FPV is highly resilient, with the capacity to survive for up to one year at room temperature in organic material. In feces, it can persist for 5 to 10 months or longer (Fischer et al., 2007). The disease has a high contagion rate

and a high mortality rate, particularly among kittens (Kabir et al., 2023). In newborn kittens, the disease progresses in an acute manner, resulting in the onset of neurological disorders such as ataxia and blindness. Kittens under 12 months of age frequently exhibit leukopenia and neutropenia as a consequence of infections of the bone marrow and lymphoid tissue, in addition to diarrhoea diarrhea resulting from the destruction of intestinal cells (Kiselev et al., 2021). Cats of all ages are susceptible to the disease, but the highest vulnerability is observed in kittens under 6 months of age (Nguyen et al., 2021). The most commonly reported symptoms in feline patients infected with the organism include vomiting, dehydration, and diarrhea (Barrs, 2019; Kabir et al., 2023).

The number of cats is increasing at a rapid rate, particularly due to the rising importation of foreign breeds. This exponential increase in the feline population is resulting in a proportional increase in the prevalence of feline diseases. A considerable number of studies have documented the high prevalence of FPV infection in cats. In Hanoi and surrounding areas, 34.93% of cats were found to be FPV-positive (Nguyen et al., 2021), and in Can Tho, the infection rate was 34.68% (Dang et al., 2023). In Ho Chi Minh City, the FPV infection rate reached 84.31% (Dinh et al., 2022). The elevated prevalence of FPV in cats can be attributed to a combination of inadequate vaccination coverage and constrained biosecurity measures. Additionally, research investigating a correlation between hematological parameters and FPV infection in cats remains limited in Vietnam. It is therefore imperative to study the prevalence, risk factors and haematological parameters to raise awareness, improve the efficacy of preventive measures, and facilitate the treatment of FPV.

2. Materials and Methods

2.1. Sample collection

The study was conducted between April and September 2024 at several K9 clinics in Ho Chi Minh City. One hundred and thirty two cats exhibiting clinical signs suggesting FPV infection, and with the information of their owners, were enrolled in the study. The cats suspected of having FPV exhibited (any stages of diseases) a range of clinical symptoms including high fever, lethargy, vomiting, generalized depression, loss of appetite, diarrhea, nasal discharge, and dehydration.

A comprehensive data set was assembled, face to face interviews with cat owners in order to collect data on potential risk factors for FPV, including breed, age, sex, lifestyle, housing, and history of clinic visits. The clinical symptoms were examined and collected by veterinarians using the appropriate diagnostic instruments.

2.2. Rapid diagnostic test kits

All cats exhibiting indications suspected of a potential FPV infection were subjected to the collection of either fecal (rectal swab) or vomit samples for rapid diagnostic testing via the FPV Ag test kit (Sensitivity: 95.6 % and Specificity: 98.1%; GenAction, Vietnam). For rectal swab, a cotton swab was inserted into the rectum about 1 - 2 inches past the anus and gently rotated the swab clockwise for 5 - 10 sec. Then, withdrew the swab without touching the skin and placed the swab and any adherent material into a swab container.

2.3. Blood sampling and hematological analysis

Two milliliters of blood were collected from the medial saphenous vein were collected in tubes

containing ethylene diamine tetra acetic acid (EDTA) for a complete blood count using the BC 5000VET hematology analyzer (19 parameters; Mindray company, China). The blood samples were used to estimate the following parameters: the White Blood Cell (WBC), Neutrophils (Neu), Lymphocytes (Lym), Monocytes (Mon), Eosinophils (Eos), Basophils (Bas), Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean

Corpuscular Hemoglobin Concentration (MCHC), and Platelets (PLT). There were 47 FPV-infected cats; however, only 15 cat owners agreed to test hematological parameters before and after treatment.

2.4. Treatment protocol

The FPV positive cats were treated in accordance with the standard protocol (Table 1) of the clinics.

Table 1. Treatment protocols for *Feline Panleukopenia* virus (FPV) infection in cats

Items	Drug administration route ¹
Secondary infection treatment: Broad-spectrum antibiotics (Sulfamethoxyridazole and Trimethoprim (Sept))	SC
Symptomatic treatment: reducing fever, alleviating vomiting (Cimetidine and Analgin - C)	SC
Supportive care, strengthened support (Catosal and B ₁₂ - ATP)	IV
Rehydration, electrolytes: (Ringer Lactate)	IV
Support for increasing leukocytes and enhancing immune system activity	IV or IM

¹SC: subcutaneous; IV: intravenous; IM: intramuscular.

A recovered cat is one that has a WBC count within the normal range (if the owners agreed to test hematological parameters after treatment), a normal appetite and defecation, behaves normally, and no longer exhibits clinical symptoms. An unrecovered cat does not show improvement in clinical symptoms, may worsen symptoms, or may die during the course of treatment.

2.5. Statistical analysis

The data were processed and calculated using Microsoft Excel, and the risk factors were

assessed using the Chi-square test with Minitab version 16.0.

3. Results and Discussion

3.1. The prevalence of FPV in cats

The results in Table 2 and Figure 1 demonstrated that 47 out of the 132 suspected cats were positive for FPV by using FPV Ag test kit, representing a prevalence of 35.61%. The non-specific symptoms of leukopenia in cats, coupled with the presentation of sick cats at various stages of the disease, can render clinical diagnostics

more challenging. The results of our study are consistent with those reported by Nguyen et al. (2021), who observed a 34.93% positivity rate for FPV in cats in Hanoi and surrounding areas. The

research conducted by Award et al. (2018) and Le et al. (2020) indicated higher FPV infection rates than those observed in our study, with figures of 40.19 and 40%, respectively.

Table 2. The proportion of Feline Panleukopenia virus (FPV)-infected cats

Items	Number of cats	Percentage (%)
FPV Negative	85	64.39
FPV positive	47	35.61
Total	132	100

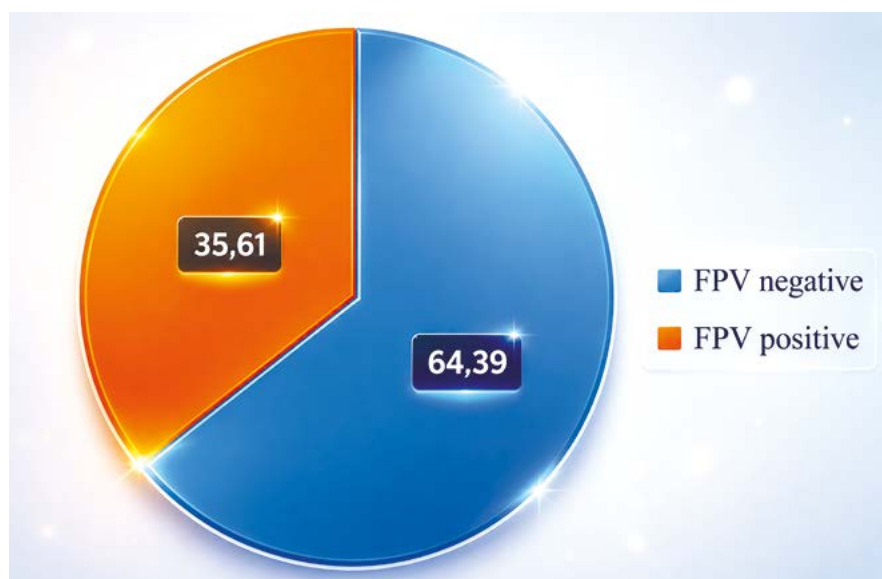


Figure 1. The proportion of *Feline Panleukopenia* virus (FPV)-infected cats.

3.2. The FPV-infected rate according to risk factors

The results presented in Table 3 indicate that the FPV positivity rate was 70.21% for domestic breeds and 29.79% for foreign breeds ($P > 0.05$). This finding is consistent with the findings of

Porporato et al. (2018), who reported that 99.4% of domestic cats and 0.6% of foreign breeds had panleukopenia. However, the report by Dinh et al. (2022) indicated a contrasting trend, with domestic breeds exhibiting a lower prevalence of 25.58%, compared to foreign breeds, which reached 74.42%.

Table 3. The prevalence of *Feline Panleukopenia* virus (FPV) infection according to risk factors

Items	Suspected cats	Infected Cats	
	132	47	Percentage (%)
Breed			
Domestic	80	33	70.21 ^a
Foreign	52	14	29.79 ^a
Gender			
Male	68	22	46.81 ^a
Female	64	25	53.19 ^a
Lifestyle			
Indoor	98	39	82.98 ^a
Outdoor	34	8	17.02 ^a
Housing			
Single-cat household	81	19	40.43 ^a
Multi-cat household	51	28	59.57 ^b
Visit to the hospital for other purposes			
Yes	37	12	25.53 ^a
No	95	35	74.47 ^a
Age			
0 to 6 months	66	29	61.70 ^a
6 to 12 months	41	15	31.92 ^a
> 12 months	25	3	6.38 ^b

^{a, b, c} Different letters in the same column indicate a significant difference ($P < 0.05$).

The data in Table 3 also indicated the FPV-infected rate between male cats (46.81%) and female cats (53.19%), suggesting that sex does not significantly affect the rate of feline panleukopenia ($P > 0.05$). The results of Awad et al. (2018) and Nguyen et al. (2021) also demonstrated comparable FPV infection rates between male and female cats. In contrast, the findings of Dinh et al. (2022) indicated a higher

prevalence of infection in male cats (76.74%) compared to female cats (23.26%).

The housing conditions (Table 3) demonstrated no statistically significant difference between the indoor group (infection rate of 82.98%) and the outdoor group (infection rate of 17.02%) ($P > 0.05$). This finding is consistent with the studies by Oğuzoğlu (2013) and Gülersoy et al. (2023), which reported infection rates of 69.23 and 88%

for indoor cats, respectively, 30.77 and 12% for outdoor cats. In contrast, Dinh et al. (2022) reported a higher infection rate among outdoor cats (76.74%) than indoor cats (23.26%).

The number of cats per household demonstrated a statistically significant difference between households with only one cat (infection rate of 40.43%) and those with two or more cats (59.57%) ($P < 0.05$). This discrepancy may be attributed to the elevated risk of disease transmission in households with multiple cats. These findings are consistent with those of Rehme et al. (2022) and Kabir et al. (2023), who observed that compared to households with only one cat, there is an elevated risk of infection in those with multiple cats.

Table 3 also indicated that 25.53% of infected cats had visited a veterinary clinic, in comparison to 74.47% of cats that had never been to a clinic. However, this difference is not statistically significant ($P > 0.05$). Consequently, the likelihood of transmission from veterinary clinics remains inconclusive. Conversely, Kabir et al. (2023) illustrated that cats attending clinics for diverse reasons exhibited a threefold elevation in infection risk compared to those that had never been brought to a clinic.

Additionally, age was identified as a risk factor for feline panleukopenia. As illustrated in Table 3, the highest infection rate was observed in cats under six months of age (61.70%), followed by those aged six to twelve months (31.92%) and cats over twelve months old (6.38%). Significant differences were identified between the groups of cats under six months and those over twelve months of age, as well as between the groups of cats aged six to twelve months and those over twelve months of age ($P < 0.05$). This trend may be attributed to the susceptibility of kittens to

feline panleukopenia as a result of the decline in maternally derived antibodies. Similarly, Khan et al. (2025) reported a comparable pattern, with a high infection rate in cats under six months (29.46%), followed by cats aged six to eighteen months (22.90%), and the lowest rate in cats over eighteen months (9.14%). Additionally, Le et al. (2020) observed the highest infection rate in cats under six months (55.56%) and the lowest in those over twelve months (5.00%), which is consistent with the findings of Nguyen et al. (2021). These findings indicate that cats under the age of six months are at an elevated risk of infection.

3.3. Hematological parameters in cats with *Feline Panleukopenia*

The results presented in Table 4 demonstrate the hematological parameters in cats that have been diagnosed with FPV. The white blood cell count exhibited a pronounced decline ($2.79 \times 10^9/L$ in comparison to the minimum threshold of $5.5 \times 10^9/L$), as did the neutrophil count ($1.77 \times 10^9/L$ in comparison to the minimum threshold of $3.12 \times 10^9/L$), whereas lymphocyte counts demonstrated a slight reduction ($0.65 \times 10^9/L$ in comparison to the minimum threshold of $0.73 \times 10^9/L$). The reduction in white blood cells results in immunosuppression, rendering FPV-infected cats more susceptible to secondary infections. It is therefore imperative that the treatment protocol includes antibiotics to prevent secondary infections and medications that can increase white blood cell count and boost the immune system in FPV-infected cats. Conversely, red blood cell counts were within the physiological range. A notable decline in platelet count was observed ($63.45 \times 10^9/L$ compared to the minimum threshold of $100 \times 10^9/L$), which elevates the risk of gastrointestinal

bleeding and necessitates the provision of additional hemostatic support. Additionally, studies conducted by Gülersoy et al. (2023) and Dishow et al. (2024) have reported reductions in analogous hematological parameters, which are consistent with the findings presented here. Similarly, research conducted by Nguyen et al. (2021) and Irgashev et al. (2023) demonstrated

a comparable trend. These studies collectively demonstrate a reduction in white blood cells, granulocytes, lymphocytes, and platelets. The presence of these subclinical symptoms is of significant diagnostic value, particularly in the development of treatment protocols for veterinary practitioners.

Table 4. Hematological parameters in cats with *Feline Panleukopenia*

Parameters	Normal values	Case group (n = 15) Range Mean ± SE
White blood cell (WBC), x 10 ⁹ /L	5.50 - 19.50	2.79 ± 1.07
Neutrophils (Neu), x 10 ⁹ /L	3.12 - 12.58	1.77 ± 0.91
Lymphocytes (Lym), x 10 ⁹ /L	0.73 - 7.86	0.65 ± 0.30
Monocytes (Mon), x 10 ⁹ /L	0.07 - 1.36	0.17 ± 0.10
Eosinophils (Eos), x 10 ⁹ /L	0.06 - 1.93	0.05 ± 0.02
Basophils (Bas), x 10 ⁹ /L	0.00 - 0.12	0.01 ± 0.01
Red blood cell (RBC), x 10 ¹² /L	4.6 - 10.2	7.24 ± 0.72
Hemoglobin (HGB), g/L	85 - 153	104.13 ± 9.95
Hematocrit (HCT), %	26 - 47	29.94 ± 2.68
Mean corpuscular volume (MCV), f/L	38 - 54	42.98 ± 1.83
Mean corpuscular hemoglobin (MCH), pg	11.8 - 18	14.45 ± 0.30
Mean corpuscular hemoglobin concentration (MCHC), g/L	290 - 360	343.60 ± 14.00
Platelets (PLT), x 10 ⁹ /L	100 - 518	63.45 ± 8.82

3.4. Clinical symptoms in FPV-infected cats

Clinical symptoms (Table 5) were frequently observed in FPV-positive cats, including foamy or yellow vomit, diarrhoea without blood, poor skin elasticity, loss of appetite, fatigue, and lethargy. Furthermore, studies by Awad et al.

(2018), Porporato et al. (2018), and Dishow et al. (2024) have also observed similar frequencies of these clinical signs. These symptoms serve as crucial indicators for cat owners and veterinarians to identify potential cases for monitoring and diagnostic purposes.

Table 5. Clinical symptoms in Feline Panleukopenia virus (FPV)-infected cats

Signs	Number of cats	Total FPV cats	Percentage (%)
Anorexia	39	47	82.98
Lethargy	41		87.23
Fever	28		59.57
Drooling	6		12.77
Dehydration	36		76.60
Pale mucous membrane	23		48.94
Diarrhea	43		91.49
Bloody diarrhea	8		17.02
Vomiting	45		95.74

3.5. Effectiveness of treatment in FPV-infected cats

The results presented in Table 6 demonstrate that the efficacy of the therapeutic intervention for cats with leukopenia is considerable, with a recovery rate of 61.70%. Currently, there is no specific treatment for feline panleukopenia (FPV). Consequently, the intervention is primarily based on supportive care and the treatment of symptoms. The survey results regarding treatment effectiveness are encouraging, mainly due to owners promptly

recognizing symptoms in their cats and bringing them to the clinic on time, administering the correct medications at the appropriate doses by the clinic's protocol, and closely implementing supportive measures. As Porporato et al. (2018) have demonstrated, the survival rate for cats with leukopenia is approximately 20.3%. In contrast, Dang et al. (2023) have reported a recovery rate of 34.88%. The cases of ineffective intervention are primarily attributable to owners detecting the disease at a late stage and failing to pursue treatment following the recommendations of the veterinary practitioner.

Table 6. Effectiveness of treatment in FPV-infected cats

Items	Number of cats	Percentage (%)
Recovered	29*	61.70
Unrecovered	18	38.30
Total	47	100.00

**Only 12 FPV-infected cats had their hematological parameters examined after treatment. The other (17) recovered cats were assessed based on clinical signs.*

4. Conclusions

The study revealed that 35.61% of cats tested positive for FPV. The number of cats per household and the age of the cats were found to be significantly associated with the occurrence of feline panleukopenia. The most commonly reported symptoms were loss of appetite, poor skin elasticity, diarrhoea, and vomiting. The FPV-positive cats exhibited a notable decline in white blood cell count, with a value of $2.76 \times 10^9/L$ for white blood cells and $1.87 \times 10^9/L$ for neutrophils. The application of supportive treatment resulted in a recovery rate of 61.70%.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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A retrospective study of myxomatous mitral valve disease:
Prevalence and predisposing factors of affected dogs

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ARTICLE INFO	ABSTRACT
Research Paper Received: February 21, 2025 Revised: April 02, 2025 Accepted: April 14, 2025 Keywords Canine Diagnostic imaging Myxomatous mitral valve disease Physical examination Prevalence *Corresponding author Tran Huynh Xuan Thao Email: thao.tran6899@gmail.com	Myxomatous mitral valve degeneration (MMVD) is the most common heart disease in small to medium-sized dog breeds (Dachshund, Miniature and Toy Poodles, Chihuahua, Pomeranian, Shih Tzu, Maltese...). The objective of this research was to study the prevalence of MMVD and predisposing factors of an affected population dogs in the Cardiology clinic at Thonglor International Pet Hospital, Bangkok, Thailand. Electronic medical records of 151 dogs confirmed with MMVD were collected. Assessment criteria included signalments (breed, age, sex, and body weight); physical examination findings (clinical signs and heart murmur grades); subjective measurement findings on diagnostic imaging (vertebral heart score (VHS), left atrial to aorta ratio (LA/Ao), normalized left ventricular internal diameter in diastole (LVIDDN), normalized left ventricular internal diameter in systole (LVIDSN), and fractional shortening (FS)). The prevalence of MMVD in the study population was 33.4%. Based on The American College of Veterinary Internal Medicine (ACVIM) consensus guidelines, the study population comprised 80 (53.0%) dogs in stage B1, 47 (31.1%) dogs in stage B2, and 24 (15.9%) dogs in stage C. The prevalence of the disease was higher in small-breed dogs over 10 years old. A high number of male dogs (58.9%) was affected by MMVD compared to female dogs (41.1%). Heart murmurs severity generally increased with disease stage. Coughing was the most common clinical sign, reported in 40.8% of MMVD population. Cardiomegaly (based on VHS), increased LA/Ao along with increased LVIDDN, and increased LVIDSN were significantly associated with ACVIM stages ($P < 0.001$).

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1. Introduction

Myxomatous mitral valve disease (MMVD) is the most common cardiovascular disease in dogs, accounting for more than 70% of heart diseases and typically reported in small-breed dogs (Atkins et al., 2009; Parker & Kilroy-Glynn, 2012). MMVD is a chronic disease characterized by progressive myxomatous degeneration of atrioventricular valves, leading to thickening and incomplete coaptation (Hezzell, 2018). The mitral valve is most affected, compared to tricuspid valves and other valves which are sometimes also involved (Orozco et al., 2019). These structural changes result in secondary mitral valve regurgitation (leakage) from the ventricle into the atrium. Over time, excessive regurgitant blood causes left atrial enlargement and left ventricular remodeling (Fox, 2012). A systolic murmur sound caused by mitral regurgitation is a hallmark sign detected on auscultation, providing information about disease severity (Ljungvall et al., 2014). In addition, compensatory mechanism for increased leaked volume can lead to elevated left atrial pressure, which transmits to the lungs and causes pulmonary edema. Although most dogs remain asymptomatic for years, some dogs experience evident disease progression, leading to chronic heart failure (CHF) associated with clinical signs and ultimately death (Hezzell, 2018).

Diagnosis of MMVD is challenging due to the long-term preclinical stage. Diagnosis requires a thorough physical examination, radiography and echocardiography to confirm the presence of MMVD (Keene et al., 2019). Previous studies showed that the prevalence and progression of the MMVD were strongly associated with breed, age, sex and body weight (Petrič, 2015; Pascon et al., 2021). Evidence of inheritance has been

reported in some highly susceptible breeds such as the Cavalier King Charles Spaniels and Dachshund although the genetic mode was not exactly clarified (Petrič, 2015). Small-sized dogs such as Poodles, Yorkshire terrier, Chihuahuas, and Shi-Tzu were most frequently affected. Furthermore, MMVD is more common in male dogs. The prevalence of MMVD markedly increases with age over 10 years old (Fox, 2012; Kenne et al., 2019). The aim of this study was to investigate the prevalence of MMVD in a practice-attending population and determine population characteristics of MMVD dogs. This information will help clinicians identify high-risk individuals and aid in prognostication of disease severity.

2. Materials and Methods

2.1. Animals

A total of 452 dogs admitted to the Cardiology unit, Thonglor International Pet Hospital, Bangkok, Thailand from November 1, 2021 to November 30, 2022 were included. Among them, 151 dogs were confirmed to have MMVD based on a thorough diagnostic evaluation, including physical examination, radiography, and echocardiography (2D mode, M-Mode, and color flow Doppler).

2.2. Data collection and assessment criteria

Physical examination including signalments (breed, age, sex, and body weight), clinical signs, and murmur grades were collected through observation and auscultation. Murmur grade was classified as soft (I - II), moderate (III), loud (IV), or thrilling (V - VI) according to the murmur grading system described by Ljungvall et al. (2014). In radiographic examination, the vertebral heart scale (VHS)

was measured in right lateral radiographic projections following the method of Gugjoo et al. (2013). Echocardiographic examinations were performed using 2D, M-mode, and Doppler modalities. In echocardiography, dogs were placed in the right lateral position for the right parasternal long axis and short axis views, in the left lateral position for the left apical long axis view. The severity of disease was classified based on the American College of Veterinary Internal Medicine (ACVIM) consensus guidelines (Keene et al., 2019). The variables collected from echocardiography were the left ventricular internal diameter at end diastole (LVIDD) and end systole (LVIDS), both normalized to body weight using the method described by Cornell et al. (2004); the ratio of left atrium diameter to aortic diameter (LA/Ao); and left ventricular shortening fraction (FS).

2.3. Statistical analysis

Statistical analyses were performed using commercial software (IBM SPSS version 24.0). Categorical variables were presented as number (n) and percentage (%). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed variables were presented as mean and standard deviation (SD) (mean $\pm$ SD); while non-normally distributed variables were presented as median and interquartile range [median (IQR)].

Categorical variables were compared among study groups using Chi-square test. Differences in radiographic and echocardiographic indices among stages were evaluated using One way ANOVA followed by a multiple comparison test for normally distributed variables or the Kruskal-Wallis test followed by a multiple comparison test for non-normally distributed variables. Statistical significance was defined as $P < 0.05$.

3. Results and Discussion

3.1. The rate of MMVD dogs in Cardiology unit and the rates of MMVD dogs among ACVIM stages

Of the 452 dogs admitted to the Cardiology unit of Thonglor International Pet Hospital in Bangkok, Thailand, 151 (33.4%) were diagnosed with MMVD. This prevalence is higher than that reported in previous study by Pascon et al. (2021) of 30.3%. This is because the prevalence of the disease typically varies based on epidemiological factors age, sex, and breed distribution. Differences in epidemiological factors of population will lead to overall prevalence differences of the disease because old, male, small-sized dogs have been reported to have a higher prevalence of MMVD. However, the observed disease progression patterns in this study align with previous reports of Franchini et al. (2021) and Pascon et al., (2021), showing incidence are closely related to specific breed, age, and body weight characteristics.

According to the ACVIM consensus guidelines (Keene et al., 2019), MMVD dogs in this study were classified in three different stages including stages B1, B2, and C. The majority of dogs with MMVD were classified as stage B1 (53.0%), followed by stage B2 (31.1%) and stage C (15.9%). Notably, no dogs were classified as stages A or D. In agreement with previous studies (Pascon et al., 2021, Trofiniak & Slivinska, 2021), stage B1 predominated in this study population when compared to stages B2 and C. This may be because our study was collected from an international pet hospital, where routine cardiac disease screenings and preoperative examinations are commonly performed. These practices likely result in a higher detection rate of asymptomatic patients (stage B dogs).

3.2. The rates of MMVD dogs according to signalments (breed, sex, age, and body weight) among stages

All 151 dogs diagnosed with MMVD in this study represented 16 different breeds (Table 1). The most prevalent breeds among MMVD-affected dogs were Chihuahuas (31.1%), followed by Pomeranian (27.2%) and Poodles (10.6%). This finding supports the research by Mattin et al. (2015) and by Pascon et al. (2021) indicating that MMVD is a common cardiovascular disease in small breed dogs. Parker & Kilroy-Glynn (2012) proposed several explanations for why small breeds are more susceptible to MMVD than large breeds. One possibility is that smaller dogs have a higher heart-to-body size ratio. Another

possibility is that if small breeds share many of the same genetic mutations responsible for their small size, they may also carry associated genes that increase their susceptibility to MMVD. Additionally, the longer lifespan of small dogs compared to larger dogs may contribute to higher diagnosis rates, as they are more likely to live long enough for the disease to develop and progress. Interestingly, despite Cavalier King Charles Spaniels being a breed known to be at high risk for MMVD at a young age in previous studies (Mattin et al., 2015, Franchini et al., 2021), they represented only 1.3% of the affected dogs in this study. This low representation may be due to the limited number of this breed presenting to the hospital during the study period, possibly reflecting breed preferences of Thai owners.

Table 1. The rate of dogs with Myxomatous mitral valve degeneration (MMVD) by breeds

Breeds	Dogs with MMVD (n)	Rate (%)
Chihuahuas	47	31.1
Pomeranian	41	27.2
Poodles	16	10.6
Mixed	14	9.3
Shih Tzu	7	4.6
Miniature Pinscher	4	2.6
Beagle	4	2.6
Golden Retriever	3	2.0
Yorkshire Terrier	3	2.0
Cavalier King Charles Spaniel	2	1.3
Pekingese	2	1.3
Spitz	2	1.3
Thai	2	1.3
French ulldog	2	1.3
Dachshund	1	0.7
Labrador Retriever	1	0.7

n: number of dogs with MMVD.

Table 2 presented demographic data for dogs diagnosed with MMVD. No signalment had statistical significance between groups ($P > 0.05$). A slight male predominance was observed, with 58.9% male dogs compared to 41.1% female dogs. The result was in line with the general trend observed in other studies (Mattin et al., 2015; Franchini et al., 2021), which have shown that MMVD are more prevalent in male dogs.

The age of an onset ranged from 8 to 17 years, with a median age of 12 years (interquartile range: 10 - 13). However, the median age in this study was higher than that of previous studies (Kim et al., 2017; Toaldo et al., 2018; Pascon et al., 2021). However, all these studies found that middle-aged and older dogs are primarily affected by MMVD, with age being a recognized predisposing factor for this disease.

Body weight in the MMVD-affected dogs ranged from 2.3 to 33.8 kg, with a median weight of 5.1 kg (interquartile range: 4 - 7.2). The large majority (94.7%) of affected dogs weighed less than 20 kg, which was consistent with previous studies (Mattin et al., 2015; Franchini et al., 2021) that have reported a higher prevalence of MMVD in small- and medium-sized breeds. However, the median value of body weight in this study was lower than the medians reported in these mentioned studies, which were 10.9 kg (interquartile range: 8.3 - 15.75) and 7.9 kg (interquartile range: 1 - 82.4), respectively. This difference was due to the greater proportion of small-sized dogs in our study. However, all these studies agreed that MMVD occurred mostly in small- to medium-sized dogs.

Table 2. Distribution of Myxomatous mitral valve degeneration (MMVD) by sex, age, and body weight among stages

Factors	Stage B1	Stage B2	Stage C	Total	<i>P</i>
Sex n (%)					0.812
Male	48 (60.0)	26 (55.3)	15 (62.5)	89 (58.9)	
Female	32 (40.0)	21 (44.7)	9 (37.5)	62 (41.1)	
Age (years)	12 (10 - 13)	11 (9 - 14)	12 (11 - 13)	12 (10 - 13)	0.157
Body weight groups n (%)					0.296
< 20 kg	75 (93.8)	45 (95.7)	23 (95.8)	143 (94.7)	
≥ 20 kg	5 (6.2)	2 (4.3)	1 (4.2)	8 (5.3)	
Body weight (kg)	4.8 (3.8 - 7.4)	5.2 (4.4 - 7.4)	4.9 (3.7 - 6)	5.1 (4 - 7.2)	0.849
Total n (%)	80 (53.0)	47 (31.1)	24 (15.9)	151 (100.0)	

n: number of dogs with MMVD.

3.3. Physical examination findings

Table 3 summarizes the physical findings observed across the different MMVD stages. A

statistically significant association was found between clinical signs and disease stages ($P < 0.001$).

Among the 151 MMVD-affected dogs, 66 cases (43.7%) were asymptomatic, while 85 cases (56.3%) exhibited clinical signs. The rate of asymptomatic cases in this study was higher than that reported in previous studies (Kim et al., 2017; Franchini et al., 2021) which recorded only 5.9 and 7% asymptomatic cases, respectively. These results may be explained by study's setting within an international pet hospital, where routine cardiac screenings and preoperative examinations are standard practice, leading to increased detection of asymptomatic MMVD. Coughing (40.8%) was the most prevalent clinical sign, consistent with previous reports (Kim et al., 2017; Franchini et al., 2021). In dogs with more advanced MMVD, the most common causes of cough were the mainstem bronchial compression due to LA dilation and concurrent airway disease (Ferasin et al., 2013). All dogs in Stage C exhibited clinical signs due to the development of CHF. Dyspnea and tachypnea (respiratory rate > 30 bpm) were most frequently observed in stage C. However, these clinical

signs can be caused by other respiratory diseases rather than CHF, particularly in stage B1 or stage B2 dogs.

Murmur intensity was strongly associated with MMVD severity ($P < 0.001$) (Table 4). Dogs in stage B1 are most often presented with soft (57.5%) or moderate (30.0%) murmurs. In stage B2, moderate (44.7%) and loud (48.9%) murmurs were predominant, with a smaller proportion of dogs exhibiting thrilling murmurs (6.4%). Notably, no stage B1 dogs had thrilling murmurs, and no stage B2 dogs had soft murmurs. In contrast, all stage C cases with CHF exhibited loud or thrilling murmurs, accounting for 29.2 and 70.8%, respectively. These differences in murmur intensity across MMVD stages were in line with previous reports (Ljungvall et al., 2014; Franchini et al., 2021). Assessing the intensity of the left apical systolic murmur is a valuable indicator for evaluating MMVD severity, as stated in the ACVIM consensus guidelines (Keene et al., 2019).

Table 3. The grading of heart murmur intensity among Myxomatous mitral valve degeneration (MMVD) stages

Clinical signs	Stage B1	Stage B2	Stage C	Total n (%)
Cough	27 (32.1)	23 (46.0)	21 (52.5)	71 (40.8)
Asymptomatic	47 (56.0)	19 (38.0)	0 (0.0)	66 (37.9)
Dyspnea	5 (5.9)	5 (10.0)	9 (22.5)	19 (11.0)
Tachypnea	4 (4.8)	3 (6.0)	7 (17.5)	14 (8.0)
Syncope	1 (1.2)	0 (0.0)	3 (7.5)	4 (2.3)
Total n (%)	84 (48.3)	50 (28.7)	40 (23.0)	174 (100.0)

n: number of clinical signs presenting in dogs with MMVD.

Table 4. The grading of heart murmur intensity among Myxomatous mitral valve degeneration (MMVD) stages

Murmur grades		Stage B1	Stage B2	Stage C	Total n (%)
I	Soft	7 (8.8)	0 (0.0)	0 (0.0)	7 (4.6)
II		39 (48.7)			39 (25.8)
III	Moderate	24 (30.0)	21 (44.7)	0 (0.0)	45 (29.9)
IV	Loud	10 (12.5)	23 (48.9)	7 (29.2)	40 (26.5)
V	Thrilling	0 (0.0)	3 (6.4)	15 (62.5)	18 (11.9)
VI			0 (0.0)	2 (8.3)	2 (1.3)
Total n (%)		80 (53.0)	47 (31.1)	24 (15.9)	151 (100.0)

n: number of dogs with stage of MMVD.

3.4. Radiographic and echocardiographic findings

Table 5 summarizes the radiographic and echocardiographic findings across the

different MMVD stages. Statistically significant associations with disease stages were observed in subjective collected variables ($P < 0.001$) These variables included VHS, LVIDDN, LVIDSN, and LA/Ao.

Table 5. Result of radiographic and echocardiographic variables in dogs with different ACVIM stages of Myxomatous mitral valve degeneration (MMVD)

Index	Stage B1	Stage B2	Stage C	Total	<i>P</i>
VHS	10.22 (9.51 - 10.52) ^a	10.89 (10.6 - 11.23) ^b	11.97 (11.14 - 12.43) ^c	10.50 (10.00 - 11.00)	< 0.001
LA/Ao	1.46 (1.22 - 1.51) ^a	1.77 (1.70 - 1.91) ^b	2.31 (2.04 - 2.54) ^c	1.60 (1.37 - 1.83)	< 0.001
LVIDDN (mm)	1.41 (1.33 - 1.54) ^a	1.77 (1.61 - 1.80) ^b	1.90 (1.82 - 2.15) ^c	1.59 (1.41 - 1.75)	< 0.001
LVIDSN (mm)	0.71 ± 0.14 ^a	0.82 ± 0.16 ^b	0.91 ± 0.18 ^c	0.77 ± 0.17	< 0.001
FS (%)	48.61 ± 8.17	50.77 ± 8.07	51.73 ± 6.85	49.78 ± 7.34	0.142

ACVIM: American college of veterinary internal medicine; VHS, vertebral heart score, LA/Ao, left atrial-to-aortic ratio; LVIDDN (cm), left ventricular internal dimension in diastole normalized to body size; LVIDSN (cm), left ventricular internal dimension in systole normalized to body size; FS (%), fractional shortening. Values represented with same superscript letters for dogs with stages of MMVD did not differ significantly ($P < 0.001$).

The VHS is an index for assessing the presence of radiographic cardiomegaly. A VHS ranging from 8.5 to 10.5 is considered normal for the majority of dog breeds (Kvart & Pedersen, 2005). The median VHS in this study was 10.50 (interquartile range: 10.00 - 11.00) and differed significantly between stages ($P < 0.001$). This value was slightly lower than the median of 11.2 (interquartile range: 8 - 13.5) reported by Franchini et al. (2021). While the median VHS for stage B2 dogs in the current study was also lower than that in Franchini et al. (2021), it remained consistent with the reference values for stage B2 recommended by the ACVIM consensus guidelines (Keene et al., 2019). Dogs in stage C had the highest median of 11.97 (interquartile range: 11.14 - 12.43) which was remarkably higher than those values in stages B1 and B2. A difference was also observed by Franchini et al. (2021). These results highlight the association between cardiomegaly and progressive cardiac remodeling, reflected by increasing VHS values with advancing disease stages.

The LA/Ao ratio is a valuable index for assessing left atrial dilatation, which typically occurs due to significant mitral regurgitation. The median value was 1.60 (interquartile range: 1.37 - 1.83), with statistically significant differences between stages. Dogs in stage C had a markedly higher median LA/Ao ratio of 2.31 (interquartile range: 2.04 - 2.54) compared to other stages ($P < 0.001$). Similarly, dogs in stage B2 had a higher LA/Ao ratio of 1.77 (interquartile range: 1.70 - 1.91) than stage B1, which had the median value of 1.46 (interquartile range: 1.22 - 1.51) ($P < 0.001$). These results are in agreement with previous studies by Franchini et al. (2021) and Trofiniak & Slivinska (2021), which also reported a progressive increase in the LA/Ao ratio with advancing ACVIM stages. This reflects

the disease progression and the severity of volume overflow that leads to structural changes in the left atrium.

The LVIDDN is a commonly used index for assessing the degree of left ventricular dilation, which occurs due to volume overload as mitral regurgitation progresses (Chetboul & Tissier, 2012). The median LVIDDN was 1.59 (interquartile range: 1.41 - 1.75) with significant differences observed across three stages ($P < 0.001$). The median LVIDDN was 1.41 (interquartile range: 1.33 - 1.54) in stage B1, 1.77 (interquartile range: 1.61 - 1.8) in stage B2, and 1.90 (interquartile range: 1.82 - 2.15) in stage C, demonstrating a progressive increase in value with disease severity. While similar results were observed by Franchini et al. (2021) and Trofiniak & Slivinska (2021), the values in the present study were lower than those reported in their study. There was a significant relationship between LVIDDN and the clinical status, with LVIDDN increasing with disease severity (left ventricular enlargement), as stated ACVIM consensus guidelines (Keene et al., 2019) for classifying preclinical MMVD dogs into stage B1 or B2 (LVIDDN ≥ 1.7).

The LVIDSN is commonly used for assessing systolic function although it is dependent on cardiac loading conditions (Menciotti & Borgarelli, 2017). LVIDSN > 1.26 can be a suggestion for left ventricular systolic dysfunction. The mean LVIDSN was 0.77 ± 0.17 , with significant differences between the study groups ($P < 0.001$). The LVIDSN in stage C dogs had the highest mean (0.91 ± 0.18) and differed statistically from stages B1 (0.71 ± 0.14) and B2 (0.82 ± 0.16) ($P < 0.001$). Similarly, the LVIDSN was also significantly higher in B2 dogs compared to those in stage B1 ($P < 0.001$). This increasing trend in mean LVIDSN values with disease

progression aligns with the findings reported by Franchini et al. (2021). However, while Franchini et al. (2021) did not find a significant difference between stages B2 and C, the present study did.

Percentage of FS is an indicator for assessing myocardial systolic function, influenced by factors such as preload, afterload and sympathetic tone. While FS values can experience a gradual increase (over 50%) during the progression of MMVD, they may return to normal levels in advanced stage (Chetboul & Tissier, 2012; Reynolds et al. 2012). In the present study, FS showed a negligible increase of values in the progression to the advanced stage of heart failure. However, no statistically significant association was found between FS values and the different MMVD stages ($P > 0.05$). This suggests that FS may be influenced by the factors mentioned.

4. Conclusions

Myxomatous mitral valve degeneration is a common heart disease in dogs with the higher prevalence of MMVD found in small-sized breeds, middle-aged to old-aged, and male dogs. Murmur intensity could be used for early disease detection in high-risk individuals. Radiographic and echocardiographic examination of MMVD dogs across different stages revealed that increases in VHS, LA/Ao, LVIDDN, and LVIDSN are clinical relevance in assessment of the progression of MMVD in dogs.

Conflict of interest

The authors declare that there is no conflict of interest.

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Chitosan from shrimp by-products as potential feed additive for improving shrimp farming efficiency in Vietnam, towards a more sustainable and circular economy

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Research Paper Received: January 23, 2025 Revised: April 22, 2025 Accepted: May 08, 2025 Keywords Chitosan Circular economy Shrimp by-products Sustainable aquaculture *Corresponding author Tran Van Ty Email: vantytran103@gmail.com	Shrimp farming in Vietnam, particularly in intensive systems, faces many challenges: disease outbreaks, environmental stress, feed quality issues, and water management, leading to high mortality and reduced efficiency. Chitosan, a natural biopolymer derived from crustaceans like shrimp and crab, offers a sustainable solution. With antimicrobial, immune-boosting, and growth-enhancing properties, chitosan has been shown to improve shrimp health by boosting immunity, reducing oxidative stress, and supporting growth, while also increasing feed efficiency and water quality. The purpose of this paper is to review the current knowledge on the potential application of chitosan in shrimp farming, particularly in Vietnam, and its role in addressing the challenges of sustainability, disease prevention, and feed efficiency in the shrimp aquaculture industry. Vietnam, as a global leader in shrimp production, possesses large quantities of shrimp by-products from processing plants and molted shells at farms, provides an abundant resource for chitosan production, creating a closed-loop solution within the shrimp industry itself. Converting this waste into chitosan supports circular economy goals and reduces reliance on antibiotics. Integrating shrimp-derived chitosan could foster a more sustainable and resilient shrimp farming industry, benefiting both the economy and the environment.

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1. Introduction

1.1. Vietnam's shrimp industry overview

The shrimp industry is a cornerstone of Vietnam's economy, particularly in the

Mekong Delta, where shrimp farming provides a livelihood for millions of people. Shrimp farming areas have expanded by an average of 5% annually, while production has grown by 8.4% per year, driven largely by improvements

in whiteleg shrimp (*Litopenaeus vannamei*) productivity. Vietnam is now among the world's top five shrimp-exporting countries, with whiteleg shrimp dominating the export market and achieving an average growth rate of 7% per year (SI, 2024; VASEP, 2024).

1.2. Disease management crisis

The management of shrimp health in intensive farming systems is a significant challenge due to their high vulnerability to diseases and environmental stress. At the most critical level, diseases like early mortality syndrome (EMS) can devastate entire populations, leading to almost 100% mortality and causing global losses of up to \$9 billion annually - about 15% of the world's shellfish production (Schryver et al., 2014). This issue is compounded in super-intensive farming environments, where 100 - 300 shrimp per square meter promote the growth of harmful pathogens such as bacteria and algae due to ineffective feed management (Nguyen, 2020). Furthermore, shrimp's inability to regulate their own body temperature makes them highly sensitive to even small environmental fluctuations. For instance, minor changes in pH, temperature shifts of 2 - 5°C, or salinity variations of 5% can cause significant stress, particularly in species like tiger prawns (Ta & Huynh, 2017; Phuong, 2021). Adding to this, shrimp lack an adaptive immune system, leaving them with very limited natural defenses (Tepbac, 2018).

1.3. Supply of raw materials for shrimp feed

The reliance on imported feed ingredients in Vietnam is significant, with approximately 70 - 80% of essential materials, such as fishmeal and soybean, sourced from abroad (WB, 2022). This dependency makes the shrimp industry vulnerable to supply chain disruptions and cost

increases. For example, between 2021 and 2022, the price of imported fishmeal rose by 6.4%, contributing to a 10 - 17% increase in production expenses (Hong, 2022; WB, 2022). These rising costs have eroded Vietnam's competitiveness, especially compared to shrimp exporters like India and Ecuador, where production costs are 30% lower (Trung, 2021).

To address this, plant-based proteins have been proposed as an alternative to fishmeal. However, this shift presents challenges that can negatively impact shrimp health and performance. Plant-based proteins often contain anti-nutritional factors (ANFs), such as protease inhibitors and phytic acid, which reduce feed efficiency, ultimately impairing shrimp growth (Gatlin et al., 2007; Glencross et al., 2007). They are also prone to mycotoxin contamination, like aflatoxins, which suppress immunity and cause organ damage (Hasan & Goncalves, 2017). Additionally, amino acid imbalances and low digestibility in plant proteins can further limit growth, reduce feed intake, and increase stress from oxidative damage (Richard et al., 2011; Tacon & Metian, 2015). Natural farming practices, which prioritize strict environmental controls and minimize antibiotic use, are emerging as key strategies to enhance shrimp health and ensure production sustainability (Xia & Yue, 2010).

1.4. Understanding chitosan

Chitosan is a biopolymer derived from chitin, the second most abundant polymer, primarily extracted from the exoskeletons of crustaceans like shrimp and crabs. The industrial production of chitosan involves the deacetylation of chitin by removing most of the acetyl groups from chitin, converting N-acetyl-d-glucosamine into β -1,4-d-glucosamine (Malerba & Cerana, 2018).

Chitosan is biodegradable, biocompatible and non-toxic, making it environmentally safe and suitable for various applications. Additionally, it possesses diverse functionalities, including antimicrobial, antioxidant, immunostimulant, and heavy metal-binding properties (Malerba & Cerana, 2018). These features make chitosan a highly valuable ingredient across numerous sectors, including food production, pharmaceuticals, and agriculture. In Vietnam, there is an abundant supply of chitosan sourced from shrimp processing by-products and molted shells from farms (Trang & Pham, 2012). Leveraging these resources not only adds value to the shrimp industry but also promotes sustainability by converting waste into a valuable biopolymer.

In aquaculture, chitosan is widely used as a feed additive to enhance shrimp's health and aquatic environment. It strengthens shrimp immunity, reduces disease outbreaks with its antimicrobial properties, and helps shrimp better handle environmental stress (Wang et al., 2017). Additionally, chitosan enhances nutrient absorption, supports gut health, promotes molting, and improves the stability of feed pellets in water, leading to greater feed efficiency. Environmentally, chitosan improves water quality by binding to and removing harmful substances like phytoplankton, organic matter, and heavy metals, resulting in cleaner, more stable water conditions in shrimp ponds (Lertsutthiwong et al., 2009). These combined effects make chitosan a valuable compound for sustainable shrimp farming.

2. Chitosan as a Functional Ingredient for Shrimp Feed

Chitosan, as a highly versatile biopolymer, varies in physicochemical and biological

properties such as degree of deacetylation (DD), molecular weight (MW), viscosity, and solubility (Pellis et al., 2022), influencing its bioactivity in aquaculture. Chitosan can be formulated as powder or liquid, and may require acidic solvents (e.g., acetic acid) for complete dissolution when used in feed applications. Therefore, selecting the appropriate form of chitosan and tailoring its properties to specific aquaculture targets are essential for maximizing its functional performance in shrimp farming.

2.1. Chitosan as an antimicrobial, immunostimulant for disease prevention and treatment

Chitosan acts as a strong natural antibacterial agent that helps shrimp combat common diseases without heavy reliance on antibiotics. First, it interacts with the negatively charged cell walls of bacteria, causing leakage and cell wall damage (Xing et al., 2015). It also forms a film on the bacterial surface, inhibiting their metabolism and nutrient exchange (Flegel, 1998). Additionally, chitosan creates chelation to specific metal ions, destabilizing the bacterial cell wall and suppressing growth. Finally, it acts as a DNA binder, preventing protein synthesis and halting bacterial replication (Xing et al., 2015).

Chitosan effectively inhibits *Vibrio* species bacteria, including *Vibrio alginolyticus* (Anas et al., 2005) and *Vibrio parahaemolyticus* (Schryver et al., 2014), which are associated with Hepatopancreatic Necrosis, and *Vibrio mediterranei* (Ruangpan, 1998), which causes Luminescent Disease. In severe cases, these pathogens can lead to mortality rates of up to 100% in shrimp (Ruangpan, 1998). By reducing pathogen prevalence, chitosan helps lower the need for antibiotics, improving shrimp health and survival (Flegel, 1998; Schryver et al., 2014).

Chitosan also boosts the shrimp's immune system, strengthening their ability to combat biotic stress. It stimulates the production of hemocytes, the primary immune cells in shrimp. Hemocytes perform key functions such as phagocytosis (engulfing pathogens), nodulation (aggregating to trap bacteria), and encapsulation (surrounding larger invaders), strengthening the shrimp's overall immune response (Wang & Chen, 2005). In the humoral immune response, hemocytes release enzymes like transglutaminase (Tgase) and prophenoloxidase (ProPO), which promote coagulation and pathogen killing through the melanization reaction, while also releasing antimicrobial peptides to inhibit or kill pathogens (Cerenius & Söderhäll, 2012; Tassanakajon et al., 2017).

By activating both cellular and humoral immune responses, chitosan significantly enhances shrimp resistance to pathogens, particularly *Vibrio* species, thereby reducing reliance on antibiotics and promoting healthier aquaculture stocks. In the study by Wang & Chen (2005), whiteleg shrimp were injected with 2 and 4 µg/g of chitosan and subsequently challenged with *V. alginolyticus* at a dose of 2×10^6 cfu/shrimp. Chitosan-treated shrimp showed significantly higher survival rates than controls, with enhanced immune responses, including total hemocyte count, and respiratory burst. This indicates that increasing chitosan up to 4 µg/g optimizes antimicrobial defenses and enhances shrimp resistance to infection.

2.2. Chitosan as an antioxidant for environmental adaptation

Chitosan plays a crucial role in reducing oxidative stress in shrimp by acting on free radicals that cause cellular damage. It works through two main mechanisms. First, chitosan

enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), as well as non-enzymatic antioxidants like glutathione (GSH), which help neutralize excess reactive oxygen species (ROS) (Niu et al., 2013). Second, chitosan directly scavenges free radicals through its amino and hydroxyl groups, protecting cells from oxidative damage (Wang et al., 2012). These combined actions allow chitosan to help maintain the health of shrimp cells (Table 1).

By alleviating oxidative stress, chitosan shields shrimp from free radical-induced damage, including genetic mutations, lipid peroxidation, and protein degradation. Shrimp treated with chitosan shows enhanced antioxidant capacity, reduced oxidative damage, and improved survival rates under stress (Rahimnejad et al., 2018). This highlights chitosan as a valuable tool for boosting shrimp resilience in aquaculture systems.

Studies have shown that dietary chitosan can greatly enhance disease resistance and stress resilience in whiteleg shrimp. Chen et al. (2020) found that chitosan at 1.0 g/kg increased SOD activity by 15%, indicating stronger antioxidant defenses and less oxidative damage, leading to a 4.7% increase in survival rates under ammonia stress. Expanding on these findings, Brol et al. (2021) explored dietary chitosan at 1, 3, and 5 g/kg under saline stress, revealing that 1 g/kg was the optimal dose. This concentration maintained SOD and GST activity at baseline, increased CAT activity in the gills by 12%, and stabilized GSH levels in the hepatopancreas. Although higher concentrations offered benefits, they showed diminishing returns without added advantages over 1 g/kg. Overall, these findings collectively establish chitosan as a powerful and sustainable supplement for boosting antioxidant defenses and improving disease resistance in shrimp aquaculture.

Table 1. Chitosan on stress and disease resistance in shrimp species

	Stress Resistance		Disease Resistance	
Object	Whiteleg shrimp	Whiteleg shrimp	Whiteleg shrimp	Whiteleg shrimp
Condition	Ammonia stress add NH ₄ Cl 2.15 mg/L	Salinity stress Sal30 (sea water) → Sal5 (sea water + fresh water)	<i>Vibrio</i> <i>alginolyticus</i> (infectious disease)	<i>Vibrio</i> <i>parahaemolyticus</i> (EMS/AHPND disease)
Chitosan property	Water-soluble chitosan	From shrimp shell DD ≈ 90% MW = 125 kDa	DD ≈ 90%	MW = 544 kDa DD = 95%
Best dosage	0.1%	1 - 3%	4%	0.025%
References	(Chen et al., 2020)	(Brol et al., 2021)	(Cheng et al., 2021)	(Liang et al., 2020)

2.3. Chitosan as a growth promoter

Chitosan promotes shrimp growth by improving both nutrition digestion, adsorption and facilitating the molting process. It enhances enzyme production and nutrient absorption, leading to better feed use efficiency (Ekaputri et al., 2018). At the same time, chitosan supports molting by accelerating the breakdown of the old exoskeleton and aiding the formation of a new, stronger one (Zhang et al., 2014). These combined effects enhance shrimp growth, feed efficiency, and overall performance in aquaculture systems.

2.3.1. Nutrient digestion and absorption

The hepatopancreas, the vital digestive organ in shrimp, plays a critical role in nutrient absorption, storage, and overall health. However, plant-based proteins or fishmeal alternatives can compromise its function, causing inflammation, structural damage, and reduced efficiency in nutrient utilization (Rahimnejad et al., 2018). Chitosan enhances hepatopancreas health by

stimulating the proliferation of embryonic cells and resorptive cells, essential for nutrient absorption and storage, while strengthening hepatopancreatic tubules and the basal membrane (Rahimnejad et al., 2018). These benefits optimize digestion, nutrient utilization, and organ function, making chitosan a crucial component in supporting low-fishmeal diets for sustainable shrimp farming.

Chitosan enhances nutrient digestion and absorption by increasing the activity of digestive enzymes like protease and trypsin (Liu et al., 2019; Tseng et al., 2021). These enzymes help break down proteins more effectively, leading to improved nutrient availability. Chitosan also improves intestinal histology by increasing fold height, which expands the surface area for nutrient absorption (Rahimnejad et al., 2018). The effectiveness of chitosan as a growth-promoting agent in shrimp aquaculture is well-supported by various studies, which consistently highlight its benefits in enhancing growth performance, specific growth rate in shrimp (Table 2).

Table 2. Effects of Chitosan on promoting growth in shrimp species

No.	Chitosan Properties	Year	Object	References
1	DD $\geq$ 75% Optimal dosage: 2.13 - 2.67 g/kg	2011	Whiteleg shrimp	(Niu et al., 2011)
2	DD $\geq$ 75% Optimal dosage: 0.1 - 0.2%	2015	Giant Tiger Prawn	(Niu et al., 2015)
3	Nano chitosan particles Optimal dosage: 1 mL/kg	2020	Giant Tiger Prawn	(Abdel-Warith et al., 2020)
4	From <i>Daphnia similis</i> source Optimal dosage: 0.4%	2021	Whiteleg shrimp	(Tseng et al., 2021)

In the 23-day investigation by Niu et al. (2011), whiteleg shrimp was fed a diet containing 2 g/kg chitosan showed a significant 39% increase in weight gain compared to the control, accompanied by a 7.5% boost in specific growth rate. The study concluded that chitosan at 2.13 - 2.67 g/kg were optimal for achieving balanced growth and nutrient absorption. Further validation from Tseng et al. (2021) in their 56-day study on whiteleg shrimp showed that shrimp fed 0.4% chitosan exhibited an 8.8% higher specific growth rate and a 22% increase in weight gain. Collectively, these highlight chitosan's potential to enhance shrimp digestion and growth, establishing it as a valuable and sustainable supplement to shrimp farming.

2.3.2. Molting process stimulation

Molting is a vital process for shrimp growth, requiring sufficient chitin and nutrients stored in the hepatopancreas. In natural environments, shrimp obtain enough chitin from sources like small shrimps and crabs. However, in intensive farming systems, high stocking densities limit chitin availability from feed, and self-synthesis

alone is insufficient. Chitosan offers a solution by supplying chitin while promoting efficient molting and stronger shells, making it essential for sustainable shrimp farming (BFM, 2024).

Chitosan aids in the shrimp growth promotion by facilitating both the breakdown of the old exoskeleton and the formation of the new one (Zhang et al., 2014). During the pre-molt stage, chitosan boosts CHT6 enzyme expression, aiding in hydrolyzing the old exoskeleton for easier shedding (Zhang et al., 2014). During molt and post-molt stages, chitosan boosts CHT3 enzyme activity to hydrolyze chitin into monomers and enhances chitin synthase activity to polymerize them, building a stronger new exoskeleton.

In the study by Tseng et al. (2021), optimal chitosan supplementation at 0.4% in whiteleg shrimp led to a significant increase in molting enzyme activity, with 1,200% and 335.7% increases in CHT3 and CHT6, respectively. Similarly, in research by Attasart et al. (2005), adding chitosan at 100 ppm (with DD = 85%) in whiteleg shrimp enhanced shell formation, increasing shell weight by 13.7% and chitin content by 0.81%. These findings show that

chitosan enhances molting efficiency and exoskeleton strength, thereby improving shrimp performance in aquaculture systems (Zhang et al., 2014).

Chitosan, chitin, and shrimp head meal each have distinct benefits and limitations in shrimp farming. Chitosan presents a promising solution, such as enhancing growth performance and boosting molting enzyme activity (Zhang et al., 2014; Tseng et al., 2021). In contrast, chitin mainly serves as a chitin source, offering little support for molting enzymes and limited applicability due to its water insolubility (Attasart et al., 2005). Shrimp head meal also have some limitations, including poor feed stability and low digestibility due to high fiber and ash content (approximately 10 and 35%, respectively), which lowers nutrient absorption and feed efficiency (Cavalheiro et al., 2007). Overall, chitosan offers a superior solution over chitin and shrimp head meal, making it the preferred choice for aquaculture systems.

2.4. Chitosan in reducing waste and protecting water quality

2.4.1. Chitosan as a feed additive to improve feed pellet durability

The durability of feed pellets in water is crucial in aquaculture as it prevents disintegration, ensuring that nutrients remain intact and available for shrimp consumption. This minimizes water pollution, and improves the overall efficiency of feeding practices (BFM, 2024). Chitosan improves the feed stability by forming a protective film around pellets. When dissolved in dilute acid, its amino and hydroxyl groups become activated, forming cross-linking bonds with feed polymers, creating a tight and durable network (Dezfooli et al., 2022). This network strengthens the pellet structure,

maintaining feed integrity longer in water (Jaroensaensuai et al., 2022).

In the study by Jaroensaensuai et al. (2022), the integration of chitosan into an egg yolk-chitosan composite demonstrated exceptional improvements in the durability and nutrient retention of shrimp feed pellets. The fortified feed maintained 85 - 87% of its dry mass after 6 h in seawater, significantly outperforming uncoated pellets, which disintegrated much faster. By preserving feed integrity, chitosan helps minimize waste, maintain water quality, and ensure more efficient nutrient delivery to shrimp.

2.4.2. Chitosan as a green chemical for water quality improvement

Intensive shrimp farming in Vietnam presents significant challenges to maintaining water quality in aquaculture ponds. Conventional chemical disinfectants can disrupt the natural microbial balance and increase the risk of disease outbreaks (Nguyen & Tran, 2019; Do et al., 2023). Chitosan acts as a sustainable flocculant, effectively aggregating organic matter, such as uneaten feed, without harming beneficial microbes. The resulting sludge can be repurposed as organic fertilizer, promoting waste management and reducing the environmental impact of shrimp farming (Lertsutthiwong et al., 2009).

Chitosan's efficiency as a flocculant stems from its cationic polymer structure. It binds to negatively charged particles via electrostatic interactions, forming flocs that are easily removed from water (Lertsutthiwong et al., 2009). Additionally, chitosan performs chelation, where its amino groups bind to heavy metal ions, capturing them for efficient removal (Zubair et

al., 2020). Through these combined mechanisms, chitosan neutralizes pollutants and enhances water quality effectively.

Lertsutthiwong et al. (2009) demonstrated that 40 mg/L chitosan achieved 85% flocculation efficiency in outdoor shrimp tanks over a 28-day period. This approach effectively reduced nitrogenous waste buildup, and promoted shrimp health. Similarly, Ruswahyuni et al. (2010) applied 200 ppm liquid chitosan in semi-traditional whiteleg shrimp ponds in Indonesia. Over three months, chitosan reduced total suspended solids by 48.5% (0.68 to 0.35 mg/L),

improving water clarity. These studies underscore chitosan's role in enhancing water quality and supporting sustainable aquaculture.

3. Research on Using Chitosan in Aquaculture in Vietnam

Several studies have explored the applications of chitosan in Vietnam's aquaculture sector, particularly for enhancing immunity, growth, and disease resistance, highlighting its potential to improve both yield and sustainability in aquaculture practices (Table 3).

Table 3. Some studies on the use of chitosan in Vietnam Aquaculture

No.	Article	Year	Object	References
1	Research on the Development and Assessment of Immune-Stimulating Effects Against Liver and Kidney Disease in Striped Catfish (<i>Pangasianodon hypophthalmus</i>) Using Oligochitosan and Oligo β -Glucan	2015	Pangasius (<i>Pangasianodon hypophthalmus</i>)	(Nguyen et al., 2015)
2	Effect of Oligochitosan and Oligo- β -Glucan Supplementation on Growth, Immunity, and Disease Resistance of Striped Catfish (<i>Pangasianodon hypophthalmus</i>)	2017	Pangasius (<i>Pangasianodon hypophthalmus</i>)	(Nguyen et al., 2017)
3	Effects of dietary chitin supplementation on the resistance of Whiteleg shrimp (<i>Litopenaeid vannamei</i>) to <i>Vibrio parahaemolyticus</i>	2019	Pangasius (<i>Pangasianodon hypophthalmus</i>)	(Bui & Nguyen, 2019)
4	Effect of dietary chitosan on non-specific immune parameters of striped catfish (<i>Pangasianodon hypophthalmus</i>)	2020	Whiteleg shrimp	(Tran et al., 2020)

Chitosan has been effectively applied in aquaculture systems in some regions of Vietnam, with studies underscoring its diverse benefits. In the study by Nguyen et al. (2015), catfish were fed diets supplemented with 0, 50, 100, and 200 mg/kg of oligochitosan for 45 days before being challenged with *Edwardsiella ictaluri*, a bacterium causing enteric septicemia and high mortality rates. The results revealed that fish receiving 100 mg/kg oligochitosan had a survival rate 436% higher than the positive control (47.62 vs. 8.89%), with significant boosts in both humoral and cellular immune responses. Supporting this, Bui & Nguyen (2019) investigated chitosan at higher concentrations (0, 1, 5, and 10 g/kg) over a 4-week period. Their findings indicated that fish fed 1 g/kg chitosan showed substantial immune improvements, including higher blood cell counts and lysozyme activity. When exposed to *E. ictaluri*, these fish exhibited the lowest mortality rate at 43.3%, a 27.8% reduction compared to the 60% observed in controls. These studies affirm chitosan as powerful immunostimulants, enhancing disease resilience and sustainability in Vietnam's aquaculture, benefiting not only striped catfish but also shrimp and other cultured species.

4. Chitosan from Shrimp By-Products in Vietnam

Vietnam, a major shrimp producer, generates significant shrimp by-products, including shells, heads, and processing waste, often discarded or underutilized. However, with growing sustainability awareness, these by-products are increasingly recognized as valuable resources, particularly for chitosan production.

According to a study by Trang & Pham (2012), the shrimp processing industry in Vietnam generates approximately 200,000 metric tons

of by-products each year. This includes shrimp shells, which constitute a major portion of the waste, and can be collected in large quantities from processing plants. For instance, intensive shrimp farming systems can generate up to 280 kg of shrimp shells per 1,500 m² pond per harvest (Pham et al., 2021). These shrimp shells, which are primarily composed of chitin, are an excellent raw material for chitosan production.

In recent years, the rapid expansion of Vietnam's shrimp industry has significantly increased the volume of by-products, with estimates suggesting an annual output of 325,000 tons, and projections reaching over 450,000 tons by 2025 - equivalent to more than 1,000 tons of waste discarded daily (MOIT, 2024). If not properly managed or utilized, these by-products pose a serious environmental concern. However, they are increasingly recognized as a valuable resource, rich in protein, chitin, and astaxanthin, which can be used to produce high-value compounds such as feed additives, fertilizers, and biopolymers.

Among these, chitosan stands out as a promising solution to enhance shrimp health, digestion, and disease resistance. It supports the adoption of low fishmeal diets while decreasing dependency on antibiotics and imported additives, making shrimp farming more sustainable and cost-effective. Leveraging shrimp by-products for chitosan production also strengthens Vietnam's circular economy, turning waste into valuable resources and reducing pollution in coastal areas (Nguyen et al., 2023). With eco-friendly production methods like enzymatic degradation, chitosan not only promotes environmental sustainability but also creates economic value, reinforcing Vietnam's role as a leader in sustainable aquaculture.

5. Conclusions

Chitosan emerges as a promising multifunctional additive that addresses several critical challenges in shrimp aquaculture. Its antimicrobial and immunostimulant properties help mitigate disease outbreaks and reduce dependence on antibiotics, thereby supporting healthier and more resilient shrimp populations. Additionally, chitosan plays a key role in alleviating oxidative stress, enhancing digestive efficiency, and stimulating molting, all of which contribute to improved shrimp growth performance and survival rates.

From a feed technology perspective, chitosan improves pellet durability and nutrient retention, while also contributing to water quality improvement through its flocculating and metal-binding capabilities. These characteristics make it an ideal additive for advancing feed efficiency and environmental sustainability in high-density shrimp farming systems.

Importantly, Vietnam holds significant potential for localized chitosan production, given its abundant shrimp by-products, including shells and molted exoskeletons, which can be converted into high-value chitosan using eco-friendly extraction methods. This not only adds economic value to what would otherwise be waste, but also supports a circular economy model that aligns with global trends in sustainable aquaculture.

Overall, integrating shrimp-derived chitosan into farming practices offers a comprehensive solution, enhancing shrimp health, improving production efficiency, reducing environmental footprint, and creating value from waste. As such, chitosan not only benefits shrimp and farmers but also positions Vietnam's shrimp industry

as a frontrunner in sustainable and responsible aquaculture development.

Conflict of interest

We hereby declare that this is our original research work. The data and results presented in this article are entirely truthful and have never been published in any previous research work by anyone.

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The situation of florae diversity on the Nong Lam University campus, Ho Chi Minh City

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ARTICLE INFO	ABSTRACT
Research Paper Received: October 17, 2024 Revised: January 25, 2025 Accepted: March 17, 2025 Keywords Flora diversity Flora management Nong Lam University campus Species *Corresponding author Vuong Thi Thuy Email: vuongthuy@hcmuaf.edu.vn	College campuses usually have diverse floral habitats and plant communities. Nong Lam University is one of the largest universities in Vietnam with an area of 118 ha. This study determined the diversity of flora on the Nong Lam University campus. This research was carried out from June to September 2024 and collected systematic data to support the management of the university's flora. There were 243 recorded species, distributed in 70 families, including 12 rare species listed in the 2024 IUCN Red List (International Union for Conservation of Nature), and 9 species recorded in the Vietnam Red Data Book (2024). The study evaluated species diversity at many taxa such as phylum, class, order, genus, species, as well as plant growth forms, and origins. The current flora management practices were analyzed. Prior to this study, no flora database existed at the Nong Lam campus, including names, years of planting, ages, etc. Hence, the study recommended that the flora should be collected and managed using geographic information systems (GIS) and integrated into the university's website (webGIS). This will inform staff and students about the campus's plant biodiversity, promote awareness of the role of plants in ecology, and encourage the protection of existing flora.

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1. Introduction

Florae on university campuses is critical for students' lives. Previous studies showed that the florae on campuses might improve the students' mental and physical health. Besides that, plants on campuses could reduce school violence (Alcock, 2014; Younan, 2016) by mitigating stress and

altering emotional states such as more happiness or less anxiety (Bowler et al., 2010; Lachowycz & Jones, 2013; Mcsweeney et al., 2015). In one research, Dymment & Bell (2008) concluded that in schools with plants, students behaved more civilly (72%), communicated more effectively (63%), were more cooperative (69%), and reinforced their interactions with teachers (69%). In addition,

there was a decline of 7% in health problems for children in classrooms with plants (Duijn et al., 2011). Besides that, students' scores significantly increased while studying in a green environment. Florae around the school buildings support raising test scores, grades, memory, work efficiency, and attention (Matsuoka, 2010). Lawns, gardens, and trees on campuses provide students with spaces for academic exchange, thus enhancing communication skills (Dymont & Bell, 2008). Moreover, flora communities support students' learning and living in a green environment and teach them a sense of responsibility for environmental sustainability (Doan, 2002).

Due to the importance of plant communities on campuses, many studies have been conducted to understand the situation and the diversity of florae on campuses. Jichun (2012) did research in three high schools in Suzhou City. The plant frequency and species richness were analyzed. The results found 144 plant species on campuses, belonging to 65 families, and 131 genera. There were 31 arbor species, 37 bush species, 73 herb species, and 3 liana species. Besides that, there were 92 cultivated species and 51 naturally growing weed species. Among them *Cynodon dactylon* and *Zoysia matrella* were the main weeds. Another study was conducted on the campus of five high schools in Chu-Shan town. There were 183 species found on these campuses. These species belonged to 138 genera and 57 families, including 2 Pteridophytes, 17 Gymnosperms, 140 Dicotyledons, and 24 Monocotyledons (Yang et al., 2011). Similarly, the Central University of Himachal Pradesh campus collected 125 plant samples, both cryptogams and phanerogams in 2019. The results showed there were 56 flora families and the dominance was *Poaceae* and *Asteraceae* (Dey et al., 2021). The diversity of woody plants on three university campuses, including Inner Mongolia University

(IMU), Inner Mongolia Agriculture University (IMAU), and Inner Mongolia Normal University (IMNU) was determined by Qing (2012). There were 85 species found in 29 families. Among them, they recorded 10 evergreen trees, 43 broadleaved trees, and 32 shrubs that belonged to 2, 20, and 13 families, respectively. IMU contained 63 plant species, which belonged to 24 families while IMAU and IMNU recorded 57, and 43 species, which belonged to 22, and 15 families correspondingly. Furthermore, due to the vital role of the flora ecosystem on campus, the plant management practice should be noticed. Barker concluded that an effective system in place to manage plants on campus was important. Suitable plant management could prevent risks, contribute to sustainable safety, and improve education (Barker, 2024). In summary, the previous studies showed that the florae on campuses were diverse and needed to be managed well. It is necessary to collect information on flora diversity on campuses and provide management planning for these communities.

Nong Lam University was established in 1955, with a campus area of nearly 120 ha, which is considered one of the largest universities in Ho Chi Minh City (Ngo, 2019). There have not been many studies on florae at the campus of Nong Lam University. It was recorded that only one research by Tran (2020) collected information on trees along main roads and lecture halls at Nong Lam University Campus. The 33 woody species were found belonging to 16 families. Thus, the study of flora diversity on the Nong Lam University Campus was still limited. The entire campus should be surveyed to determine the flora diversity. In addition, groups of habits, such as trees, shrubs, herbs, climbers, lilies, and bamboo, should be analyzed to build a comprehensive plant catalog for Nong Lam University. These results

may serve as the cornerstone for management and provide research and learning environment for lecturers and students. Therefore, the study on flora diversity on the Nong Lam University campus was conducted.

2. Materials and Methods

The main campus of Nong Lam University is located on an area of 118 ha that belongs to Linh Trung Ward, Thu Duc City, and Di An City, Binh Duong Province ($10^{\circ}52'16.6''\text{N}$ $106^{\circ}47'29.8''\text{E}$), belongs to the Southeast region so it has all the terrain, climate, and soil characteristics of the region. This region has a tropical monsoon climate (Köppen, 1948), high temperatures all year round, with a deep differentiation between the rainy and

dry seasons, and a predominance of rainfall from May to November. The terrain is mainly semi-plains, midlands, and low mountains below 1000 m above sea level. The main soil in the research area is primarily ancient alluvial gray soil and basalt (Nguyen et al., 2024). The Nong Lam University campus contains 13 sub-zones (Figure 1). The survey was carried out in the flowerbed, roads, gardens, interior, and between the open areas of the buildings, and nurseries of the entire campus from June 2024 to September 2024 (Figures 1 and 3). The data collection method included observation, information records, photography, and sample collection. These methods were conducted based on the documents of Klein & Klein (1970) for unidentified species. Figure 2 illustrates some of the collected samples.

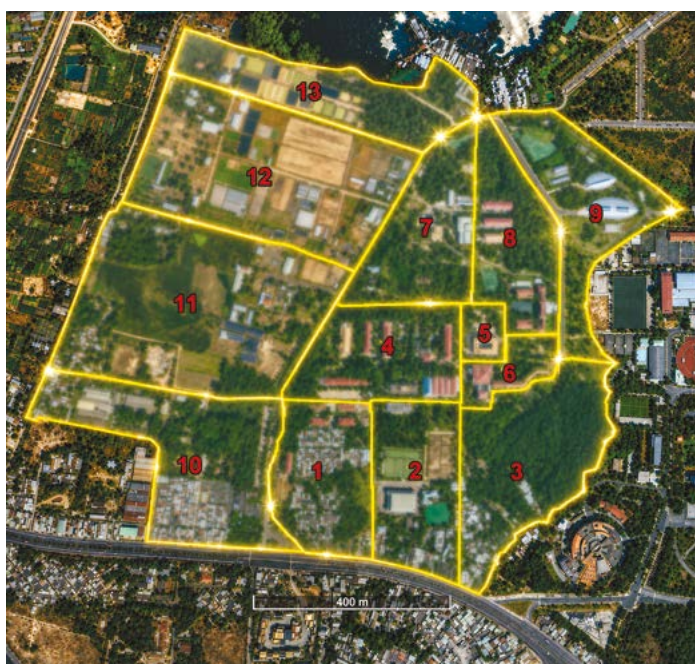


Figure 1. Map of the study areas. (1. Tam Giang area and Guest house, 2. Co May dormitory and stadium, 3. Area behind Rang Dong lecture hall, 4. Dormitory A, B, C, D, E, 5. Administrative building, 6. Rang Dong lecture hall, 7. Water pavilion area, Cam Tu building and Faculty of Mechanics, 8. Phuong Vy, Cat Tuong, Tuong Vy, Huong Duong building, 9. Faculty of Forestry, Faculty of Biology, 10. Kiosk and sawmill area, 11. Faculty of Environment & Natural Resources and Nguyen Thai Binh park, 12. Faculty of Agronomy nursery, 13. Aquatic experimental farm and Landscaping and environmental horticulture nursery).

The collected samples, photos, and descriptions of floras were compared morphologically with species in books, including “An Illustrated Flora of Vietnam” (Pham, 2000), “500 Useful Plants in Landscape Design” (Dinh, 2021), and “1001 Garden plants in Singapore” (Boo et al., 2006) to identify the species' botanical names. The updated scientific names of collected floras were updated according to the website (<http://www.worldfloraonline.org/>, 2024). The rarity of the species was verified in the

IUCN (International Union for Conservation of Nature) Red List categories (IUCN, 2024) and Vietnam Red Data Book - Part II Plants (MOST, 2024). The collected data were synthesized in Microsoft Excel 2015. In addition, an in-depth interview was conducted to understand the management situation of flora at the Nong Lam campus. The purposive sampling method was chosen. The participant was an officer who worked at the Facility Management office.

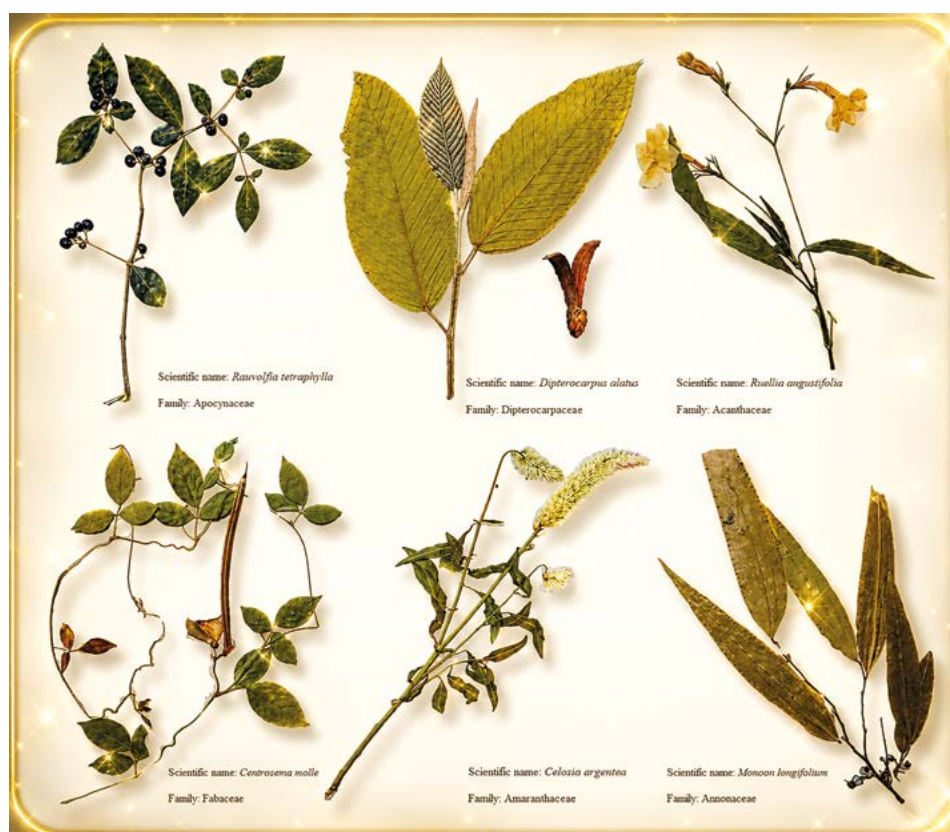


Figure 2. Specimens of some plant species at Nong Lam University.



Figure 3. Some survey areas in Nong Lam University campus. a) Zone 1 - Tam Giang area and Guest house b) Zone 4 - Dormitory c) Zone 6 - Rang Dong lecture hall d) Zone 7 - Water pavilion area, Cam Tu building and Faculty of Mechanics e) Zone 8 - Phuong Vy, Cat Tuong, Tuong Vy, Huong Duong building f) Zone 12 - Faculty of Agronomy nursery.

3. Results and Discussion

3.1. Inventory of floras at the Nong Lam University campus

Taxonomic diversity by phylum

The 243 flora species were recorded, belonging to 197 genera, 70 families, 30 orders, and 4 classes (Table 1).

Table 1. Taxon distribution by phylum and class

Phylum		Class		Order		Family		Genus		Species	
		Rate		Rate		Rate		Rate		Rate	
		Number	(%)	Number	(%)	Number	(%)	Number	(%)	Number	(%)
Polypodiophyta	Polypodiopsida	1	25.0	1	3.3	1	1.4	1	0.5	1	0.4
Pinophyta	Pinopsida	1	25.0	1	3.3	2	2.9	2	1.0	2	0.9
Magnoliophyta	Magnoliopsida	2	50.0	22	73.3	53	75.7	151	76.2	192	98.7
	Liliopsida			6	20.0	14	20.0	43	22.3	48	
Total		4	100	30	100	70	100	197	100	243	100

Most taxa belonged to the phylum Magnoliophyta with 240 species (98.7%), 194 genera (98.4%), 67 families (95.7%), 28 orders (93.3%), and 2 classes (50%). Two phyla accounted for a very small proportion: Pinophyta with 2 species (0.9%), 2 genera (1.0%), 2 families (2.9%), 1 order (3.3%) and 1 class (25%); Polypodiophyta with 1 species (0.4%), 1 genus (0.5%), 1 family (1.4%), 1 order (3.3%), and 1 class (25%). These results were reasonable since the Nong Lam University campus is in Binh Duong Province and Thu Duc City, in the Southeast region (DFI, 2023) with a sub-equatorial climate and high temperatures year-round (EMP, 2017) is suitable for most species of the Magnoliophyta phylum.

Taxonomic diversity by class

There were differences in the distribution of taxa among three phyla, including

Magnoliophyta (Magnoliopsida class and Liliopsida class), Pinophyta (Pinopsida class), and Polypodiophyta (Polypodiopsida class) (Table 1). The Magnoliopsida class has 192 species, 151 genera, 53 families, and 22 orders. The Liliopsida class has 48 species, 43 genera, 14 families, and 6 orders. The Pinopsida class has the number of taxa at species, genus, family, and order levels 2, 2, 2, and 1, respectively. The Polypodiopsida class has the lowest number.

Taxonomic diversity by family

Among 70 flora families surveyed, the *Fabaceae* family had the highest number of species (35 species counted for 14.4%). The *Fabaceae* is a big plant family in Vietnam with about 600 species (Nguyen, 2003), making it the third largest flowering flora family following *Asteraceae* and *Orchidaceae* (Judd

et al., 2002). The *Fabaceae* family is suitable for campus cultivation. Additionally, other families were considered noteworthy such as *Arecaceae* (15 species), *Asteraceae* (12 species),

and *Apocynaceae* and *Poaceae* (11 species for each). Each remaining family accounted for 1 - 8 species. The number of species for each flora family was shown in Figure 4.

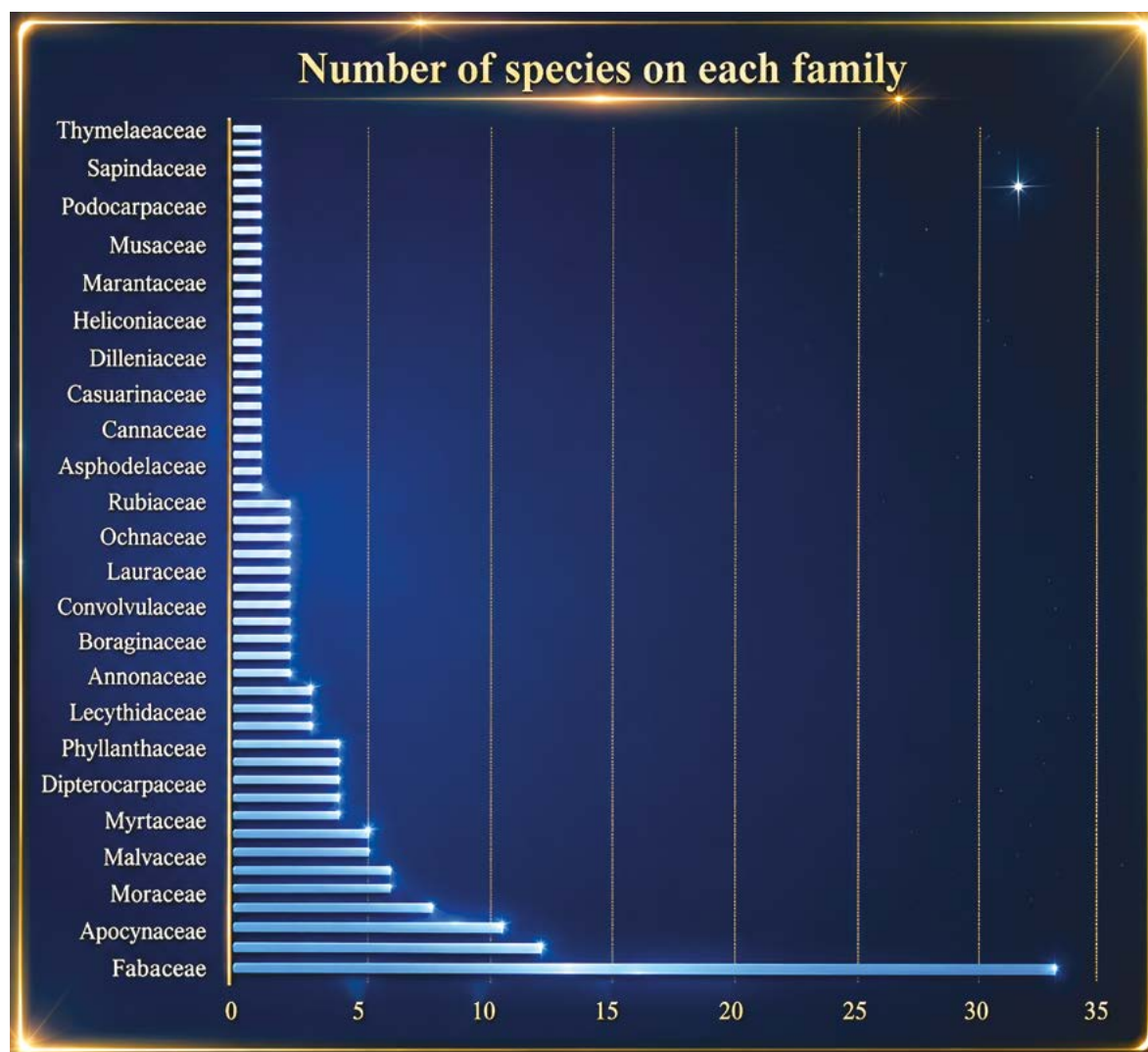


Figure 4. Number of species by families.

Taxonomic diversity of genus

A total of 197 genera were recorded at the Nong Lam University campus, of which 9 genera

(with 3 - 6 species per genus), accounted for 4.7%. The remaining genera were 1 - 2 species per genus (Figure 5).

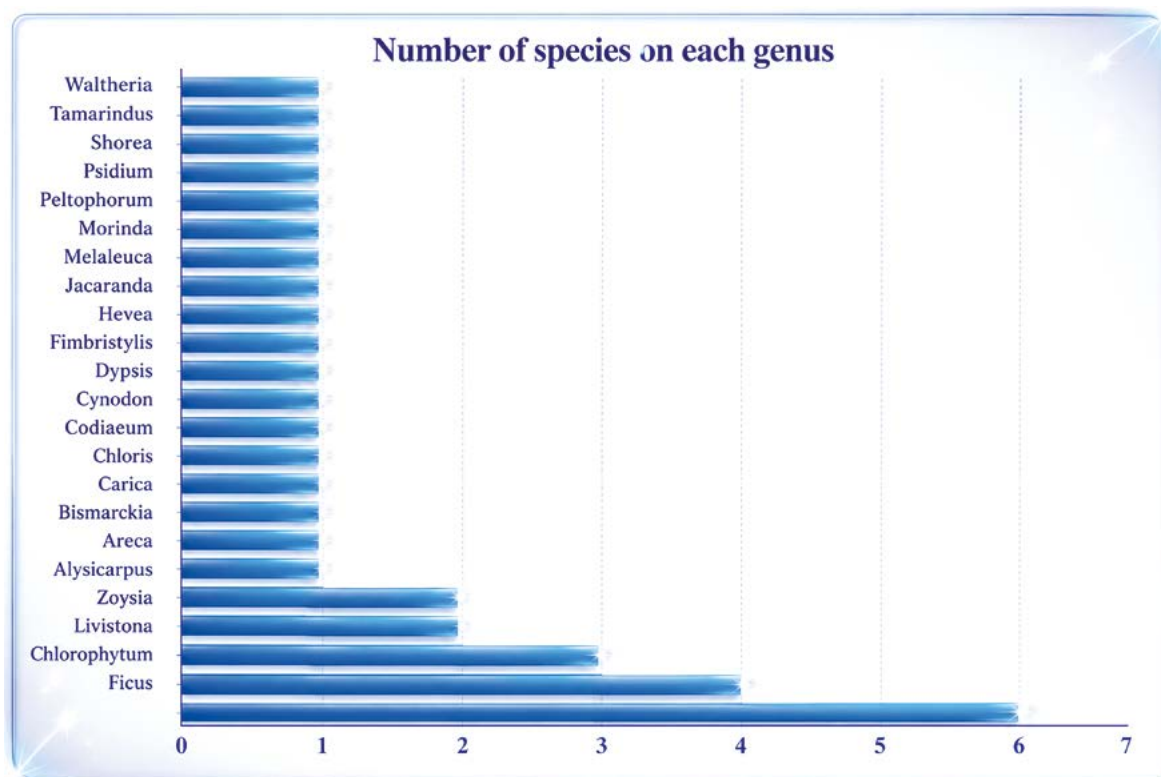


Figure 5. Number of species by genus.

Taxonomic diversity of species and their situation

The study recorded 243 plant species on the Nong Lam University campus. Among them, 12 species (5.1%) were listed in the IUCN Red List (IUCN, 2024), and 9 species (3.7%) were included in the Vietnam Red Data Book (MOST, 2024) (Table 2). *Aquilaria crassna*, *Diospyros mun*, and *Dalbergia oliveri* were classified as critically endangered (CR) in the IUCN Red List (IUCN, 2024); meanwhile, *Aquilaria crassna* and *Dalbergia oliveri* were endangered (EN), and *Diospyros mun* was vulnerable (VU) in the Vietnam Red Data Book (MOST, 2024). The numbers of individuals recorded were 14, 2, and 4, respectively. Most of these individuals were cultivated on the campus.

Pterocarpus indicus, *Pterocarpus macrocarpus*, *Anisoptera costata*, and *Swietenia macrophylla* were listed as EN species in the IUCN Red List (IUCN, 2024), with 17, 3, 9, and 1 individuals, respectively. All had been planted as ornamentals. Additionally, *Pterocarpus macrocarpus* and *Anisoptera costata* were also listed as EN species in the Vietnam Red Data Book (MOST, 2024).

Dipterocarpus alatus, *Hopea odorata*, *Madhuca pasquieri*, *Dalbergia tonkinensis*, and *Khaya senegalensis* were classified as vulnerable (VU) in the IUCN Red List (IUCN, 2024), with 533, 84, 1, 2, and 565 individuals, respectively. These species had also been cultivated for ornamental purposes. Moreover, *Madhuca pasquieri* and *Dalbergia tonkinensis* were categorized as EN species in the Vietnam Red Data Book (MOST, 2024).

Similarly, *Sindora siamensis* (2 individuals) and *Chukrasia tabularis* (7 individuals) were listed as EN and VU species, respectively, in the Vietnam Red Data Book (MOST, 2024). These results indicate that rare and valuable species comprised only a small proportion of the total recorded flora. Representative plant photographs are presented in Figure 6.

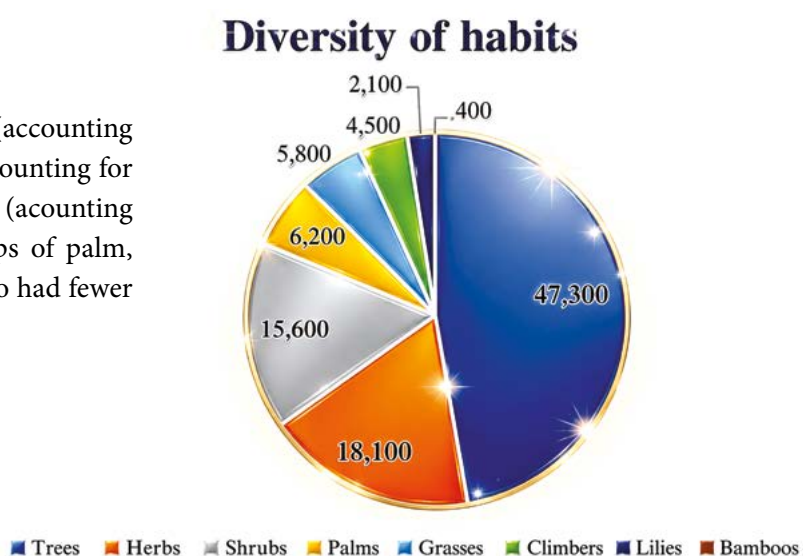
Table 2. List of species belonging to the IUCN Red List categories (2024) and the Vietnam Red Data Book (2024)

No.	Name	Family	Origin	Quantity	IUCN Red List (2024)	Vietnam Red Data Book (2024)
1	<i>Aquilaria crassna</i>	Thymelaeaceae	Cultivated	14	CR	EN
2	<i>Diospyros mun</i>	Ebenaceae	Cultivated	2	CR	VU
3	<i>Dalbergia oliveri</i>	Fabaceae	Cultivated	4	CR	EN
4	<i>Pterocarpus indicus</i>	Fabaceae	Cultivated	17	EN	
5	<i>Pterocarpus macrocarpus</i>	Fabaceae	Cultivated	3	EN	EN
6	<i>Anisoptera costata</i>	Dipterocarpaceae	Cultivated	9	EN	EN
7	<i>Swietenia macrophylla</i>	Meliaceae	Cultivated	1	EN	
8	<i>Dipterocarpus alatus</i>	Dipterocarpaceae	Cultivated	533	VU	
9	<i>Hopea odorata</i>	Dipterocarpaceae	Cultivated	84	VU	
10	<i>Madhuca pasquieri</i>	Sapotaceae	Cultivated	1	VU	EN
11	<i>Dalbergia tonkinensis</i>	Fabaceae	Cultivated	2	VU	EN
12	<i>Khaya senegalensis</i>	Meliaceae	Cultivated	565	VU	
13	<i>Sindora siamensis</i>	Fabaceae	Cultivated	2	LC	EN
14	<i>Chukrasia tabularis</i>	Meliaceae	Cultivated	7	LC	VU
Total				1,244		

*Memecylon caeruleum* Jack*Loropetalum chinense* (R. Br.)
Oliv.*Eurycoma longifolia* Jack*Lagerstroemia indica* L.*Tridax procumbens* L.*Waltheria indica* L.**Figure 6.** Photographs of selected plant species at Nong Lam University.

Diversity of grow habits

There were 115 tree species (accounting for 47.3%), 44 herb species (accounting for 18.8%), and 38 shrub species (accounting for 15.6%). In addition, groups of palm, grass, climber, lily, and bamboo had fewer than 15 species (Figure 7).

**Figure 7.** Plant growth habits.

Origin of the species

Most of the surveyed species in this study had been planted (180 species, 74.1%). They were mainly distributed in area 4, 5, 6, 7 and 8; which concentrated the large impervious surfaces such as offices and lecture halls. The other 63 nature species (25.9%) were mainly found in area 2, 3, 9, 12 and 13 which had the less impervious surfaces (Figure 8). Besides that, the dominant portion of

flora in Nong Lam campus was native, occupying 57.2% while 42.8% was imported species. The proportion of imported species was quite high, which might negatively affect the population of native species such as changing the composition and function of the microbiome, affecting the nutrient cycle, and reducing native biodiversity (Scharfy et al., 2010).

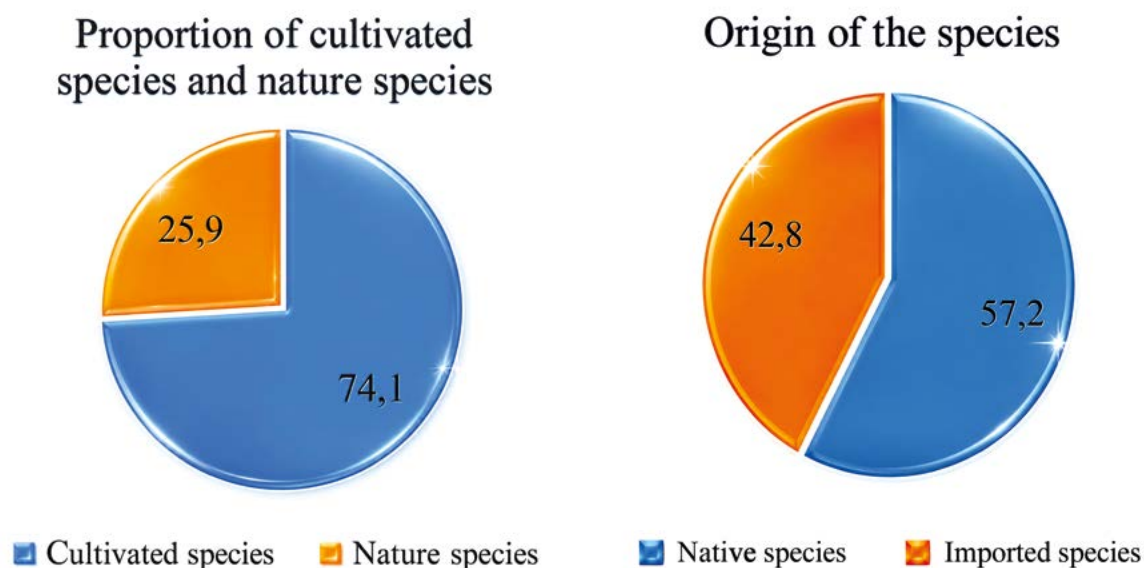


Figure 8. Origin of the species.

Management situation

Flora at the Nong Lam campus has not been assigned to any specialized functional office. The Facility Management office took charge of the flora system at the general level. Contractors conducted maintenance processes such as pruning, fertilizing, weeding, and watering. The big trees were pruned before the rainy season but there was no specific schedule for each year. The pruning, fertilizing, and weeding processes were applied for shrubs, flowers, and grass at some zones such as Nguyen Thai Binh park,

Phuong Vy area, Administration Building, and Huynh Anh Square. Only name tags showed scientific names and families of the big trees at the Administrative Building zone. In addition, no flora database at Nong Lam campus such as name, year of planting, age, etc. was managed by software.

3.2. Recommendations for flora management at the Nong Lam university campus

The results of the interview show that the flora in the Nong Lam campus need a sustainable

management system. To monitor and manage more effectively in the future, floras on the Nong Lam University campus should be collected and established a geodatabase. Hence, flora could be handled by space (planting location and growing space) and attribute characteristics (origin, growth, development characteristics, etc.). This could be accomplished by implementing a tree management system on specialized software such as Geographic Information Systems. Integrating the mentioned tree management software with the university's web page (webGIS) could provide information for students and staff about floras biodiversity on the campus, and foster the love of nature and environmental protection awareness. Particularly, it was crucial to have a geodatabase of 1,244 trees in the Vietnam Red Data Book (MOST, 2024) and IUCN Red List (IUCN, 2024) (Table 3). They were perennial trees and had historical and cultural values associated

with Nong Lam University. Hence, they should be marked as preserved groups. The preserved group should be photographed annually for monitoring. Trees should be pruned for vitality and safety. The management plan should control pests periodically every 6 months.

Vegetation on the Nong Lam University campus should be planned in separate planting areas with the plant labeled. The collection planting should focus on rare and native species to boost the richness of the local flora. The precious shading trees with beautiful canopies and flowers were suggested. In the dormitory and lecture hall areas, plants with large leaves that could prevent dust, and plants that repel insects should be chosen. The shrubs could be selected if they adapt to low-care conditions or can be pruned. Some species were suggested in Table 3.

Table 3. List of suggested tree species for future planting at Nong Lam University

No.	Scientific name	Family	Proposed planting area
1	<i>Gluta laccifera</i> (Pierre) Ding Hou	Anacardiaceae	Dormitory
2	<i>Pauldopia ghorta</i> (Buch.-Ham. ex G.Don) Steenis	Bignoniaceae	Lecture hall, Dormitory
3	<i>Terminalia calamansanai</i> (Blanco) Rolfe	Combretaceae	Dormitory
4	<i>Pentacme siamensis</i> (Miq.) Kurz	Dipterocarpaceae	Dormitory
5	<i>Parashorea stellata</i> Kurz	Dipterocarpaceae	Dormitory
6	<i>Shorea roxburghii</i> G. Don	Dipterocarpaceae	Dormitory
7	<i>Dellenia obovata</i> (Blume) Hoogland	Dilleniaceae	Lecture hall, Dormitory
8	<i>Diospyros mollis</i> Griff.	Ebenaceae	Dormitory
9	<i>Dalbergia cochinchinensis</i> Laness.	Fabaceae	Lecture hall
10	<i>Dalbergia tonkinensis</i> Prain	Fabaceae	Lecture hall
11	<i>Pterocarpus echinatus</i> Pers.	Fabaceae	Lecture hall
12	<i>Huchimingia ichthyochtona</i> (Drake) Z.Q.Song	Fabaceae	Lecture hall
13	<i>Millettia ichthyochtona</i> Drake	Fabaceae	Lecture hall

14	<i>Sindora siamensis</i> Teysm. ex Miq.	Fabaceae	Dormitory
15	<i>Saraca indica</i> L.	Fabaceae	Dormitory
16	<i>Saraca dives</i> Pierre	Fabaceae	Dormitory
17	<i>Schizolobium excelsum</i> Vogel	Fabaceae	Lecture hall
18	<i>Xylia xylocarpa</i> (Roxb.) W.Theob.	Fabaceae	Dormitory
19	<i>Peltophorum dasyrrhachis</i> (Miq.) Kurz	Fabaceae	Lecture hall
20	<i>Dalbergia cochinchinensis</i> Pierre	Fabaceae	Lecture hall
21	<i>Archidendron clypearia</i> (Jack) I. C.Nielsen	Fabaceae	Dormitory
22	<i>Brownea coccinea</i> Jacq.	Fabaceae	Lecture hall
23	<i>Dalbergia fusca</i> Pierre	Fabaceae	Lecture hall
24	<i>Fagraea fragrans</i> Roxb.	Gentianaceae	Dormitory
25	<i>Lagerstroemia loudonii</i> Teijsm. & Binn.	Lythraceae	Lecture hall
26	<i>Magnolia fordiana</i> (Oliv.) Hu	Magnoliaceae	Lecture hall
27	<i>Dysoxylum loureiri</i> Pierre	Meliaceae	Lecture hall, Dormitory
28	<i>Lagerstroemia floribunda</i> Jack	Lythraceae	Dormitory
29	<i>Lagerstroemia lecomtei</i> Gagnep.	Lythraceae	Lecture hall, Dormitory
30	<i>Lagerstroemia reginae</i> Roxb.	Lythraceae	Lecture hall
31	<i>Koelreuteria paniculata</i> Laxm.	Sapindaceae	Lecture hall
32	<i>Madhuca pasquieri</i> (Dubard) H. J. Lam	Sapotaceae	Lecture hall
33	<i>Vitex ajugiflora</i> Dop	Verbenaceae	Lecture hall

4. Conclusions

It can be concluded that flora diversity was notable on the Nong Lam University campus. The data collection showed that the phylum Polypodiophyta, Pinophyta, and Magnoliophyta were diverse in habits such as herbs, shrubs, trees, and climbers. The study recorded 243 species, belonging to 197 genera and 70 families. Among them, 12 rare species were recorded in the IUCN Red List categories (2024) and 9 species were listed in the Vietnam Red Data Book. Most of them are cultivated and imported plants. Hence, the future planting of flora at Nong Lam University should be planned with an emphasis on rare and native species. The results showed that plant management should be delegated to

a specialized unit. Spatial and attribute data of plants should be collected and managed using appropriate software. The maintenance plans should be based on the monitoring results. The oncoming research may develop professional software for flora management.

Conflict of interest

The authors declare that they have no conflict of interest.

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Assessing agricultural household vulnerability to climate change: A case study in Chau Thanh district, Ben Tre province, Vietnam

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ABSTRACT

Climate change significantly impacts the livelihoods of agricultural households, increasing their vulnerability. This study developed 49 indicators grouped into 12 main components to assess the livelihood vulnerability index (LVI) of agricultural households in Chau Thanh district, Ben Tre province. Using data from a survey of 300 farm households, complemented by secondary data on climate factors, a composite index was calculated to compare variations in vulnerability across the region. The overall LVI for Chau Thanh district was 0.32, indicating moderate vulnerability. Among the 12 main components, livelihood strategies, land, water, natural disasters and climate variability, and finance and income were identified as the most vulnerable dimensions, whereas housing was the least vulnerable. Using the LVI developed based on the intergovernmental panel on climate change (IPCC) framework, Giao Long commune was found to be more vulnerable than the other communes. To address these challenges, solutions based on the human, natural, and financial capitals of the livelihood efficiency index (LEI) were proposed. These solutions aim to enhance the adaptive capacity of agricultural households while mitigating the impacts of climate change, thereby fostering sustainable livelihoods in the district.

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1. Introduction

Vietnam is recognized as one of the countries in Southeast Asia most vulnerable to the impacts of climate change (Yusuf & Francisco,

2009), particularly in recent years (VMONRE, 2021). The two largest deltas in Vietnam, the Red River Delta and the Mekong River Delta, are experiencing significant disruption to the lives and livelihoods of their inhabitants, especially

farmers (VNASTI, 2006; Entzinger & Scholten, 2016). Although numerous climate change adaptation strategies have been implemented, the situation continues to evolve in complexity, particularly in the Mekong River Delta, the nation's largest agricultural region (VNASTI, 2006). Key climate-related challenges in this area include prolonged droughts and increasingly severe saltwater intrusion.

Ben Tre is a province situated in the lower Mekong River basin, bordering the East Sea, and is formed by four major rivers: the Tien, Ba Lai, Ham Luong, and Co Chien rivers. The province has 179,885 ha of agricultural land, representing 75.59% of the total area, with 19,101 ha (8.03%) designated for paddy fields and 33,692 ha (14.16%) for aquaculture (BTSO, 2023). In 2022, the province's gross regional domestic product (GRDP) at current prices was 63,585 billion VND, with the agriculture, forestry, and fishery sector contributing 35.37% (BTSO, 2023). Agriculture serves as a key driver of economic development in Ben Tre. Chau Thanh, the gateway district of Ben Tre province, boasts fertile alluvial soils deposited by the Mekong River branches, making it highly suitable for agricultural production, including fruit orchards and vegetable cultivation. However, agricultural activities in Chau Thanh are increasingly impacted by climate change, particularly saltwater intrusion (Thien et al., 2023). Climate change is a gradual yet persistent disaster that impacts agriculture through increased pest and disease outbreaks, more frequent and severe landslides, droughts, floods, reduced crop yields, crop failures, and higher livestock mortality rates (Harvey et al., 2014). Farmers' livelihoods are especially vulnerable, as they rely heavily on climate-sensitive sectors like agriculture, particularly in developing countries (Sujakhu et

al., 2018). Poor and agrarian communities in these regions are disproportionately affected by climate change due to their limited adaptive capacity and restricted access to alternative production methods (IPCC, 2007; Skoufias et al., 2012). Livelihood vulnerability assessments are crucial for developing strategies, evaluating policies, and guiding programs at various levels (Sujakhu et al., 2018). The concept of vulnerability remains ambiguous, with its definition varying across different disciplines (Gallopín, 2006). This study aimed to assess the climate change vulnerability of farming households in Chau Thanh district, Ben Tre province, Vietnam, using the livelihood vulnerability index (LVI) developed by Hahn et al. (2009), which can help mitigate adverse impacts.

2. Materials and Methods

2.1. Study site

Chau Thanh district in Ben Tre province consists of 20 communes and one town (Figure 1). The district has a population of 177,159, with 171,511 people (96.81%) residing in rural areas (BTSO, 2023). Chau Thanh covers an area of 22,489 ha, of which 16,060 ha (71.41%) are agricultural land (BTSO, 2023). The district is known for its specialization in fruit cultivation, with key crops including coconut, rambutan, durian, grapefruit, cocoa, and mangosteen (PCCT, 2023). However, agricultural practices are largely spontaneous and small-scale, with uneven product quality that lacks competitiveness in comparison to agricultural products from neighboring regions (Nguyen et al., 2022).

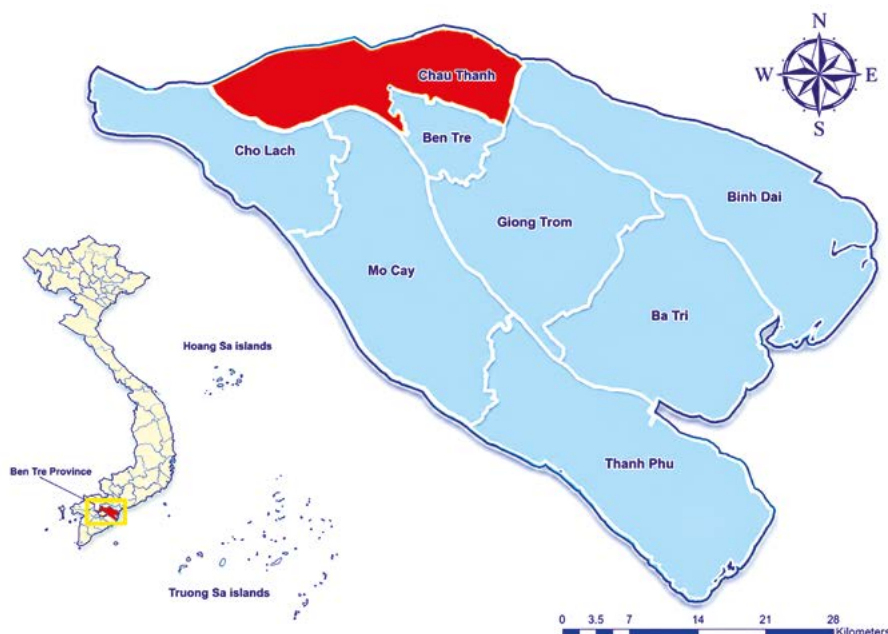


Figure 1. The map of the study site.

2.2. Data collection

This study employed a combination of methods, including secondary data analysis and structured interviews. Secondary data collection focused on gathering reports, documents, policies, and resolutions related to agricultural development, natural disasters and climate variability in Chau Thanh district. Simultaneously, primary data was obtained through structured interviews conducted during fieldwork.

Three communes, Phu Tuc, Tan Phu, and Giao Long, were purposefully selected within Chau Thanh district. For the household survey, 100 households were interviewed in each commune. The communes were selected based on the varying impacts of saline intrusion on agricultural cultivation (Thien et al., 2023). Additionally, in terms of geographical location,

Tan Phu, Phu Tuc, and Giao Long communes are situated along the Tien river from west to east, following the river's flow toward its mouth. Respondents were household heads or their spouses, predominantly farmers who had been engaged in agriculture and aquaculture for at least five years. The interviews were conducted in August 2024 using a structured questionnaire designed to gather information on topics such as health, knowledge and skills, food security, natural resources, disasters, social issues, finance, infrastructure, and livelihoods. Throughout the research, detailed field notes were meticulously recorded, capturing key information, conversations, interactions, and significant events (Stake, 2010). Structured interviews were chosen because they reduce the cognitive demands of information processing (Pettersen & Durivage, 2008). Additionally, the time allocated for each household interview was determined by the scope of the information being sought, making it

easier to control the interview duration. However, it is important to note that structured interviews resemble oral examinations, which may remind respondents they are being evaluated. As a result, respondents might attempt to present themselves in a more favorable light, potentially filtering the information they provide (Pettersen & Durivage, 2008).

2.3. Data analysis

This study employed three types of analysis: (1) developing the LVI main components and indicators, (2) calculating the LVI based on the IPCC framework (IPCC, 2007), and (3) calculating a livelihood effect index (LEI). Additionally, a t-test was used to compare the mean scores of the main components and indicators across the three communes.

2.3.1. Developing the LVI

The methodology applied for calculating vulnerability to climate variability was based on previous studies by Hahn et al. (2009), Shah et al. (2013), and a case study in Vietnam by Huong et al. (2018). However, modifications were made to tailor the method to this specific case study. The set of main components from Huong et al. (2018) was adopted, but three new indicators were introduced: one under knowledge and skills and two under natural disasters and climate variability (Table 1). The LVI framework in this study includes 12 components and 49 indicators of livelihood vulnerability. Each main component is composed of multiple indicators, and each indicator and main component contributes equally to the overall LVI score (Sullivan et al., 2002).

Table 1. The developed livelihood vulnerability index framework.

Capitals	Main components	Indicators	Units	Data sources	References
Human	Health	(1) Households with at least one member suffering from chronic diseases	%	Survey	Hahn et al. (2009)
		(2) Households with at least one member suffering from communicable diseases	%	Survey	
		(3) Households with at least one member missing work or school due to illness in the past month	%	Survey	
		(4) Average time to the nearest health care centre	minutes	Survey	

Knowledge and skills	(5) Unlettered household head	%	Survey	Hahn et al. (2009)
	(6) Households that have not yet received any training to cope with climate change	%	Survey	Mendoza et al. (2014)
	(7) Household members have not received any vocational or higher training	%	Survey	
	(8) Households with less than 10 years of farming experience	%	Survey	Can et al. (2013)
	(9) Households that do not know how to apply measures to mitigate or avoid the consequences of saltwater intrusion	%	Survey	New
Food	(10) Households that depend on their own family farm for food	%	Survey	Hahn et al. (2009)
	(11) Average Crop Diversity Index	index	Survey	
	(12) Households that do not sell their agricultural products	%	Survey	Shah et al. (2013)
Livelihood	(13) Household members working outside the community	%	Survey	Hahn et al. (2009)
	(14) Average Agricultural Livelihood Diversification Index	index	Survey	
	(15) Households that depend solely on agriculture as a source of income	%	Survey	
	(16) Households' nonfarm activities affected by climate change	%	Survey	Huong et al. (2018)
	(17) Households with no jobs during extreme weather events	%	Survey	
	(18) Households that depend on (exploiting) natural resources	%	Survey	Shah et al. (2013)
	(19) Landless households	%	Survey	Shah et al. (2013)

	(20) Households with small farmland (less than 1 ha)	%	Survey	Can et al. (2013)
	(21) Households with land degradation due to climate-related extremes over the past 10 years	%	Survey	Lamichhane (2010)
Water	(22) Households that have heard about any water-related conflicts in the community	%	Survey	Hahn et al. (2009)
	(23) Households that collect water directly from rivers, streams, or canals	%	Survey	
	(24) Households that do not have a reliable water supply	%	Survey	
Natural disasters and climate variability	(25) Number of floods, erosions, and droughts in the past 5 years	events	Secondary data	
	(26) Number of months affected by saltwater intrusion in the past 5 years	months	Secondary data	New
	(27) Households affected by saltwater intrusion in the past 5 years	%	Survey	New
	(28) Households with an injury or death resulting from the most severe natural disaster in the past 5 years	%	Secondary data	Hahn et al. (2009)
	(29) Households that did not receive any warning about impending natural disasters	%	Survey	
	(30) Households that suffered property damage due to natural disasters or extreme climate events in the past 5 years	%	Secondary data	Shah et al. (2013)
	(31) Mean standard deviation of the daily average maximum temperature by month over the past 5 years	°C	Secondary data	Hahn et al. (2009)

		(32) Mean standard deviation of the daily average minimum temperature by month over the past 5 years	°C	Secondary data	
		(33) Mean standard deviation of average precipitation by month over the past 5 years	mm	Secondary data	
Social	Socio-demographic profile	(34) Dependency ratio	ratio	Survey	
		(35) Female-headed households	%	Survey	
		(36) Number of household members	persons	Survey	Huong et al. (2018)
		(37) Poor households	%	Secondary data	
	Social networks	(38) Receive:Give ratio	ratio	Survey	Hahn et al. (2009)
		(39) Borrow:Lend money ratio	ratio	Survey	
		(40) Households that have not received any assistance from local government	%	Survey	
		(41) Households that do not participate in any organizations	%	Survey	Huong et al. (2018)
		(42) Households without TVs	%	Survey	Panthi et al. (2016)
		(43) Households without internet	%	Survey	
Financial	Finance and income	(44) Households with debt	%	Survey	Huong et al. (2018)
		(45) Households with a net income lower than VND 10M	%	Survey	Can et al. (2013)
		(46) Households that cannot access any financial services	%	Survey	Huong et al. (2018)
Physical	Infrastructure	(47) Average time to reach the nearest bus station	min	Survey	
		(48) Average time to reach the nearest post office	min	Survey	
	Housing	(49) Households with non-solid houses	%	Observation	Shah et al. (2013)

2.3.2. LVI calculation

Since the indicators are measured on different scales, they were first standardized into indices using the following equation:

$$\text{Index}_{s_i} = \frac{s_i - s_{\min}}{s_{\max} - s_{\min}}$$

Where s_i is the original value of the indicator, and $s_{\min}$ and $s_{\max}$ are the minimum and maximum values, respectively, determined from the data across the three communes. After standardization, the indicators were averaged using the formula:

$$M_i = \frac{\sum_{i=1}^n \text{index}_{s_i}}{n}$$

Where M_i represents one of the 12 main components, and n is the number of indicators within that component. The overall LVI score was calculated as a weighted average of the main components:

$$\text{LVI} = \frac{\sum_{i=1}^{12} w_{M_i} M_i}{\sum_{i=1}^{12} w_{M_i}}$$

Here, the weights w_{M_i} are determined by the number of indicators in each main component to ensure equal contribution to the final LVI score (Sullivan et al., 2002). The LVI is scaled from 0 (least vulnerable) to 0.5 (most vulnerable) (Hahn et al., 2009).

2.3.3. LVI-IPCC calculation

An alternative approach to calculating the LVI was developed using the IPCC framework (Hahn et al., 2009; Shah et al., 2013; Huong et al., 2018). The 12 main components were reorganized according to the IPCC framework, which categorizes vulnerability into exposure,

sensitivity, and adaptive capacity. Exposure is measured by the number of natural disasters and the average variability in maximum and minimum monthly temperatures and precipitation over the past five years. Adaptive capacity includes demographic factors, livelihood strategies, knowledge and skills, infrastructure, housing, and social networks, while sensitivity is determined by food and water security, land use conditions, financial status, and health. The same indicators and equations from 2.3.2 were used, but the main components were combined into contributing factors:

$$CF = \frac{\sum_{i=1}^n w_{M_i} M_i}{\sum_{i=1}^n w_{M_i}}$$

Where CF is one of the IPCC-defined contributing factors (exposure, sensitivity, or adaptive capacity). Finally, the LVI-IPCC was calculated as:

$$\text{LVI-IPCC} = (e-a)*s$$

Where e is exposure, a is adaptive capacity, and s is sensitivity. The LVI-IPCC is scaled from -1 (least vulnerable) to 1 (most vulnerable) (Hahn et al., 2009).

2.3.4. LEI calculation

The LEI was calculated based on five capital groups: human, natural, social, physical, and financial capital. The 12 main components were grouped into these livelihood assets, and the main components for each asset were averaged using the following equation:

$$\text{LEI} = \frac{\sum_{i=1}^5 w_{M_i} M_i}{\sum_{i=1}^5 w_{M_i}}$$

Where LEI represents the vulnerability index for one of the five livelihood assets. The LEI

is scaled from 0 (least vulnerable) to 1 (most vulnerable) (Huong et al., 2018).

3. Results and Discussion

3.1. Livelihoods of farming households

Rice cultivation in Chau Thanh district, Ben Tre province has ceased since 2019, as farmers

have transitioned from rice paddies to more valuable fruit crops like pomelo and coconut, or repurposed the land for residential or production use. By 2022, the district's fruit farming area totaled 7,554 ha (Table 2).

Table 2. Agricultural statistics for Chau Thanh district, Ben Tre province in 2022

	2018	2019	2020	2021	2022
Planted area of rice (ha)	22	0	0	0	0
Planted area of maize (ha)	51	24	4	6	2
Planted area of cassava (ha)	5	0	0	0	0
Planted area of vegetables and beans (ha)	218	93	16	26	20
Planted area of coconut (ha)	7,989	7,987	7,990	8,038	7,989
Planted area of cocoa (ha)	210	107	79	74	74
Planted area of orange and mandarin (ha)	185	184	185	186	163
Planted area of lemon (ha)	52	58	67	76	84
Planted area of pomelo (ha)	3,331	3,440	3,594	3,742	3,683
Planted area of banana (ha)	490	477	479	452	434
Planted area of mango (ha)	11	11	14	18	16
Planted area of longan (ha)	254	211	190	150	137
Planted area of rambutan (ha)	2,074	1,857	1,789	1,397	1,307
Planted area of durian (ha)	951	1,105	1,093	1,212	1,314
Planted area of mangosteen (ha)	127	120	109	106	98
Number of cows (head)	4,114	3,915	3,927	3,836	3,070
Number of pigs (head)	26,796	12,085	10,764	12,147	8,831
Number of poultry (head)	698	673	716	722	748
Number of goat and sheeps	22,696	20,685	21,547	19,454	19,848
Area of aquaculture (ha)	611	465	457	390	280

Source: BTSO (2023).

Although the fruit-growing area has shown a declining trend from 2018 to 2022, Chau Thanh remains the district with the largest fruit acreage in the province. The coconut plantation area spans 7,989 ha, with an estimated annual yield of 70,196 tons, of which approximately 70% is used for oil production and 30% for fresh consumption. Through various project programs, seminars, and technical training provided by local authorities, farmers' practices have gradually improved, leading to better productivity, quality, and efficiency in coconut farming. While the district boasts the largest area for key fruit crops like grapefruit and bananas in the province, these crops are dispersed across the district and have not yet been developed into specialized, concentrated farming zones based on land and water conditions. To maximize land-use efficiency, increase economic returns, and improve the livelihoods of farmers, it is crucial to devise development strategies and appropriately restructure agricultural production based on the district's land and water potential. In addition to fruit farming, the district has emphasized livestock development, with 3,070 cows, 8,831 pigs, and 748 poultry, mostly raised on small household farms (Table 2). In recent years, there has been a gradual shift toward larger farm-based operations. Aquaculture also plays a significant role in the district, covering 280 ha in 2022. This includes intensive farming, mainly *Siluriformes* (catfish) for export, as well as extensive and cage farming, with total aquaculture production reaching 39,252 tons.

3.2. LVI

The LVI values for the 12 main components, calculated using 49 indicators for the communes of Phu Tuc, Tan Phu, and Giao Long, are presented in a spider diagram (Figure 2) and

Table 3. The diagram employs a scale with 0.1-unit increments, ranging from 0 (least vulnerable) at the center to 0.5 (most vulnerable) at the outer edge. Overall, Phu Tuc and Giao Long recorded higher LVI scores (0.33) compared to Tan Phu (0.31). Among the 12 main components, all communes displayed the least vulnerability in housing (0.01 - 0.03), as most agricultural households possess solid structures that are less susceptible to climate change. However, agricultural households in Chau Thanh district were found to be more vulnerable in the areas of livelihoods, land, water, natural disasters and climate variabilities, and finance and income. When compared to northwest Vietnam (Huong et al., 2018), agricultural households in Chau Thanh district were less vulnerable. LVI in northwest Vietnam showed higher vulnerability in housing and social networks, explaining the higher overall vulnerability compared to Chau Thanh district. The LVI of Chau Thanh district also closely aligns with Thanh Phu district (Ben Tre province) in 2018 - 2019, which recorded a score of 0.355 (Can et al., 2020).

While the overall differences in vulnerability among the three communes were relatively minor, more detailed analysis reveals important variations. In the knowledge and skills sector, the LVI for Chau Thanh District ranged from 0.18 to 0.27, lower than northwest Vietnam (0.41 - 0.62) due to a lower percentage of unlettered household heads and farmers' extensive experience in agriculture. However, the lack of effective training on climate change adaptation, particularly regarding saltwater intrusion, remains a significant issue (Thien et al., 2023). Specifically, in Giao Thuy Commune, 31% of farming households lacked knowledge on implementing measures to mitigate or avoid the consequences of saltwater intrusion, which

coincided with the highest percentage (64%) of households that had not received any training on climate change adaptation. In contrast, the proportion of farming households lacking this knowledge was significantly lower in Tan Phu (6%) and Phu Tuc (2%), contributing to the reduced vulnerability of these communes in the knowledge and skills component. In the food dimension, low crop diversity due to climatic conditions and saltwater intrusion has pushed farmers to cultivate high-value crops like pomelo and durian, leading to decreased production of traditional crops such as rice, cassava, maize, vegetables, and cocoa (Table 2). Phu Tuc had the lowest average crop diversity index, which significantly increased its vulnerability in the

food sector, resulting in the highest LVI score for food (0.33). Additionally, 24% of households in Phu Tuc relied solely on their own family farms for food, and 31% did not sell their agricultural products, further contributing to its high vulnerability in this sector. Livelihood vulnerability was notably higher in Phu Tuc (0.51), where more households had members working outside the community than in Tan Phu (0.34) or Giao Long (0.37). This aligns with the rural-to-urban migration trend seen in northwest Vietnam (Huong et al., 2018) and central Vietnam (Nguyen et al., 2015). Over the past decade, agricultural households have shifted toward specializing in singular livelihoods, often with income solely dependent on agriculture.

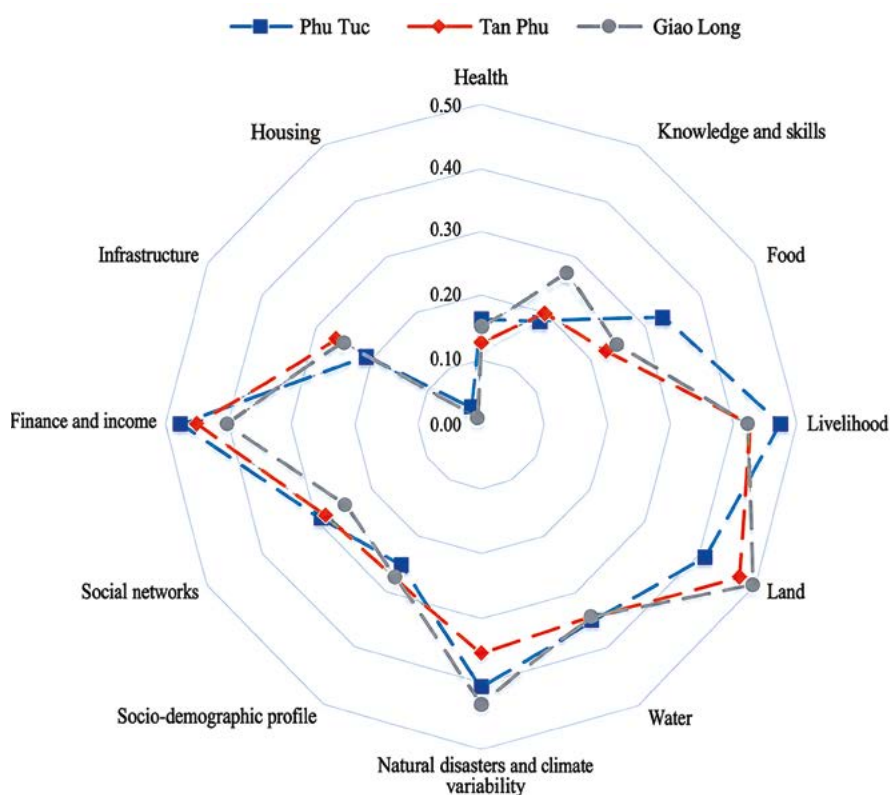


Figure 2. Vulnerability spider diagram depicting the 12 main components of the livelihood vulnerability index for three communes in Chau Thanh district, Ben Tre province.

Table 3. Results of the capitals, main components, and indicators for three communes in Chau Thanh district, Ben Tre province

Capitals	PT	TP	GL	Main components	PT	TP	GL	Indicators	PT	TP	GL
Human	0.30	0.26	0.29	Health	0.16	0.13	0.15	(1)	0.12	0.08	0.18
								(2)	0.06	0.03	0.18
								(3)	0.30	0.21	0.09
								(4)	0.17	0.18	0.15
				Knowledge and skills	0.18	0.20	0.27	(5)	0.09	0.06	0.03
								(6)	0.61	0.59	0.64
								(7)	0.09	0.15	0.12
								(8)	0.11	0.14	0.26
								(9)	0.02	0.06	0.31
				Food	0.33	0.23	0.25	(10)	0.24	0.21	0.12
								(11)	0.45	0.38	0.38
								(12)	0.31	0.09	0.24
				Livelihood	0.47	0.43	0.43	(13)	0.51	0.34	0.37
								(14)	0.48	0.48	0.50
								(15)	0.59	0.77	0.68
								(16)	0.89	0.80	0.90
								(17)	0.24	0.15	0.06
								(18)	0.13	0.03	0.04
Natural	0.40	0.38	0.43	Land	0.41	0.47	0.50	(19)	0.05	0.02	0.01
								(20)	0.40	0.53	0.58
								(21)	0.78	0.87	0.90
				Water	0.35	0.34	0.34	(22)	0.03	0.03	0.01
								(23)	0.97	0.97	0.94
								(24)	0.05	0.03	0.08
				Natural disasters and climate variability	0.41	0.35	0.43	(25)	0.44	0.44	0.56
								(26)	0.37	0.37	0.38
								(27)	0.98	0.91	1.00
								(28)	0.01	0.00	0.02
								(29)	0.05	0.02	0.01
								(30)	0.77	0.41	0.88
								(31)	0.39	0.39	0.39
								(32)	0.33	0.33	0.33
								(33)	0.32	0.32	0.32
Social	0.28	0.28	0.26	Socio-demographic profile	0.25	0.28	0.27	(34)	0.33	0.53	0.49
								(35)	0.29	0.18	0.21
								(36)	0.34	0.34	0.36
								(37)	0.05	0.05	0.03

				Social networks	0.29	0.28	0.25	(38)	0.34	0.22	0.19
								(39)	0.26	0.15	0.15
								(40)	0.21	0.27	0.25
								(41)	0.62	0.88	0.76
								(42)	0.08	0.03	0.00
								(43)	0.24	0.15	0.14
Financial	0.48	0.45	0.40	Finance and income	0.48	0.45	0.40	(44)	0.11	0.09	0.12
								(45)	0.94	0.91	0.94
								(46)	0.38	0.35	0.15
Physical	0.15	0.18	0.17	Infrastructure	0.21	0.26	0.25	(47)	0.27	0.36	0.34
								(48)	0.15	0.17	0.17
				Housing	0.03	0.01	0.01	(49)	0.03	0.01	0.01

PT: Phu Tuc; TP: Tan Phu; GL: Giao Long. The order of the indicators corresponds to Table 1.

Land emerged as the most vulnerable component, with scores of 0.50 in Giao Long, 0.47 in Tan Phu, and 0.41 in Phu Tuc. Between 78 and 90% of farmers experienced land degradation due to climate-related extremes, particularly saltwater intrusion (Thien et al., 2023). In addition, most farmers owned agricultural land, but its small size and degradation from saltwater intrusion and erosion, particularly in Giao Long (90%), exacerbated vulnerability. Water vulnerability was moderate (0.34 - 0.35), as many farmers directly relied on river water for irrigation, making them highly susceptible to drought and saltwater intrusion. Natural disasters and climate variability vulnerability scores were consistent with Thanh Phu District (Can et al., 2020), primarily driven by extreme weather events such as erosion, drought, and saltwater intrusion. Among the communes, Giao Long (0.43) was the most vulnerable to these

impacts, followed by Phu Tuc (0.41) and Tan Phu (0.35) which aligns with Thien et al. (2023). The financial and income sector also showed high vulnerability (0.40 - 0.48) due to low agricultural household incomes.

The LVI-IPCC contributing factors and overall vulnerability scores for the three communes are summarized in Table 4. The LVI-IPCC values for Phu Tuc, Tan Phu, and Giao Long were 0.039, 0.021, and 0.044, respectively. Based on LVI-IPCC, Giao Long was the most vulnerable to climate change, with the highest exposure (0.432) and low adaptive capacity (0.292). Conversely, Tan Phu was the least vulnerable (0.021), aligning with findings by Thien et al. (2023), as its geographic location in the western part of the district and well-developed irrigation infrastructure mitigate the impacts of saltwater intrusion.

Table 4. Calculation of livelihood vulnerability index-intergovernmental panel on climate change (LVI-IPCC) contributing factors for the three communes in Chau Thanh district, Ben Tre province

IPCC contributing factors to vulnerability	Phu Tuc	Tan Phu	Giao Long
Exposure	0.407	0.354	0.432
Adaptive capacity	0.290	0.288	0.292
Sensitivity	0.335	0.311	0.317
LVI-IPCC	0.039	0.021	0.044

3.3. LEI and solutions

The LEI results for the five capitals - human, natural, social, financial, and physical are presented in Figure 3. According to the results, agricultural households in Chau Thanh district were most vulnerable in the human (0.29), natural (0.40), and financial (0.44) capitals. To address these vulnerabilities and help agricultural households adapt to climate change while mitigating its impacts, the following solutions are proposed in order of priority:

3.3.1. Financial capital

Low-interest loans and credit access: Establish low-interest loan programs and credit facilities to enable farmers to invest in adaptive measures, such as salt-tolerant crop varieties, efficient irrigation systems, and post-harvest storage technologies. This is particularly crucial in Phu Tuc (38%) and Tan Phu (35%), where a significant proportion of farmers lack access to financial services.

Crop insurance: Develop and promote affordable crop insurance schemes to protect farmers from financial losses caused by extreme climate events, including droughts, floods, and saltwater intrusion.

Savings programs: Organize community-based savings groups or cooperatives to help farmers build financial reserves for emergencies and enhance resilience.

3.3.2. Natural capital

Soil improvement measures: Implement strategies to improve soil quality, particularly in Giao Long (90%) and Tan Phu (87%) communes degraded by saline intrusion, through organic amendments, soil testing, and the introduction of salt-tolerant crops.

Crop diversification: Promote the planting of diverse crops to enhance soil health, reduce pest outbreaks, and increase resilience to climate variability, especially in Phu Tuc commune (because of the lowest average crop diversity index).

Freshwater storage systems: Encourage the construction of freshwater storage systems, such as ditches and ponds, to support irrigation and agricultural activities during dry seasons.

Rainwater harvesting: Develop systems for rainwater collection and storage to provide water for irrigation and household use during drought periods.

Efficient irrigation systems: Introduce modern water-saving irrigation techniques, such as drip irrigation and sprinkler systems, to minimize water wastage.

Desalination technology: Establish small-scale desalination units in areas affected by saltwater intrusion to supply fresh water for agricultural use.

3.3.3. Human capital

Training programs: Conduct training sessions on sustainable natural resource management and climate adaptation strategies, tailored to local conditions, particularly in Giao Long commune, where 31% of households lacked knowledge on mitigating the impacts of saltwater intrusion.

Workshops on climate risk management: Provide practical workshops to help farmers manage climate risks, diversify their agricultural practices, and adopt climate-resilient crops and technologies.

Financial literacy education: Train farmers in financial management, focusing on creating budgets, managing debts, and planning long-term investments for climate adaptation.

By addressing these key vulnerabilities in the financial, human, and natural capitals, agricultural households in Chau Thanh can build resilience to climate change impacts, improve their livelihoods, and ensure sustainable agricultural practices.

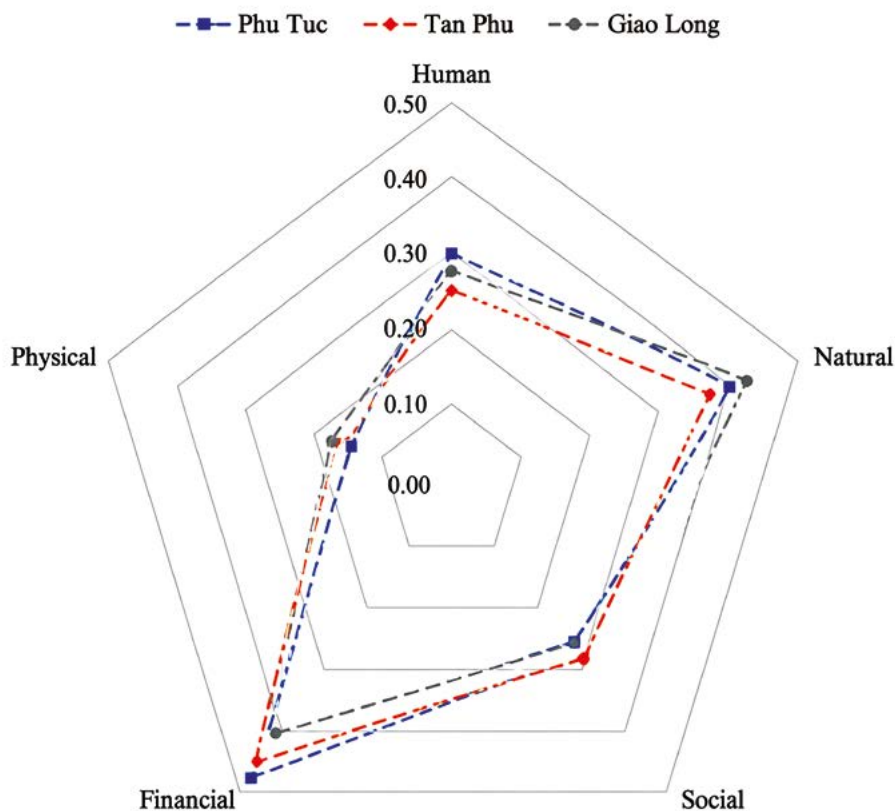


Figure 3. Vulnerability spider diagram depicting the 5 capitals of the livelihood efficiency index for three communes in Chau Thanh district, Ben Tre province.

4. Conclusions

This study assessed the vulnerability of agricultural households in Chau Thanh district, Ben Tre province, to climate change using the livelihood vulnerability index framework. With data collected from 300 households and complemented by secondary climate data, the overall livelihood vulnerability index of Chau Thanh district was calculated to be 0.32, indicating moderate vulnerability. Among the 12 main components, livelihoods, land, water, natural disasters, and financial resources were identified as the most vulnerable sectors, whereas housing exhibited the least vulnerability.

The livelihood vulnerability index and intergovernmental panel on climate change results highlighted that Giao Long commune had the highest vulnerability due to greater exposure and lower adaptive capacity compared to Tan Phu and Phu Tuc communes. Key drivers of vulnerability included land degradation caused by saltwater intrusion, limited crop diversity, reliance on river water for irrigation, and insufficient adaptive capacity to cope with extreme climate events.

Solutions to mitigate these vulnerabilities were proposed based on the livelihood effect index, focusing on strengthening financial, human, and natural capitals. These included improving access to financial services, providing low-interest loans, and introducing crop insurance; organizing training programs on climate risk management and sustainable farming practices; and adopting sustainable land and water management strategies, such as rainwater harvesting and soil improvement techniques.

Addressing these key vulnerabilities will not only enhance the resilience of farming households in Chau Thanh district but also provide a framework for similar communities in Vietnam and beyond to adapt to climate change impacts. Future studies should explore the long-term effectiveness of proposed solutions and their scalability to other regions.

Conflict on interest

The authors declare no conflict of interest.

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Application of unmanned aerial vehicles (UAV) to establish land use map of the Ring Road 2 project in Phuoc Long B ward, Thu Duc city (now Phuoc Long ward), Ho Chi Minh City

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ARTICLE INFO	ABSTRACT
Research Paper Received: May 06, 2025 Revised: September 03, 2025 Accepted: September 10, 2025 Keywords Land use map Site clearance compensation UAV *Corresponding author Le Ngoc Lam Email: lengoclam@hcmuaf.edu.vn	Establishing land use map is a mandatory requirement for all projects involving land use. Mapping the current land use status will use different methods, which depend on factors such as purpose, requirements and scale of the project ... The Ho Chi Minh City Ring Road 2 project passes through many areas from urban to agricultural areas. The scope of the project has a total length of 6 km and a width of 120 m. The total area is more than 61 ha, passing through 6 wards of Thu Duc city, Ho Chi Minh City. The research objective was to establish a high-precision land use status map to serve the work of compensation and site clearance. The study used unmanned aerial vehicle (UAV), which had captured 637 images in the project area with a spatial resolution of 2 cm. The images were combined and adjusted to the VN2000 coordinate system. From the orthomosaic images, the study digitized and directly interpreted the images to extract information about land use layers in the study area. From that result, the study established a map of the current land use status in 2024. The statistical results showed that the total area to be recovered for the project is 17.66 ha, mainly urban residential land.

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1. Introduction

Traditional data collection applying a total station is limited by time and safety and thus often leads to a lack of necessary information for

monitoring and mapping (Nguyen et al., 2020). Unmanned aerial vehicle (UAV) is an important and innovative tool in the field of surveying, measuring and mapping. Currently, traditional measurement methods are often combined with

UAV imagery methods. From there, it will bring high efficiency and accuracy. The result is the creation of digital elevation models or Image maps quickly and accurately. That helps designers choose optimal route options, suitable for site clearance and project proposal preparation (Cao et al., 2016). In the field of cadastral mapping, previous experimental research results show that many land use maps have been created from UAV image data. These maps can meet the accuracy requirements in cadastral mapping in Vietnam according to current regulations. At the same time, land use status maps from UAV images also ensure the criteria of quality, accuracy, and level of detail in land classification for commune level, which are required by Circular 28/2014/TT-BTNMT of the Ministry of Natural Resources and Environment (Phan et al., 2018). The existing map georeferencing method requires longer time, cost and manpower to conduct a survey and produce a map. The output might be inaccurate due to the lower resolution of satellite and cadastral data (Stalin & Gnanaprakasam, 2020). Another study by Phan et al. (2023) on the application of UAVs in map creation for site clearance showed that the quality of the product maps was assessed as satisfactory. Including requirements on form, completeness of content, consistency of land type symbols, accuracy of land plots on the map, and deviation of land area between the map and data in cadastral records. In particular, the results of this topic have contributed to monitoring assets on land, which serves the work of site clearance in the research area (Phan et al., 2023). In addition, with the advantages of high resolution (cm), very small image center error, the use of UAV technology is considered very suitable for establishing cadastral maps in areas with low difficulty levels, especially for agricultural land with annual crops. For residential areas and

areas with higher difficulty levels, it is necessary to combine with other methods such as Global Navigation Satellite System (GNSS) or electronic total stations to bring the best results (Nguyen et al., 2022). High-resolution UAV images show detailed ground objects and should be used to create land use maps and land cover maps. The results show that the images obtained from UAV have a higher level of accuracy with an accuracy of 93,80% compared to 86,67 (IKONOS) and 83,89% (Quickbird). This proves that using UAV to study land use/land cover changes is completely reasonable and highly reliable (Jumaat, 2018). UAV remote sensing is no longer just a cheap substitute for satellite remote sensing in industrial and agricultural applications. Instead, it is now being used to solve problems that were previously difficult to address using satellite remote sensing, thanks to their flight platform and sensor advantages. As a result, non-traditional research fields such as forestry, artificial buildings, precision agriculture, and the natural environment have received increased attention in recent years (Zhang & Zhu, 2023).

The Ring Road 2 project in Thu Duc city, Ho Chi Minh City with a total length of 6.04 km. The project is 120 m wide with an area of 61.15 ha, which passes through 6 wards with three main intersections. The research area has places that are difficult to access from the ground surface. The project route passes through many prime areas, so the inventory of current status is very important. At the same time, the map results require high accuracy and are implemented in a short time to serve as a basis for compensation and site clearance for project implementation. In this study, unmanned aerial vehicles (UAVs) were used to create a map of current land use status of the Ring Road 2 project in Phuoc Long B ward, Thu Duc city (now Phuoc Long ward). The study

aimed to effectively support compensation and site clearance for Ho Chi Minh City.

2. Equipment and Methods

2.1. Equipment

This study used the DJI Mavic 3 drone (Figure 1). The DJI Mavic 3 is one of DJI's most advanced drone lines, with some features: Flight

range of 15 km; camera model M3E (12.29 mm); resolution 5280 x 3956; focal length 12.29 mm; pixel size 3.36 x 3.36 μm .

Thanks to the integration of many modern technologies, the Mavic 3 has improved accuracy to the centimeter (cm) level. At the same time, with the ability to capture images in real time, it ensures accuracy and is suitable for research areas with high population density.



Figure 1. DJI Mavic 3 enterprise drone.

2.2. Methods

Data collection survey method: Many data, documents, map data, survey data to serve as input data for the study. Data sources were collected from the Department of Natural Resources and Environment of Thu Duc City, the Compensation and Site Clearance Board, the People's Committee of Phuoc Long B ward (now Phuoc Long ward, Figure 2).

Image processing method: Images created after the UAV flight will be processed according to the image processing process using Agisoft Metashape software. The steps include: Creating

an image project; correcting image blocks; converting the coordinates of the center of the image to the VN2000 system; establishing a point cloud; creating a digital elevation model and creating an image map.

Methods for creating land use status maps: Digitize imagegrammetry, correct errors, create regions, assign land type attributes, edit land use status maps, calculate areas and statistics of land types. At the same time, the study also collects and processes relevant maps including: cadastral maps, previous land use status maps (2019), planning maps and other types of maps for additional comparison.

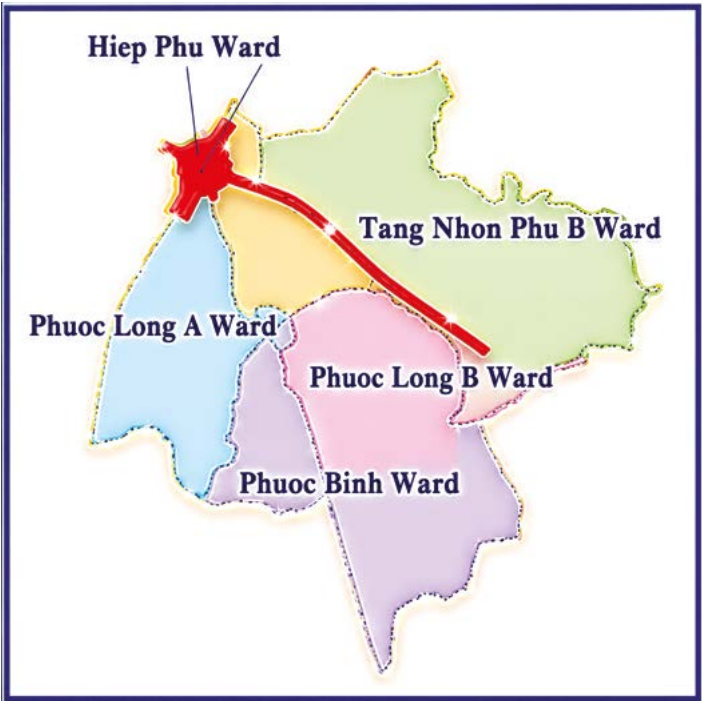


Figure 2. Study area location.

The process of establishing a land use status map was applied according to Circular 07/2021/TT-BTNMT of the Ministry of Natural Resources and Environment, which regulates the techniques for collecting and processing digital image data from unmanned aerial vehicles. At

the same time, the study also applies Circular 08/2024/TT-BTNMT of the Ministry of Natural Resources and Environment, which regulates land statistics, inventory and mapping of land use status (Figure 3).

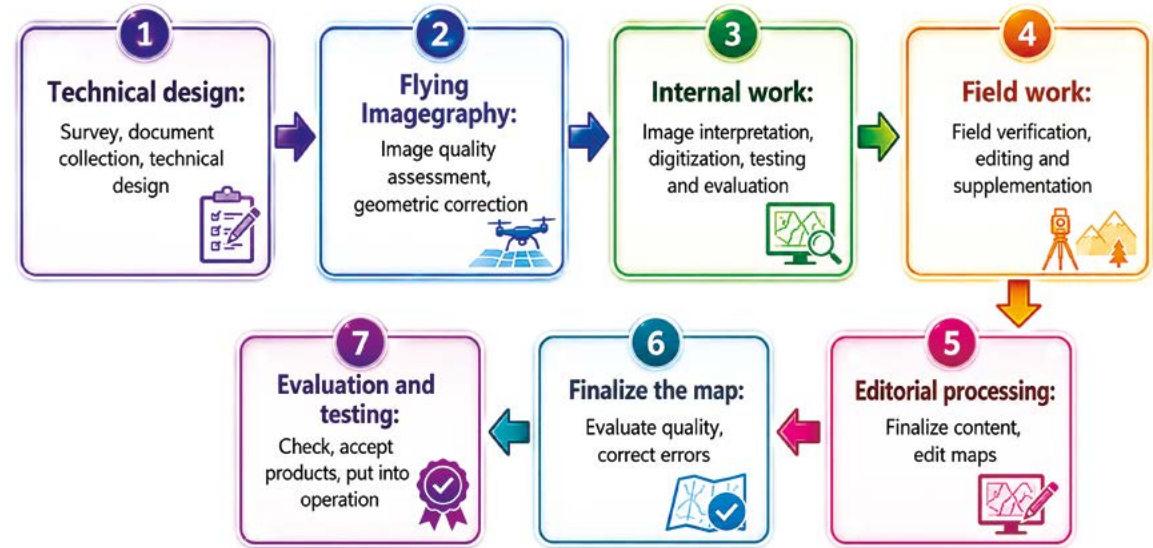


Figure 3. Mapping process using unmanned aerial vehicle in the research area.

3. Results and Discussion

3.1. Technical design and processing of flight results

3.1.1. Technical design

The survey area had a relatively flat terrain, so the grid flight mode (mapping) was selected. In this study, the coverage was selected at the following level: vertical coverage 70%

and horizontal coverage 60% (AEROTAS, 2020). The selected flight altitude was 74.5 m, corresponding to a ground image resolution of 2 cm. The measurement area was 0.554 km², with a linear shape of 2 km. Therefore, the total number of selected ground control points (GCPs) is 6 points and 2 base station points (Figure 4). The locations of the GCPs are shown in Figure 3. The coordinates of the GCPs are shown in Table 1.

Table 1. Coordinates of ground control points (GCPs)

Point ID	X Coordinate	Y Coordinate	Z Elevation
GCP-01	1198823,933	611195,262	11,982
GCP-02	1198405,186	610924,810	5,976
GCP-03	1198359,700	611252,437	6,857
GCP-04	1198255,128	611504,397	7,944
GCP-05	1197954,360	612013,889	7,756
GCP-06	1197696,455	612418,559	4,978
Base-01	1198351,434	611258,568	6,590
Base-02	1197694,311	612421,682	4,983

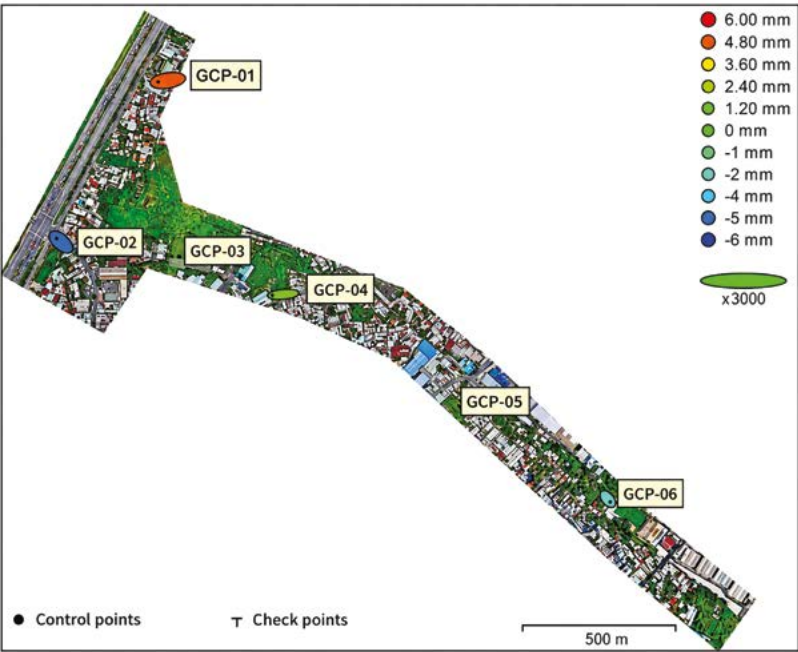


Figure 4. Location of ground control points (GCP).

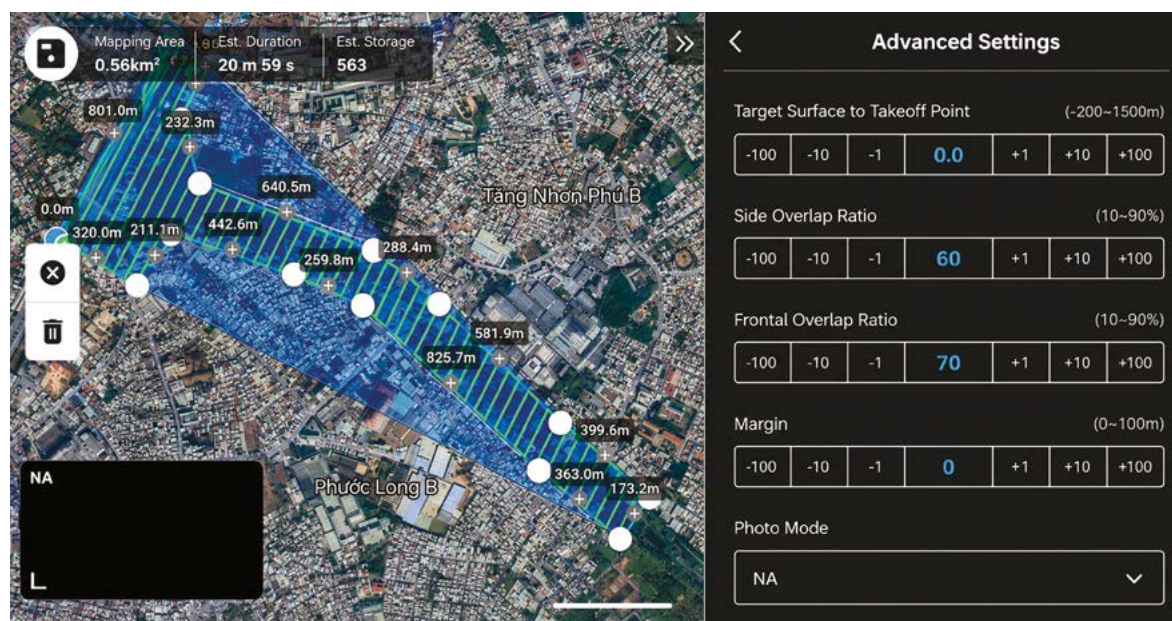


Figure 5. Flight diagram of the study area.

3.1.2. Mapping flight

The mapping flight was conducted at 9:00 on August 23, 2024, with the flight diagram shown in Figure 5. The parameters were set and calculated on the DJI Flot 2 flight control software. Continuous capture mode was used in this study. The flight time estimated by the software was 22 min. The flight was carried out in accordance with flight safety standards and the flight plan designed according to Circular 07/2021-BTNMT. The mapping flight resulted in 637 images, those in *.JPG format covered the flight diagram as designed.

3.1.3. Post-flight image processing

Image quality was evaluated using Agisoft Metashape software. Accuracy estimation was based on the Estimate Quality images tool. The test results showed that 637/637 images were of good quality (100%). The images were linked into image blocks with a mean square error of 2.01 pixels. The coordinates of the photo center were converted to the VN2000 coordinate system with a 3-degree projection zone. The Ho Chi Minh City axis meridian was 105°45'. The mean square error of the field Ground Control Points involved in the processing was 0,015 m, which ensured accuracy. The number of point clouds in the flight area was 2.5 billion points (Figure 5). From the point cloud set, the classification results are shown in Figure 6.

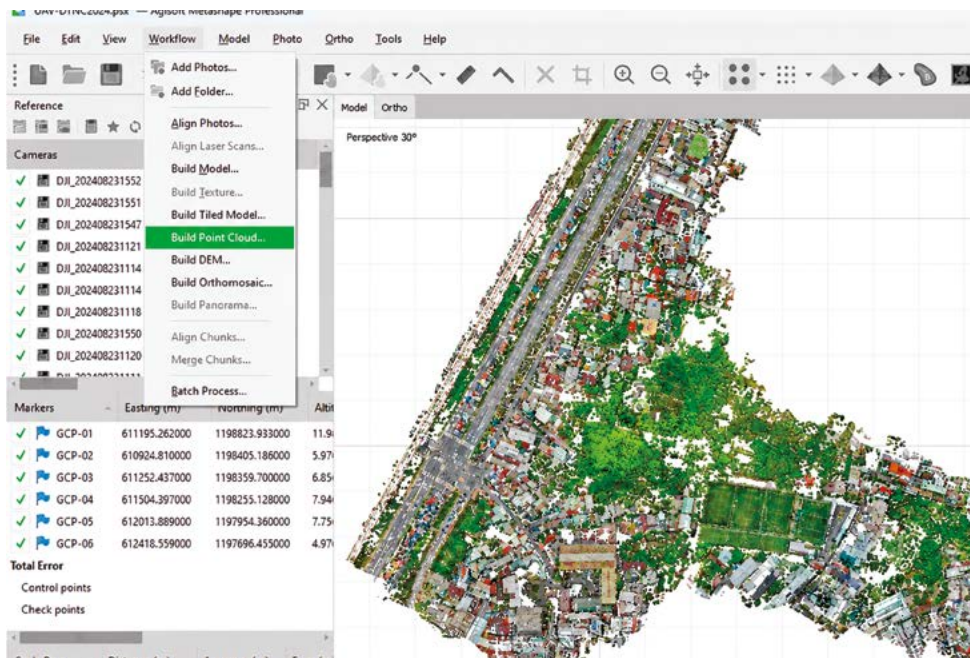


Figure 6. Point cloud creation.

3.2. Establishing maps from images

After the images were processed to ensure the required accuracy, the study digitized them according to the interpretation boundaries on the images. At the same time, the field investigation was combined to accurately determine the types

of land in the study area (Figure 7). The results digitized 415 land plots, located on 14 maps at a scale of 1/500. Finally, the study established a land demarcation map. This was the basis for statistical analysis on the area of land use types and for editing the current land use map (Figure 8).

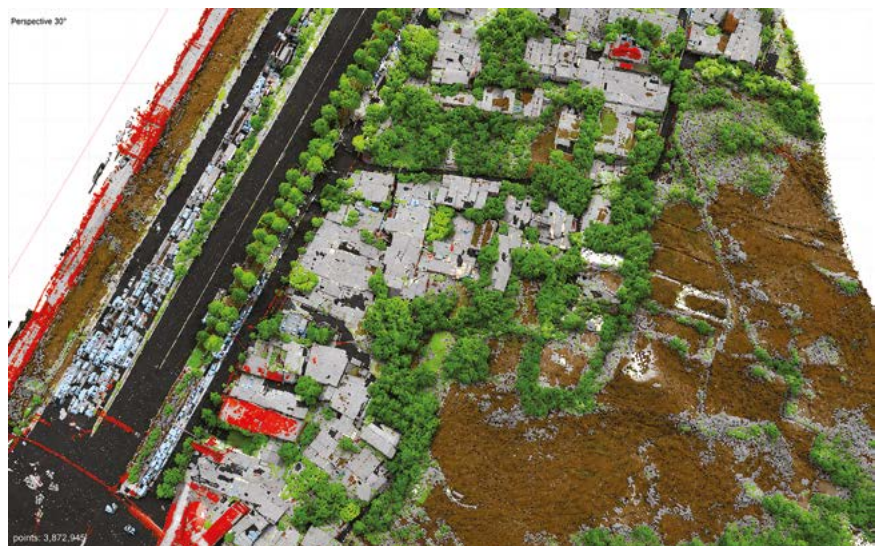


Figure 8. Digitization of land boundaries and structures on land.



Figure 9. Land use map of Ring road 2.

3.3. Land area statistics

Based on the land use map, the land use types are automatically counted on the Gcadas software. The statistical results showed that

within the project, there were 7 types of land that need to be recovered. Specifically, the land types were shown in Table 2. In the total current measured area of 17,66 hectares, it was mainly urban residential land (ODT).

Table 2. Area of land types

STT	Symbol	Land type	Area (ha)
1	CLN	Land for perennial crops	3.79
2	DGT	Traffic land	1.23
3	DTL	Irrigation land	0.03
4	SKC	Land for non-agricultural production facilities	0.08
5	HNK	Other annual crop land	4.55
6	NTD	Cemetery or graveyard land	0.12
7	ODT	Urban residential land	11.62
Total			17.66

3.4. Map quality assessment and discussion

The results of image block processing show that the mean square error is 2,01 pixels. The mean square error of field ground control points was 0.015 m. This result ensures the accuracy according to the regulations in Circular 07/2021/TT-BTNMT. The results of establishing the current land use map of the study area ensure compliance with the regulations according to Circular 08/2024/TT-BTNMT. The map meets the requirements on: presentation form, symbols of land types, and completeness of information. The results of field comparison show that 415/415 land plots ensure the accuracy of land types and plot boundaries. From there, it showed that the current land use map of the Ring Road 2 project in Phuoc Long B ward (now Phuoc Long ward) ensured quality, which can serve the work of compensation and site clearance.

Using UAVs provided better support for land clearance compensation. Instead of having to walk to each parcel, the survey team can collect comprehensive aerial data. This not only shortens the project implementation time but also limits errors due to human. The images and coordinate data obtained from UAVs are a solid basis for mapping the land use types and valuing assets fairly and transparently. The application of UAVs significantly reduces labor costs and field time. Instead of having to mobilize a large team for survey work, only a few technicians can operate UAVs to collect data. This is especially useful for areas with complex, difficult-to-access terrain, or large-scale and fast-implementation projects such as the Ring Road 2 project. The cost of operating a UAV is also much lower than using other traditional means of transport. The image and video data obtained from the UAV is visual, irrefutable evidence of the current condition of

the property before clearance. This helps people easily check and compare information, ensuring that compensation is carried out in accordance with reality. It also helps local management agencies closely monitor the process, prevent fraudulent and negative behaviors, thereby increasing people's trust in the government.

4. Conclusions

The study applied UAV technology to establish a 1/500 scale land use status map, which is within the boundary of Ring Road 2 in Phuoc Long B ward, Thu Duc City (now Phuoc Long ward). The research results showed that the land use status map was of good quality, which complied with the technical standards prescribed by the Ministry of Natural Resources and Environment. The results of the study confirmed that UAV technology can be applied in the establishment of large-scale maps. It was especially effective in areas that were difficult to access. Through the study, it can be seen that the application of UAV technology for ground monitoring had advantages such as: shortening the time to establish maps, ensuring high accuracy and limiting errors. Through the research process, it shows that the higher the image resolution, the longer the image processing time and the higher the computer configuration requirements. Therefore, depending on the purpose of using the image and the accuracy requirements, the researcher needs to choose the appropriate image resolution.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgements

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Processing of premixed puree from *Syzygium cumini* fruit
and Nipa palm syrup (*Nypa fruticans*)

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ARTICLE INFO	ABSTRACT
Research Paper Received: April 02, 2025 Revised: May 17, 2025 Accepted: May 21, 2025 Keywords Nipa palm syrup Processing Puree <i>Syzygium cumini</i> *Corresponding author Nguyen Minh Xuan Hong Email: nmxhong@hcmuaf.edu.vn	This study investigated the processing parameters for the formulation of a premixed puree incorporating <i>Syzygium cumini</i> and Nipa palm syrup, with the objective of developing a natural, nutritionally rich, and palatable product. <i>Syzygium cumini</i>, a fruit abundant in Vietnam's Mekong Delta, is recognized for its high antioxidant and polyphenol content but is under-utilized due to its susceptibility to damage. Nipa palm syrup, a natural sweetener with low-glycemic index, was integrated to enhance both the sensory attributes and nutritional profile of the puree. The research examined key processing factors, including blanching duration, the ratio of <i>Syzygium cumini</i> puree to Nipa palm syrup, pectin concentration, and pasteurization conditions. Experimental results indicated that blanching for 2 min effectively preserved the fruit's characteristic purple color and sensory qualities. A puree formulation with a <i>Syzygium cumini</i>-to-Nipa palm syrup ratio of 7:3 achieved optimal sensory acceptability. The addition of 0.75% pectin resulted in desirable viscosity while maintaining cost efficiency. Pasteurization at 94°C for 4 min was found to ensure microbial stability without inducing significant nutrient degradation. These findings offer a foundation for optimizing processing of a functional puree with enhanced sensory and nutritional properties.

Cited as: Nguyen, Q. D., Dang, D. T., Nguyen, D. V. T., & Nguyen, H. M. X. (2026). Processing of premixed puree from *Syzygium cumini* fruit and Nipa palm syrup (*Nypa fruticans*). *The Journal of Agriculture and Development* 25(3), 116-134.

1. Introduction

The growing awareness of health and nutrition among consumers has led to a substantial increase in demand for functional foods and natural-origin products. This shift has emphasized the necessity of developing food sources rich in

bioactive compounds, particularly antioxidants, which play a crucial role in promoting health and preventing disease. In response to these trends, the movement toward "clean eating and green living" has gained prominence, fostering the production of food items that exhibit high

bioactivity, minimal synthetic additive content, and enhanced environmental sustainability (Siró et al., 2008). Among the diverse native fruit species available in Vietnam, *Syzygium cumini* emerges as a promising yet underutilized resource, warranting further exploration for its potential applications in health-oriented food development.

Syzygium cumini is found in several Vietnamese regions, particularly in the Mekong Delta. In addition to a wealth of vitamins and minerals, it includes a range of bioactive substances, including anthocyanins, flavonoids, tannins, and saponins. These elements serve as antioxidants, strengthen the immune system, and protect against diabetes and other chronic illnesses (Baliga et al., 2011; Sharma et al., 2012). Along with its therapeutic benefits, *Syzygium cumini* has a unique flavor and an attractive deep purple color that make it a great choice for processed goods like juices, jams, syrups, and most notably puree, which can be used as a flexible intermediate ingredient in the food and beverage sector. Previous studies on fruit puree formulations have explored various fruits to develop nutrient-rich products (Kong et al., 2010), but just a few focused on under-utilized species like *Syzygium cumini* (Diep & Ngo, 2023). Moreover, its industrial application has been limited due to its short shelf-life, extremely seasonal character, and lack of standardized processing technologies. This highlights a research gap and emphasizes how important it is to create a suitable puree processing strategy in order to make the most of this local resource. Therefore, the aim of this study was to develop a premixed *Syzygium cumini* puree that preserves the raw material's maximal sensory qualities, nutritional value, and biological activity while

simultaneously increasing the product's shelf life. From a theoretical perspective, the study contributes additional scientific data on the chemical composition and bioactivities of *Syzygium cumini* under Vietnamese conditions, enriching the knowledge on this indigenous species.

Another natural ingredient with high potential in the food industry but limited current utilization is Nipa palm syrup, an extract derived from the male flower stalks or flower clusters of the Nipa palm (*Nypa fruticans*), a typical mangrove species in the Mekong Delta. Nipa palm syrup features a light, unique aroma and is rich in natural sugars (primarily sucrose, glucose, and fructose), minerals (such as potassium and magnesium), and a high level of antioxidants. Compared to refined sugar, Nipa palm syrup has a lower glycemic index and contains numerous health-benefiting compounds, making it a suitable natural sweetener aligned with current healthy eating trends (Teklemariam et al., 2024). The combination of *Syzygium cumini*, rich in anthocyanins and bioactive compounds and Nipa palm syrup, a health-friendly natural sweetener, is expected to result in a puree product with balanced flavor, appealing natural color, high nutritional value, and diverse application potential in the food industry. Using Nipa palm syrup instead of refined sugar is also consistent with the development of clean-label, organic, and functional food products. Practically, the research findings may assist food processing facilities in developing new products from local raw materials, reducing post-harvest losses, enhancing the economic value of regional agricultural products, and meeting modern consumer trends.

2. Materials and Methods

2.1. Sample collection and preparation

Syzygium cumini were purchased from An Giang province. After being purchased, the fruits were selected to remove bruised or rotten and washed to remove dirt.

Nipa palm syrup was purchased at the VietNipa company.

2.2. Chemicals

Plate count agar (standard methods agar) was obtained from HiMedia Laboratories Pvt. Ltd. (India). Sodium hydroxide, potassium chloride, potassium dihydrogen phosphate, sodium

carbonate, sodium chloride, sodium acetate trihydrate, and L(+)-ascorbic acid were obtained from Xilong Scientific Co., Ltd. (China). Hydrochloric acid was obtained from J.T. Baker® (USA). Gallic acid and Folin-Ciocalteu's phenol reagent (2 N) were obtained from Sigma-Aldrich (Merck, Germany). The DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Alfa Aesar (UK). All chemicals are of analytical grade.

2.3. Experimental design

The processing procedure of puree from *Syzygium cumini* and Nipa palm syrup and the experimental design of this study are presented in Figure 1.

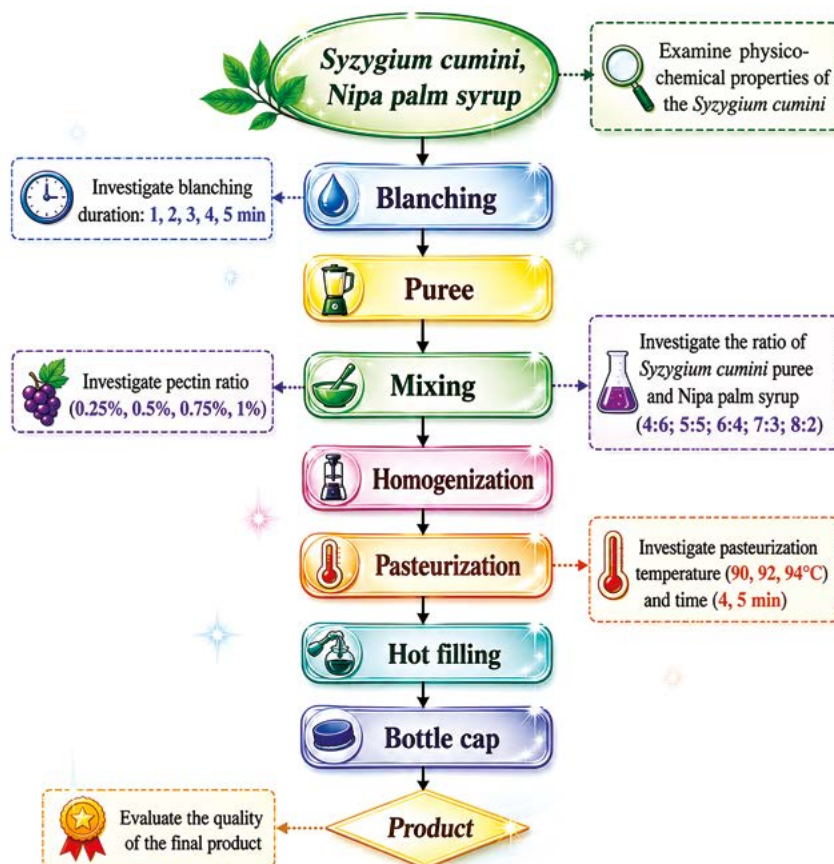


Figure 1. Processing procedure of puree from *Syzygium cumini* and Nipa palm syrup and the experimental design of this study.

2.3.1. Examination of the physicochemical properties of the *Syzygium cumini*

Syzygium cumini fruits were subjected to a washing process using clean water to remove surface contaminants. Damaged, bruised, and improperly ripened fruits were identified based on color assessment and subsequently discarded. The selected fruits were then analyzed for key physicochemical parameters, including moisture content, color, total soluble solids (TSS), acidity, vitamin C, anthocyanin, total phenolics, and antioxidant activity. These analyses were conducted to evaluate the compositional attributes and functional properties of the fruit, providing a basis for further processing and product development.

2.3.2. Investigation of the effect of blanching duration to the quality of puree

The experiment was designed to evaluate the effect of blanching duration on the physicochemical properties of *Syzygium cumini* puree. Blanching was conducted at five different time intervals: 1, 2, 3, 4, and 5 min. Following blanching, the fruits were rapidly cooled using clean water, drained to remove excess moisture, and subsequently processed into puree. The resulting puree samples were analyzed and compared across treatments to determine the optimal blanching time. Evaluations were performed based on recovery efficiency, TSS, total acidity, pH, color, and sensory attributes.

2.3.3. Investigation of the effect ratio of *Syzygium cumini* puree and Nipa palm Syrup on sensory and chemical properties of the product

The experiment was designed to evaluate the effect of varying ratios of *Syzygium cumini* puree

to Nipa palm syrup on the physicochemical and sensory properties of the final product. Five different formulations were prepared with puree-to-syrup ratios of 4:6, 5:5, 6:4, 7:3, and 8:2. Following preparation, the mixtures were homogenized to ensure uniform consistency. The resulting puree samples were then analyzed based on key parameters, including TSS, pH, acidity, and sensory attributes, to determine the optimal formulation.

2.3.4. Investigation of the effect of pectin ratio on the viscosity of the product

In this experiment, pectin was incorporated at varying concentrations of 0, 0.25, 0.5, 0.75, and 1% to assess its effect on the viscosity of the final product. After preparation, the puree samples were subjected to viscosity measurements at intervals of 1 week during 4 weeks to evaluate changes in consistency over time.

2.3.5. Investigation of the effect of pasteurization time and temperature on the quality of the product

In this experiment, the puree underwent heat treatment at six different temperature-time conditions: 90°C for 4 min, 92°C for 4 min, 94°C for 4 min, 90°C for 5 min, 92°C for 5 min, and 94°C for 5 min. After thermal processing, the puree was hot-filled into containers, and the lids were securely sealed to ensure proper preservation. Subsequent analysis was conducted to evaluate physicochemical parameters, followed by the enumeration of aerobic microorganisms after one month to assess microbial stability.

2.3.6. Evaluation of the quality of the final product

Following processing under the established parameters, the product underwent

comprehensive analysis, including chemical composition, microbiological safety, and sensory evaluation. Comparative analysis was conducted with a commercially available product to assess differences in physicochemical properties, microbial stability, and sensory attributes, providing insights into its potential market viability.

2.4. Methods for analysis

2.4.1. Determination of moisture content

Moisture content determination was conducted in accordance with TCVN 10788:2015 using a dryer (UF110, Memmert, Germany). The sample mass was recorded before and after drying at a temperature of 105°C until a constant weight was achieved. Moisture content (MC) was measured according to Equation 1, where m_1 is the sample mass (g) before drying, and m_2 is the sample mass (g) after drying.

$$MC (\%) = (m_1 - m_2) / m_1 \times 100 (\%) \text{ (Equation 1)}$$

2.4.2. Determination of color parameters

The color parameters (L^* , a^* , and b^* values) were measured by using a colorimeter (CR-400, Konica Minolta, Japan) followed principles developed by Hunter & Harold (1987). L^* value corresponds to lightness and varies from 0 to 100, a^* value measures redness when positive and greenness when negative, b^* value means yellowness when positive and blueness when negative.

2.4.3. Determination of pH

The pH determination was conducted using pH meter (pH 7110, WTW, Germany). The principle this method involves the electromotive force generated on the glass surface, which is

uniform and consistent (Harris, 2010). Electrodes on the inside and outside of the glass electrode can exchange with H^+ ions, creating a double layer on the surface of the glass and solution interface. This process generates an electromotive force inside and outside the electrode, which is then translated into the pH scale.

2.4.4. Determination of total titrable acidity

The acidity of the food sample was determined using the titration method described by Paul et al. (2010). A 5 g portion of the sample was mixed thoroughly with purified water in a 100 mL volumetric flask. Subsequently, a 20 mL aliquot from the volumetric flask was transferred to an Erlenmeyer flask, followed by the addition of 1 - 2 drops of 1% phenolphthalein solution as an indicator. The sample was then titrated with 0.1N NaOH solution until a stable pale pink color was observed. This method is based on the principle that the alkaline solution neutralizes all acidic components present in the food sample, with phenolphthalein serving as the visual indicator for endpoint determination. The total acid content (TAC, %) is calculated according to the Equation 2, where 0.0064 is the citric acid conversion index, V is the volume of 0.1 N NaOH used (mL); V_0 is the volume of mixture of sample and water (100 mL), V_1 is the volume of sample aspirated for titration (20 mL), and m is the mass of sample (5 g).

$$TAC (\%) = (0.0064 \times V \times V_0 \times 100) / (V_1 \times m) \text{ (Equation 2)}$$

2.4.5. Determination of total soluble solids (TSS)

TSS were quantified using a refractometer (RSA-BR80, 0-80°Brix, Cole-Parmer, USA) and followed by AOAC method (2005). A drop of the

test sample was placed onto the prism surface, and the prisms were pressed together to ensure proper contact. The eyepiece was adjusted to obtain a sharp dividing line between the dark and light regions of the field of view. This method is based on the principle that light undergoes refraction when transitioning from air, which has a lower refractive index, to a liquid medium with a higher refractive index. The extent of light deflection was measured to determine the solute concentration, allowing for the calculation of its percentage in the sample.

2.4.6. Determination of vitamin C content

Vitamin C content in the sample was determined using the iodine titration method described by Nielsen (2024). A 5 g portion of the sample was weighed and ground in a ceramic mortar with 5 mL of a 5% hydrochloric acid (HCl) solution to facilitate extraction. The ground mixture was then transferred into a volumetric flask containing 50 mL of distilled water and shaken thoroughly until a uniform solution was obtained. A 20 mL aliquot of the prepared solution was subsequently titrated with 0.01 N iodine solution, using starch as an indicator. The titration was carried out until the endpoint, marked by the appearance of a stable blue coloration, was achieved. The method is based on the principle that iodine reacts with vitamin C, allowing for quantification based on the amount of iodine required for complete reaction. The vitamin C content (X, mg/100 g) in the sample was calculated by the Equation 3, where V is the volume of 0.01 N I₂ used (mL), V₁ is the volume of sample (50 mL), V₂ is the volume of aspirated for titration (20 mL), W is the mass of sample for determination (5 g), 0.00088 is the gram of vitamin C in 1 mL of iodine.

$$X = (V * V_1 * 0.00088 * 100) / (V_2 * m) * 100$$

(Equation 3)

2.4.7. Extraction of bioactive compound

Syzygium cumini extracts for anthocyanin, total phenolic content (TPC), antioxidant activity, and total reducing sugar were analysed according to Faria et al. (2011), with some modifications. Aliquot of 10 g of fruit was extracted with 90 mL of 80% methanol for 30 min at room temperature. After that, samples were filtered through the qualitative filter paper.

2.4.8. Determination of anthocyanin content

Anthocyanin content was quantified using the pH differential method described by Lee et al. (2005), with modifications in accordance with TCVN 11028:2015 (MST, 2015a). Briefly, a 1 mL aliquot of the extracted sample was diluted using 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). The dilution factor was established when absorbance readings at 520 nm were below 1.2. Absorbance measurements were obtained at 520 and 700 nm using a spectrophotometer (Jenway 7305, Bibby Scientific Ltd., UK) equipped with a calibrated 1 cm cuvette, with distilled water serving as the blank reference. The difference in absorbance between pH values and wavelengths was calculated as in Equation 4.

$$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 1.0} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 4.5} \quad (4)$$

The concentration (mg/L) of monomeric anthocyanin pigment was calculated following Equation 5, where MW is the molecular weight of cyanidin-3-glucoside (449.2 Da), df is the dilution factor, ϵ is the molar absorptivity derived from a full wavelength scan (26900), l is the standard 1-cm path length.

Monomeric anthocyanin = $(A * MW * df * 1000) / (\epsilon * l)$ (Equation 5).

2.4.9. Determination of total phenolic content (TPC)

The total phenolic content was measured according to Chan et al. (2007) with some modifications. In a test tube, shake well a mixture of 0.3 mL of extract and 1.5 mL of Folin-Ciocalteu reagent 10%, then add 1.2 mL of 7.5% Na_2CO_3 and shake well. After 30 min of reaction at room temperature, the samples were measured for absorption at 765 nm. A calibration curve was built using gallic acid, and the results were displayed as milligrams of gallic acid (GAE) per 100 g of dry matter. The total phenolic content (TPC, mg GAE/100 g DM) was determined by Equation 6, where y is OD of sample, b is OD at intercept point between standard curve with y-axis, V is volume of extracted liquid (mL), DF is dilution factor (mL), a is slope obtained from the most appropriate linear calibration, m is mass of sample, and MC is the moisture content (%).

$$\text{TPC} = ((y - b) * V * \text{DF} * 100) / (a * m * (100 - \text{MC}) * 1000)$$
 (Equation 6)

2.4.10. Determination of antioxidant activity

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by Thaipong et al. (2006). A standard curve was constructed using ascorbic acid concentrations ranging from 10 to 80 mg/L. To prepare the DPPH reagent, 3.94 mg (0.0039 g) of DPPH was dissolved in 100 mL of 100% methanol. A 0.2 mL aliquot of diluted sample extract was transferred into a test tube, followed by the addition of 4 mL of 0.1 mM DPPH solution. The mixture was incubated at room temperature in darkness for 30 min. Absorbance measurements were

recorded at 517 nm using a spectrophotometer (Jenway 7305, Bibby Scientific Ltd., UK), with methanol serving as the blank. The total antioxidant capacity (TAC, mg AAE/g) was calculated according to the Equation 7, where x is the concentration from the standard curve (mg AAE/L), V is the volume of the extract used (mL), df is the dilution factor, and m is the mass of extract used (g).

$$\text{TAC} = (x * V * \text{df}) / (m * 1000)$$
 (Equation 7)

2.4.11. Determination of viscosity

Viscosity was measured using a Brookfield RVDV-E viscometer (USA), which operates based on the principle of rotational viscometry followed by Steffe (1996). This method evaluates the liquid's viscosity by assessing the resistance encountered by a submerged spindle rotating at a constant speed. The rotation induces shear stress within the liquid, and the resulting torque is directly proportional to its viscosity. To conduct the measurement, the test sample was poured into an appropriately sized container, ensuring that spindle S04 was immersed to the designated level. The viscometer's control panel was used to set the rotational speed to 30 rpm, a configuration typically adjusted via speed selection buttons or the menu interface. Once the speed setting was confirmed on the display, the spindle was allowed to rotate for approximately 30 - 60 sec to achieve stability. Viscosity readings were then recorded.

2.4.12. Determination of total aerobic microorganisms

Aerobic microorganism enumeration in *Syzygium cumini* puree was performed using the pour plate method, in accordance with TCVN 11039-1:2015 (MST, 2015b). In this procedure, the bacterial cells were mixed with melted agar to ensure uniform distribution throughout the

medium. The mixture was then poured into an empty Petri plate and allowed to solidify. Following incubation, discrete bacterial colonies were observed both on the surface and within the agar matrix. A 1 mL aliquot of the serially diluted liquid sample was thoroughly combined with approximately 15 mL of a specific molten agar medium, maintained at a temperature of 44 - 47°C (not exceeding 50°C), in a Petri plate. The medium was permitted to solidify before undergoing incubation at 37°C for 24 - 48 h. After the incubation period, viable microbial colonies were quantified based on the total count of colony-forming units (CFUs). The number (N) of CFU/g or mL of the test sample is calculated following Equation 8, where C is the sum of colonies on all plates counted, v is the volume applied to each plate, n1 is the number of plates counted at the first dilution; n2 is the number of plates counted at the second dilution, and d is the dilution from which the first count was obtained.

$$N = C/v (n1 + 0.1 n2) * d \text{ (Equation 8)}$$

2.4.13. Sensory evaluation

The final product used the quality assessment method according to the scoring test ranging

from 1 to 5 points with 10 panellists followed by Meilgaard et al. (1999) with some modifications.

2.4.14. Statistical analysis

The experiment was repeated 3 times; the value presented in this article is the mean value. The data was processed, statistically analyzed, and graphed using Minitab Statistical 2021 and Microsoft Excel 2019 software.

3. Results and Discussion

3.1. Chemical properties of *Syzygium cumini*

The chemical composition of *Syzygium cumini* fruit, as presented in Table 1, indicated that it had a high moisture content, making it a juicy fruit. The total soluble solids relate to its high sweetness level, while total acidity gives insight into its tartness. The anthocyanin concentration suggests it's rich in pigments that contribute to its deep purple color. These compounds are also known for their health benefits. Antioxidant activity and total phenolic content reflect its potential in combating oxidative stress, and the presence of vitamin C makes it even more beneficial for immunity and overall wellness.

Table 1. Chemical composition of *Syzygium cumini*

Criteria	Value
Moisture content (%)	78.55 ± 1.17
Total soluble solid (oBrix)	16.67 ± 0.58
Total acidity (%)	1.39 ± 0.04
Anthocyanin (mg/100 g)	7.206 ± 0.17
Antioxidant activity (mg AAE/g)	7.2 ± 0.0003
Vitamin C content (mg/100 g)	9.1 ± 0.5
Total phenolic content (mg/100 g)	84.69 ± 0.42

3.1.1. Effect of blanching duration to the quality of puree

Tables 2 and 3 illustrate significant differences in the physicochemical properties and bioactive compounds of *Syzygium cumini* puree across blanching durations (1 - 5 min).

Table 2. Physicochemical properties of puree at different blanching durations

Blanching duration	Total soluble solids (°Brix)	Total acidity (%)	pH	Puree yield
Control	16.67 ± 0.58 ^b	1.84 ± 0.04 ^a	3.57 ± 0.06 ^{ab}	120.67 ± 1.16 ^a
1 min	16.67 ± 0.58 ^b	1.65 ± 0.04 ^b	3.73 ± 0.06 ^a	110.33 ± 0.58 ^b
2 min	19.33 ± 0.58 ^a	1.62 ± 0.03 ^b	3.67 ± 0.12 ^{ab}	110.00 ± 2.00 ^b
3 min	11.00 ± 1.00 ^c	1.41 ± 0.07 ^c	3.60 ± 0.00 ^{ab}	84.67 ± 1.53 ^c
4 min	9.00 ± 0.00 ^d	1.22 ± 0.07 ^d	3.57 ± 0.06 ^{ab}	73.67 ± 0.58 ^d
5 min	9.00 ± 0.00 ^d	1.12 ± 0.08 ^d	3.50 ± 0.00 ^b	69.67 ± 0.58 ^e
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001

^{a,b,c,d,e} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

In Table 2, the 2-min blanching sample exhibited the highest total soluble solids at 19.33 °Brix, compared to 16.67 °Brix (control) and a sharp decline to 9.00 °Brix at 4 - 5 min ($P < 0.05$), indicating optimal sugar retention at 2 min. Total acidity decreased progressively from 1.84% (control) to 1.12% at 5 min, reflecting thermal

degradation of organic acids, as noted by Gao et al. (2000). Puree yield also varied significantly, dropping from 120.67 g (control) to 69.67 g at 5 min, suggesting increased juice loss with longer blanching, consistent with (Rausch & Singh, 2014).

Table 3. Bioactive compounds of different blanching times

Blanching duration	Anthocyanin (mg/100 g)	Total phenolics (mg/100 g)	Antioxidant activity (mg/100 g)
Control	6.79 ± 1.35 ^a	68.12 ± 0.43 ^d	128 ± 0.03 ^b
1 min	6.09 ± 0.10 ^b	92.72 ± 0.28 ^b	133 ± 0.01 ^b
2 min	6.89 ± 2.35 ^a	104.93 ± 0.29 ^a	157 ± 0.01 ^a
3 min	6.23 ± 0.67 ^b	86.66 ± 0.28 ^c	123 ± 0.06 ^b
4 min	5.27 ± 0.34 ^c	46.19 ± 0.35 ^e	93 ± 0.09 ^c
5 min	4.65 ± 1.19 ^d	32.20 ± 0.22 ^f	82 ± 0.18 ^c
<i>P</i>	< 0.001	< 0.001	< 0.001

^{a,b,c,d,e} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

In Table 3, the 2-min blanching sample had the highest anthocyanin content (6.89 mg/100 g), total phenolic content (TPC) (104.93 mg/100 g), and antioxidant activity (157 mg AAE/100 g), outperforming the control and longer blanching

durations ($P < 0.05$). Prolonged blanching (4 - 5 min) reduced anthocyanin to 4.65 mg/100 g and TPC to 32.20 mg/100 g, likely due to thermal instability, as reported by Patras et al. (2010).

Table 4. The color of puree samples at different blanching durations

Blanching duration	L*	a*	b*
Control	31.29 ± 0.98 ^a	18.98 ± 0.54 ^{ab}	-2.75 ± 0.09 ^b
1 min	28.84 ± 0.13 ^b	19.32 ± 2.49 ^{ab}	-1.91 ± 0.49 ^a
2 min	31.38 ± 0.54 ^a	16.33 ± 0.79 ^b	-2.95 ± 0.15 ^b
3 min	31.16 ± 0.61 ^a	19.13 ± 0.23 ^{ab}	-4.82 ± 0.11 ^d
4 min	32.30 ± 1.05 ^a	19.84 ± 0.32 ^a	-4.87 ± 0.25 ^d
5 min	31.22 ± 0.23 ^a	18.72 ± 0.23 ^{ab}	-4.02 ± 0.22 ^c
<i>P</i>	< 0.001	0.03	< 0.001

^{a,b,c,d,ee} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

As presented in Table 4, the blanching had a statistically significant effect on the L*, a*, and b* values of *Syzygium cumini*. It was also observed that increasing the duration of the blanching caused changes in color and intensity, whilst a short blanching duration maintained the natural purple color of the *Syzygium cumini*. Changes in pH altered the stability of anthocyanin, one of the primary pigments responsible for the purple color. The increase of pH (more basic) leads to color degradation, and lower pH means greater stability of anthocyanin. While the 2-min blanched sample had the lowest recovery compared to the rest of the samples, the textual and coloration characteristics of the 2-minute blanched sample reached the general preferences of the sensory panellists. The anthocyanin is the major pigment causing the purple colour in

Syzygium cumini, which also affects the a* value. Higher values of a* yield redder colors, while lower a* values can result in a blue/purplish tint or the color becoming lighter. Also, the b* index (from blue to yellow axis) may also have an effect, but mainly the purple color brilliance. Hence, for the monitoring of the purple coloration of *Syzygium cumini*, it is required to check the a* index changes during processing and storage.

Figure 2 presented the sensory evaluation results for *Syzygium cumini* puree across different blanching durations (0 - 5 min). The 2-min blanching sample consistently received the highest sensory scores across all attributes, outperforming the control (unblanched) and longer blanching durations (3 - 5 min). Overall, results obtained from biological and sensory activities showed that the 2-min blanching

sample retained the characteristic color of the raw material and received the highest score, so

it was chosen as the optimal blanching time to perform the following experiments.

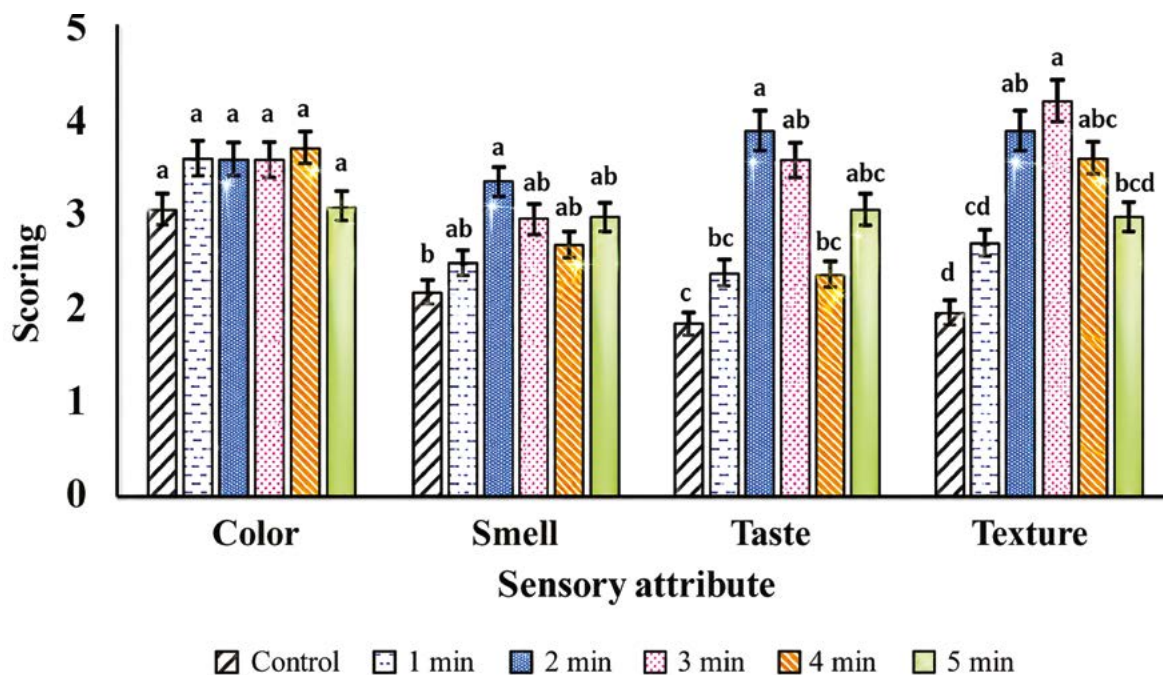


Figure 2. Sensory evaluation of puree samples at different blanching durations.

a,b,c,d Bars with different letters are significantly different from each other at $P < 0.05$.

3.2. Effect of the ratio of *Syzygium cumini* puree and Nipa palm syrup on sensory and chemical properties of the product

Table 5 illustrated the impact of varying *Syzygium cumini* puree to Nipa palm syrup ratios (4:6, 5:5, 6:4, 7:3, 8:2) on the physicochemical and sensory properties of the puree. The 7:3 ratio exhibited the highest sensory acceptability (Figure 5), with a balanced TSS of 29.67 °Brix,

total acidity of 1.88%, and pH of 3.57. This ratio also yielded a high total phenolic content of 96.47 mg/100 g and antioxidant activity of 7.38 mgAAE/g, though slightly lower than the 8:2 ratio (101.12 mg/100 g and 7.57 mg AAE/ g, respectively). The 4:6 and 5:5 ratios, with higher syrup content, showed elevated TSS (47.00 and 41.33 °Brix) but lower acidity (0.90 and 1.06%) and TPC (70.84 and 86.47 mg/100 g), likely due to dilution of fruit-derived bioactive compounds.

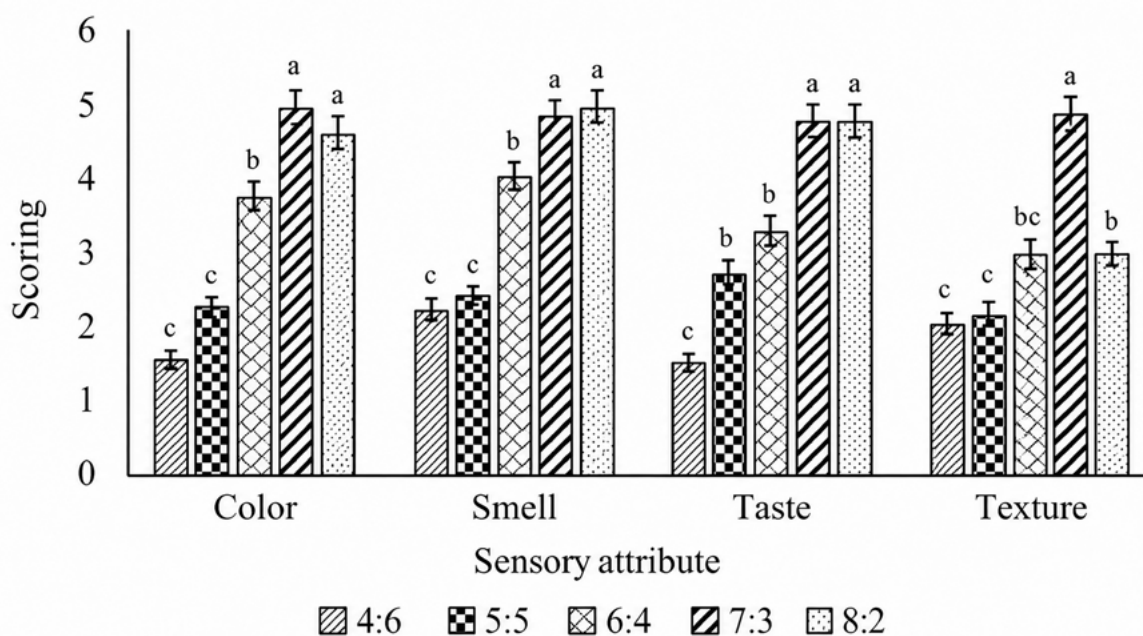
Table 5. Physicochemical properties of each ratio between *Syzygium cumini* puree and Nipa palm Syrup

Ratio	Total soluble solids (°Brix)	Total acidity (%)	pH	Total phenolics (mg/100g)	Antioxidant activity (mg AAE/g)
4:6	47.00 ± 0.00 ^a	0.90 ± 0.07 ^d	3.97 ± 0.06 ^a	70.84 ± 0.16 ^d	6.66 ± 0.03 ^d
5:5	41.33 ± 0.58 ^b	1.06 ± 0.08 ^d	3.83 ± 0.06 ^a	86.47 ± 0.29 ^c	7.20 ± 0.04 ^c
6:4	35.67 ± 0.58 ^c	1.30 ± 0.07 ^c	3.63 ± 0.06 ^b	93.85 ± 0.28 ^b	7.22 ± 0.04 ^c
7:3	29.67 ± 0.58 ^d	1.88 ± 0.03 ^a	3.57 ± 0.06 ^b	96.47 ± 0.22 ^b	7.38 ± 0.06 ^b
8:2	20.67 ± 0.58 ^e	1.64 ± 0.08 ^b	3.53 ± 0.05 ^b	101.12 ± 2.93 ^a	7.57 ± 0.07 ^a
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

^{a,b,c,d,e} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

Both ratios 7:3 and 8:2 received high sensory scores without significant differences ($P < 0.05$) (Figure 3). However, the 8:2 ratio, while richest in bioactive compounds, had lower TSS (20.67

°Brix) and less favorable sensory scores, possibly due to excessive tartness. As a result, the ratio 7:3 was chosen as the appropriate mixing ratio for the next experiments.

**Figure 3.** Sensory evaluation of puree samples with different ratios.

^{a, b, c} Bars with different letters are significantly different from each other at $P < 0.05$.

3.3. Effect of pectin ratio on sensory value and viscosity of the product

Table 6 showed that viscosity increased with higher pectin levels. Viscosity decreased over time in all samples, with the most significant

drop by week 4, attributed to structural breakdown and water separation, as noted by Kader et al. (1997). The 0.75% and 1% samples showed the slowest viscosity decline, indicating effective stabilization by pectin, which forms a gel network to retain water.

Table 6. Viscosity (cP) of different ratios of pectin each week

Sample	Week 1	Week 2	Week 3	Week 4	Week 5
Control	2,027 ± 0.58 ^{eB}	2,113 ± 0.00 ^{eA}	1,867 ± 0.00 ^{eC}	1,822 ± 0.58 ^{eC}	1,780 ± 0.00 ^{eD}
0.25%	2,645 ± 0.58 ^{dA}	2,683 ± 0.58 ^{dA}	2,552 ± 0.58 ^{dB}	2,246 ± 0.58 ^{dC}	1,867 ± 0.58 ^{dD}
0.5%	2,965 ± 0.58 ^{cB}	3,073 ± 0.58 ^{cA}	2,940 ± 0.00 ^{cB}	2,642 ± 1.15 ^{cC}	2,480 ± 0.58 ^{cD}
0.75%	4,792 ± 1.00 ^{bB}	5,042 ± 0.58 ^{bA}	4,600 ± 0.58 ^{bC}	4,313 ± 0.00 ^{bD}	4,253 ± 0.00 ^{bE}
1%	5,952 ± 0.58 ^{aA}	5,977 ± 0.58 ^{aA}	5,511 ± 0.58 ^{aB}	5,447 ± 0.00 ^{aC}	5,260 ± 0.00 ^{aD}

a, b, c, d, e Values with different superscript letters within the same column indicate significant difference ($P < 0.05$) between pectin ratios.
A, B, C, D, E Values with different superscript letters within the same row indicate significant difference ($P < 0.05$) between storage time.

Figure 4 revealed that sensory texture scores for the 0.75 and 1% samples were comparable, with no significant difference, suggesting that 0.75% achieves desirable texture while being

more cost-effective. Overall, the 0.75% pectin concentration optimally balances viscosity retention and sensory acceptability while minimizing additive costs.

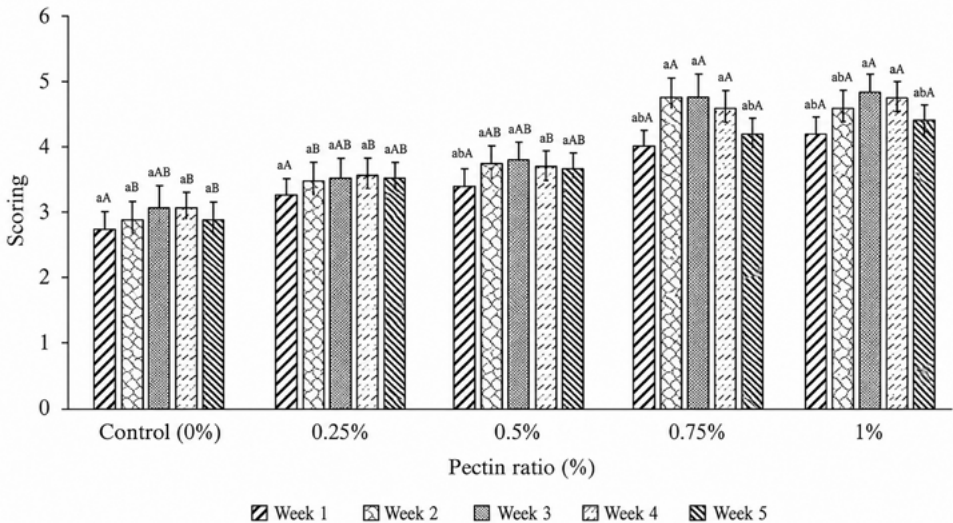


Figure 4. Sensory evaluation of the product texture of samples with different pectin ratios after 5 weeks. Different lowercase letters (a–b) indicate significant differences among storage times within the same pectin ratios, whereas different uppercase letters (A–B) indicate significant differences among pectin concentrations within the same storage time ($P < 0.05$).

3.4. Effect of pasteurization time and temperature on the quality, chemical properties and sensory properties of the product

Tables 7 and 8 compared the physicochemical properties of *Syzygium cumini* puree immediately after pasteurization (Table 7) and after one month of storage (Table 8) across different temperature-time conditions (90°C, 92°C, 94°C for 4 or 5 minutes). In Table 7, the 94°C/5-min condition showed the highest total soluble solids (TSS) at 38 °Brix, pH at 3.90, and lowest acidity at 0.36%,

compared to 90°C/4-min (35 °Brix, pH 3.77, acidity 0.64%) ($P < 0.05$). No layer separation was observed across all conditions, indicating effective stabilization. After one month (Table 8), TSS, pH, and acidity values remained largely unchanged, with 94°C/5-min retaining 38 °Brix, pH 3.90, and 0.36% acidity, and 90°C/4-min at 35 °Brix, pH 3.77, and 0.64% acidity. However, layer separation emerged in some samples, with 90°C/4-min showing 0.2 cm and 94°C/4-min showing none, suggesting better stability at higher temperatures.

Table 7. Physicochemical properties of the product after heat treatment

Time	Temperature	Total soluble solids (°Brix)	pH	Acidity (%)	Layer separation (cm)
4 min	90	35 ^c ± 0.00	3.77 ^b ± 0.06	0.64 ^a ± 0.06	0
	92	35 ^c ± 0.00	3.83 ^{ab} ± 0.06	0.58 ^{ab} ± 0.00	0
	94	35 ^c ± 0.00	3.80 ^{ab} ± 0.00	0.55 ^{ab} ± 0.04	0
5 min	90	35 ^c ± 0.00	3.83 ^{ab} ± 0.06	0.49 ^b ± 0.04	0
	92	36 ^b ± 0.00	3.90 ^a ± 0.00	0.47 ^{bc} ± 0.04	0
	94	38 ^a ± 0.00	3.90 ^a ± 0.00	0.36 ^c ± 0.04	0

^{a, b, c} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

Table 8. Physicochemical properties of the product after 1 month of storage

Time	Temperature	Total soluble solids (°Brix)	pH	Acidity (%)	Layer separation (cm)
4 min	90	35 ^c ± 0.00	3.77 ^b ± 0.00	0.64 ^a ± 0.04	0.2
	92	35 ^c ± 0.00	3.83 ^{ab} ± 0.06	0.58 ^{ab} ± 0.04	0.1
	94	35 ^c ± 0.00	3.80 ^{ab} ± 0.06	0.55 ^{ab} ± 0.04	0
5 min	90	35 ^c ± 0.00	3.83 ^{ab} ± 0.06	0.49 ^b ± 0.04	0.1
	92	36 ^b ± 0.00	3.90 ^a ± 0.06	0.47 ^{bc} ± 0.00	0
	94	38 ^a ± 0.58	3.90 ^a ± 0.06	0.36 ^c ± 0.06	0

^{a, b, c} Values with different superscript letters within the same column are significantly different ($P < 0.05$).

Table 9 presented the total aerobic microorganism counts in *Syzygium cumini* puree after one month of storage under different pasteurization conditions (90, 92, 94°C for 4 or 5 min).

Table 9. Total aerobic microorganisms after 1 month of storage

Time	Temperature	Total aerobic microorganisms (log10 CFU/mL)
4 min	90°C	5.1 ^a ± 0.02
	92°C	3.12 ^b ± 0.02
	94°C	< LOD
5 min	90°C	2.28 ^b ± 0.27
	92°C	1.79 ^b ± 0.18
	94°C	< LOD

LOD: limit of detection. a, bValues with different superscript letters within the same column are significantly different ($P < 0.05$).

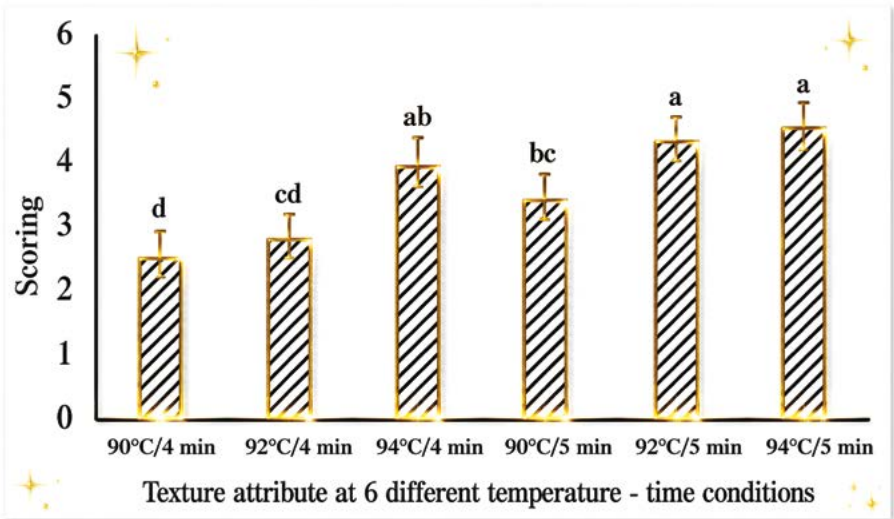


Figure 5. Sensory evaluation of texture at different temperatures and times. ^{a,b,c,d}Bars with different letters are significantly different from each other at $P < 0.05$.

The results showed significant differences in microbial load ($P < 0.05$). The 94°C/4-min and 94°C/5-min conditions achieved zero colony-forming units (CFU/mL), meeting the QCVN 6-2:2010/BYT standard of ≤ 100 CFU/mL for non-alcoholic beverages (MOH, 2010). The sample 94°C/4-min condition is optimal, as it

achieves microbial safety without the extended heating of 5 min, which could degrade bioactive compounds.

For the sensory results of the texture index presented in Figure 5, it also shows that the sensory panellists rated the highest scores for

the 92°C/5 min and 94°C/5 min samples, but the 94°C/4 min sample also gave results with no significant difference. Therefore, choosing the 94°C/4 min sample meets the requirements of food safety and hygiene and helps reduce processing costs.

The sample 94°C/4 min was sent to Eurofins Hai Dang Chromatography to evaluate microbiological indicators, followed by QCVN 6-2:2010/BYT (MOH, 2010). The result after testing was presented in Table 10. After 1 month of storage, no microbial growth was detected.

Table 10. Microbiological indicators of the product after 1 month of storage

No.	Parameters	Unit	<i>Syzygium cumini</i> puree and Nipa Palm Syrup	QCVN 6-2:2010/BYT
1	<i>Clostridium perfringens</i>	Cfu/mL	Not detected	Impossible
2	<i>Staphylococcus aureus</i>	Cfu/mL	Not detected	Impossible
3	<i>Pseudomonas aeruginosa</i>	Cfu/mL	Not detected	Impossible
4	<i>Escherichia coli</i>	Cfu/mL	Not detected	Impossible
5	Fecal streptococci	Cfu/mL	Not detected	Impossible
6	Total aerobic microorganism	Cfu/mL	Not detected	100
7	Coliforms	Cfu/mL	Not detected	10
8	Total yeast and mold	Cfu/mL	Not detected	10

3.5. Evaluation of the quality of the final product

Table 11. Physicochemical and bioactive content of final product

Parameters	Blueberry crush	<i>Syzygium cumini</i> puree with Nipa palm syrup
Moisture content (%)	51.70	66.91
Total soluble solids (oBrix)	45	36
Total acidity (%)	0.40	0.51
Vitamin C (mg/100 g)	9.32	11.1
Anthocyanin (mg/100 g)	45.98	20.82
Total phenolic content (mg/100 g)	66.43	104.46
Antioxidant activity (mg AAE/100 g)	80	149

The final product was compared with a current blueberry product on the market, with results shown in Table 11. The *Syzygium cumini* puree exhibits higher moisture content (66.91 vs. 51.70%), reflecting a less concentrated product, possibly due to minimal processing to retain natural fruit characteristics. Its total soluble solids (°Brix) are lower (36 vs. 45 °Brix), indicating less sugar addition compared to the commercial product. *Syzygium cumini* contains higher vitamin C (11.1 vs. 9.32 mg/100 g), total phenolic content (104.46 vs. 66.43 mg GAE/100 g), and antioxidant activity (149 vs. 80

mg AAE/100 g). The use of Nipa palm syrup, a low-glycemic sweetener, enhances the *Syzygium cumini* puree's health benefits over the blueberry crush sugar-based formulation. These results suggest that the *Syzygium cumini* puree offers a more nutrient-dense, less processed alternative to commercial products.

To accurately assess the nutritional value of *Syzygium cumini* puree, the final product was sent to Eurofins Hai Dang Chromatography for nutritional assessment, the results of which are shown in Table 12.

Table 12. Nutritional value of the final product

No.	Parameters	Unit	Results
1	Dietary fiber	g/100 g	3.09
2	Moisture	%	63.7
3	Ash	g/100 g	1.34
4	Sodium (Na)	mg/kg	1370
5	Carbohydrates (excluding dietary fiber)	g/100 g	30.8
6	Energy value (calculated according to Carbohydrates excluding Dietary fiber)	kcal/100 g	134
7	Protein	g/100 g	1.06
8	Total fat	g/100 g	Not detected
9	Total sugars	g/100 g	16.0

The puree contains 3.09 g/100 g dietary fiber, supporting digestive health and aligning with recommendations for functional foods. Its moisture content (63.7%) reflects a hydrated product, consistent with fruit-based purees, though slightly lower than the 66.91% reported in Table 11, possibly due to analytical variations. The ash content (1.34 g/100 g) indicates a moderate mineral presence, with sodium at 1370 mg/kg (0.137 g/100 g), which is relatively low and suitable for low-sodium diets. Carbohydrates (excluding dietary fiber) are 30.8 g/100 g, contributing to

an energy value of 134 kcal/100 g, making it a moderate-energy food ideal for health-conscious consumers. Protein content is low (1.06 g/100 g), typical for fruit-based products, while total fat is undetectable, enhancing its appeal as a low-fat option. Total sugars (16.0 g/100 g) are moderate, reflecting the use of Nipa palm syrup, a low-glycemic sweetener. This puree offers a balanced nutritional profile with high fiber, low fat, and moderate energy, positioning it as a competitive functional food product.

4. Conclusions

This study developed a processing procedure for premixed puree from *Syzygium cumini* fruit and Nipa palm syrup. The optimal parameters for the process include blanching duration at 2 min, the *Syzygium cumini*-to-Nipa palm syrup ratio of 7:3, the inclusion of 0.75% pectin, and pasteurization at 94°C for 4 min. The product has good sensory attributes with a balanced nutritional profile and the microbial index that meets the requirements of QCVN 6-2:2010/BYT for non-alcoholic beverages (MOH, 2010). These findings provide a foundation for the optimized processing of a functional puree with enhanced sensory and nutritional properties. Further research should be carried out to implement this procedure in an industrial scale.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Xanthan gum/Lysozyme nanoparticles (XG/Ly NPs) formulation and its application in stabilizing pickering emulsions

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ABSTRACT

This study investigated the formulation of nano xanthan gum (XG) through a hydrolysis reaction catalyzed by the enzyme lysozyme (Ly). Different reaction conditions of the xanthan gum cleavage process were studied including reaction times of 15, 30, and 45 min; pH levels of 8, 10, and 12; and reaction temperatures of 60, 80, and 100°C. Reaction efficiency was evaluated based on dextrose equivalent (DE) and XG/Ly particle size. Field emission scanning electron microscopy (FE-SEM) results showed that nano XG/Ly particles could be formulated with an average particle size of 500 nm when the reaction was operated at a pH of 12 and a temperature of 80°C for 15 min. Moreover, these nano polysaccharide particles were successfully applied in preparing pickering emulsions and improved their stability.

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1. Introduction

Xanthan gum (XG) is an anionic extracellular polysaccharide produced by the microorganism *Xanthomonas bacteria* (Cai et al., 2020). The molecular weight of xanthan gum is approximately two million g/mol and the main structure of this polysaccharide is quite similar to cellulose (Zhang et al., 2019; Cai et al., 2020). The backbone of xanthan gum is mainly linear bonds composing 1,4-linked β -D-glucose residues. Xanthan gum is a common food additive and has a long history of application in the food and pharmaceutical industry. Although this

polysaccharide was mainly applied as a strong thickening agent, recent studies suggested that nano xanthan gum particles were also potential materials for stabilizing advanced colloidal dispersions (Xu et al., 2015).

Among various pathways of nano polysaccharides preparations, enzymatic hydrolysis using lysozyme is an appropriate choice. Lysozyme, also known as muramidase or N-acetylmuramic hydrolase, is a small monomeric protein stabilized by four disulfide bonds among the eight cysteine residues of its polypeptide chain (Vu et al., 2019). This enzyme

is often found in large quantities in human and plant organs, tissues, and secretions (spleen, placenta, skin milk, tears, saliva, serum, etc.). Some studies successfully applied lysozyme to hydrolyze polysaccharides such as xanthan gum but reaction conditions for this process have not yet been optimized. Besides, the applications of nano xanthan gum particles are also very limited.

Currently, the emulsion system is an important step in the pesticide industry, because it helps improve the homogenization of solvent systems, additives, and biologically active ingredients. Among advanced colloidal dispersions, emulsions stabilized by solid particles (commonly known as pickering emulsions) are the choice of many recent researchers due to their low toxicity compared to synthetic surfactants adding tiny solid particles to the emulsions, they could be adsorbed at the oil/water interface and provide an effective barrier to limit the unwanted coalescence between droplets (Chen et al., 2023). The solid particles commonly used in pickering emulsions are polysaccharides, proteins, and lipids (Phan et al., 2015; Tu & Ha, 2015; Do et al., 2016).

In this study, xanthan gum will be hydrolyzed with the enzyme Lysozyme to form XG/Ly solid particles. Then, these novel solid nanoparticles will be utilized for preparing pickering emulsion systems. Various advanced technologies including FE-SEM and static light scattering will be applied to evaluate the effectiveness of hydrolysis reaction as well as the stability of pickering emulsions.

2. Materials and Methods

2.1. Materials

Chemical substances for silver nanoparticle

synthesis and analysis included: xanthan gum (XG, Mw = 1.3×10^7 Da), Lysozyme (CAS#12650-88-3), sunflower oil (Rossianka, Russia), 3,5 - Dinitrosalicylic acid (DNS) (Himedia, India), $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (Xilong Scientific, China), D-Glucose (Xilong Scientific, China). All chemical reagents were in the analytical grade.

2.2. Methods

2.2.1. Synthesis of XG/Ly

Xanthan gum and lysozyme solutions (25 mg/mL) were stirred with distilled water at room temperature for 6 and 2 h, respectively. Xanthan gum/lysozyme mixture with a mass ratio of 1:1 was prepared after the pH was adjusted to 12. The mixture was further heated at 80°C for 15 min and cooled naturally to room temperature for 24 h. Then the pH of the xanthan gum/lysozyme nanoparticle solution was adjusted to 7 using 1 M HCl. Impurities were removed by the filtration using an 80 μm membrane, yielding a solution containing XG/Ly nanoparticles. Finally, XG/Ly powder was obtained by freeze-drying for 72 h.

The effect of pH solution on XG/Ly particle morphology: changing reaction solution pH at various including 8, 10, and 12. Reaction temperature and time were kept at 80°C and 15 min, respectively. Then, based on the maximum dextrose equivalent (DE_{max}) of samples, choose the most suitable treatment for the next experiments.

The effect of reaction temperature on XG/Ly particle morphology: The best pH result from the previous experiment was kept at different temperatures of 60, 80, and 100°C using a water bath (Mettler, Germany for 15 min). Then the most appropriate reaction temperature was selected based on the DE_{max} of samples.

The effect of reaction time on XG/Ly particle morphology: Similar to previous experiments, the pH solution and the reaction temperature were fixed. Reaction time was evaluated in a range of 15 - 45 min. Then the most appropriate reaction time was selected based on the DE_{max} of samples.

2.2.2. The determination of dextrose (DE, dextrose equivalent)

The reducing sugar equivalent DE of commercial RMD and maltodextrin (MD) was determined according to the Miller (1959) method with some modifications. The reducing sugar of the sample produces color with dinitrosalicylic acid (DNS) reagent. Based on the standard curve for glucose, the reduced sugar content in the analyzed sample is calculated.

Determination of DE: Take 25 mL sample, then add 15 mL of DNS and dilute to 50 mL with distilled water, cover, shake well, boil for 45 min, take out, cool to room temperature, and then measure color at wavelength 540 nm. The blank sample is 15 mL of DNS, titrated with distilled water to the 50 mL mark. The amount of reducing sugar in the sample is determined by the glucose standard curve at the ABS value measured at wavelength 540 nm after subtracting the ABS value of the blank sample.

DE (Dextrose equivalent) is determined by the formula:

$$DE = \frac{C \times V}{m \times 100}$$

C: reducing sugar concentration in the sample (mg/mL)

V: rated volume (50 mL)

m: mass of powder sample (mg)

2.2.3. Characterization of XG/Ly

The microstructure of XG particles was observed using a polarized light microscope (DM2500P, Leica, Germany) and FE-SEM (SU8000, Hitachi, Japan).

2.2.4. Preparation of pickering emulsion

The XG NP solutions (0.5%, w/w) and sunflower oil (10%, w/w) were dispersed into the aqueous phase. Oil-in-water (O/W) pickering emulsions (10% oil/water, w/w) were prepared by dispersing sunflower oil into XG NPs solutions using an ultrasound machine (Biologics - VT150, USA) with a frequency of 20 kHz for 1 min. The oil droplet sizes of the emulsions were determined using the Static Light Scattering (SLS) method (Mastersizer 3000, UK).

2.2.5. Statistical analysis

All experiments were conducted in triplication. One way, ANOVA was applied to evaluate the effect of reaction conditions on the formation of silver nanoparticles. Statistical analyses were conducted on the statistical software (Statgraphic, IBM, USA) with a 95% confidence interval.

3. Results and Discussion

3.1. Optimizing the synthesis of XG/Ly

For optimizing enzymatic reactions, pH is one of the most important factors. When changing the pH of the solution during the chain-cutting reaction, the DE equivalent of the product XG/Ly also changed and the difference between samples was significant ($P < 0.05$). Obtained results also revealed that the DE of samples XG/Ly showed an upward tendency to the increase pH solution (Figure 1a). Among the investigated pH (8, 10,

and 12), pH 12 showed the highest DE value and this parameter was applied for further tests.

Figure 1b depicted the effect of reaction temperature (60, 80, and 100°C) on the quality of XG/Ly particles at pH 12 with a reaction time of 15 mins. As hypothesized, the DE equivalent of products achieved the maximum value at the temperature of 60°C. No significant difference was recognized by ANOVA analysis between samples reacted at higher temperatures ($P > 0.05$). Because enzymes are very sensitive to thermal treatment, a too-high reaction temperature can lead to unwanted protein denaturation resulting in decreasing the enzyme activities.

The effect of reaction time (15, 30, and 45 min) on the hydrolysis reaction was summarized

in Figure 1c. The combination of obtained results of DE values and ANOVA analysis showed that the heating time had a very limited effect on the formulation of nano XG/Ly ($P > 0.05$). Because the maximum value was quickly recognized after 15 min, further prolonging the reaction was not the appropriate choice in considering heating costs.

In general, the optimization conditions for the xanthan gum hydrolysis reaction catalyzed by Lysozyme were the pH solution of 12 and the reaction temperature of 60°C for 15 min. Obtained nano XG/Ly particles achieved a DE value of 12,371 (two times higher than native XG granules).

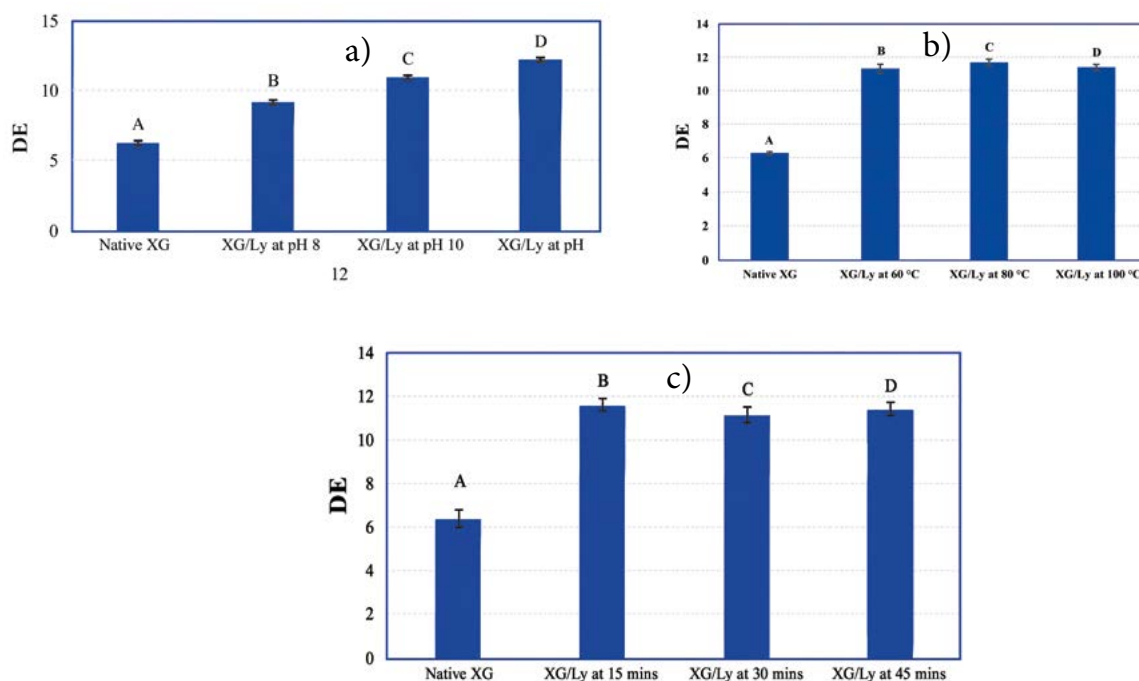


Figure 1. The dextrose equivalent of the xanthan gum/lysozyme (XG/Ly) sample when changing pH (a), temperature (b), and reaction time (c). Values within the different letters (A, B, C, and D) are significantly different ($P < 0.05$) between means of samples.

3.2. The results of XG/Ly

The morphology of XG before and after the enzymatic reaction was observed using polarized light microscopy and scanning electron microscopy and the images were depicted in Figures 2 and 3, respectively. Normally, the native XG particles are long polysaccharide fibers with a size of 100 - 500 μm and their morphology is quite

clumpy including the clusters of polysaccharides cluster together (Figure 2). However, their size was significantly reduced due to the hydrolysis process catalyzed by Lysozyme at 60°C and pH of 12 for 15 min. According to the FE-SEM image (Figure 3), XG particles after the reaction had a size of 200 - 500 nm and therefore they could be considered as solid nanoparticles.

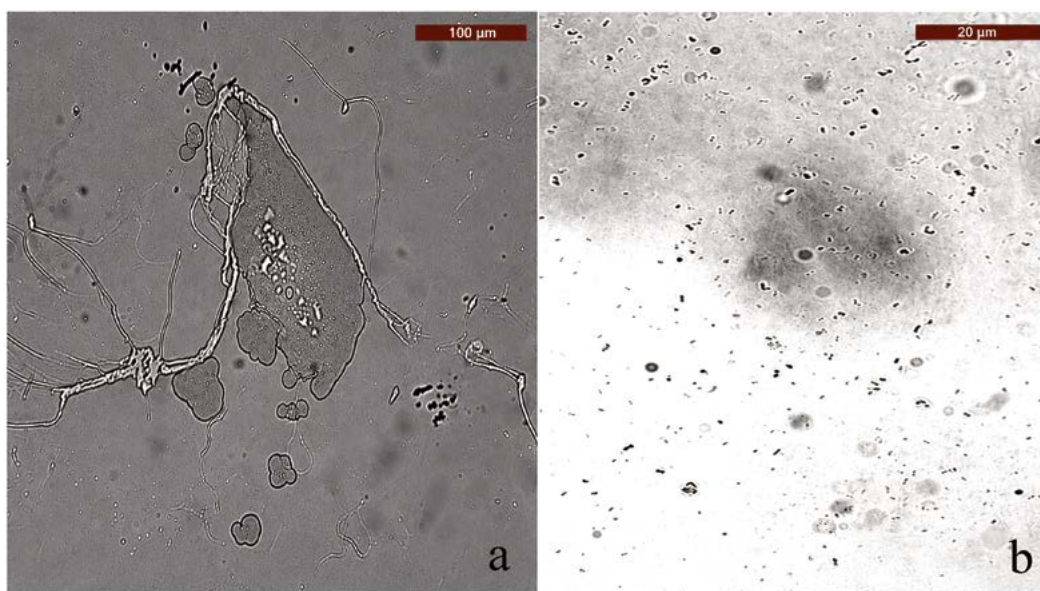


Figure 2. Image of native xanthan gum (a) and nano xanthan gum/lysozyme particles (b).

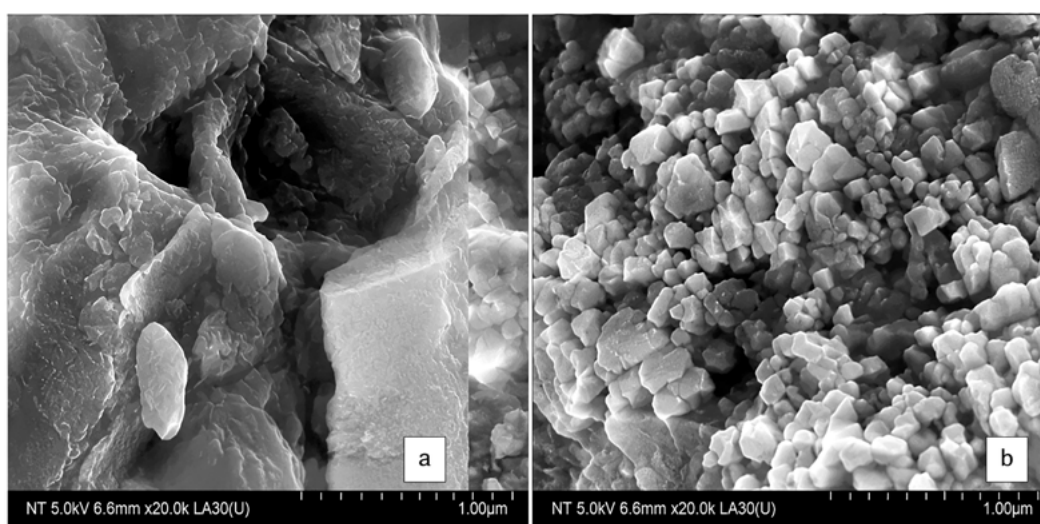


Figure 3. Field emission scanning electron microscopy image of native xanthan gum (a) and nano xanthan gum/lysozyme particles (b).

3.3 Pickering emulsions stabilizing by XG/Ly nanoparticles

The microstructure of pickering oil-in-water emulsion stabilized by XG/Ly nanoparticles was illustrated in Figure 4 while their droplet size distribution was displayed in Figure 5. Firstly, it can be seen that micro pickering emulsions with droplet sizes in a range of 10 - 100 μm successfully were produced. As expected, XG/Ly nanoparticles were located at the O/W interfaces,

forming a firm barrier protecting dispersed oil droplets. After 14 days of storage, these oil droplets tended to gather resulting in increasing the size of the emulsion particles. However, the dispersions still retained their color as well as the stable state, and no precipitation was observed (Figure 6). Therefore, it can be confirmed that the obtained XG/Ly particles after the hydrolysis reaction were small enough to form a highly stable pickering emulsion.

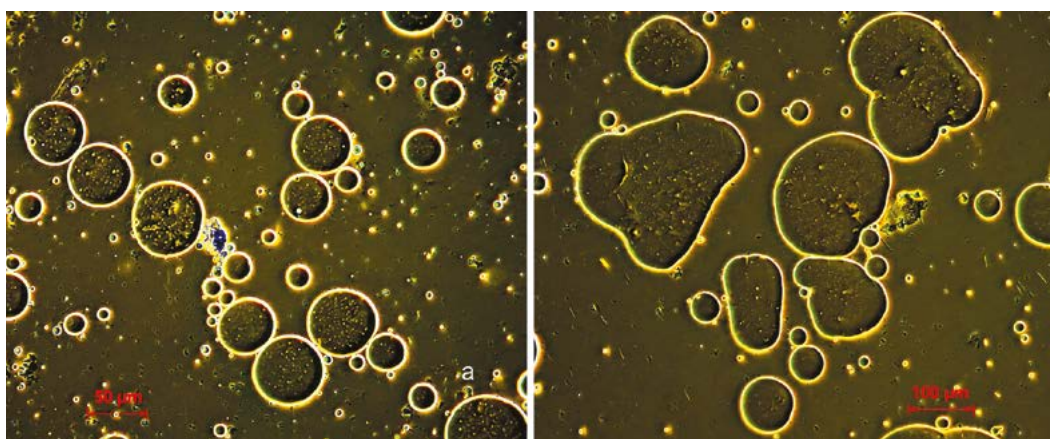


Figure 4. Light microscopic images of pickering emulsions with different concentrations of xanthan gum nanoparticles (a) after 1 day; (b) after 14 days.

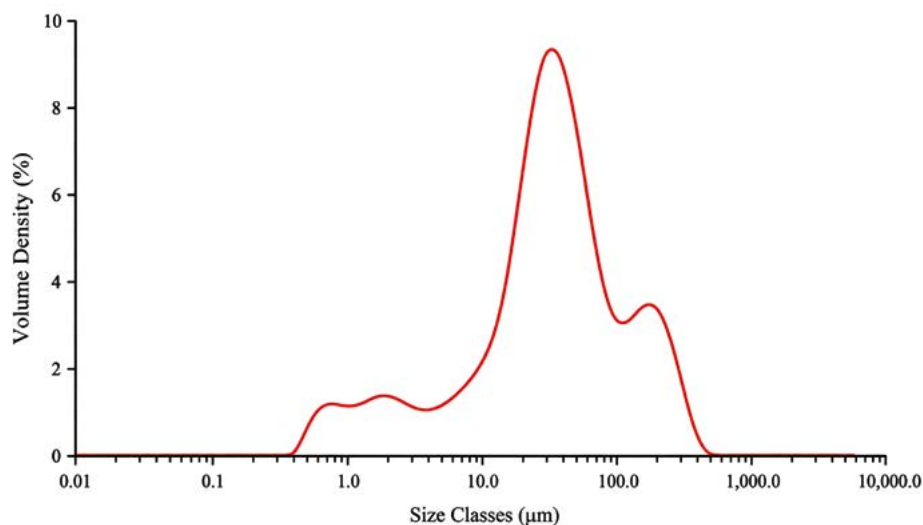


Figure 5. Droplet size distributions of xanthan gum nanoparticles pickering emulsions.

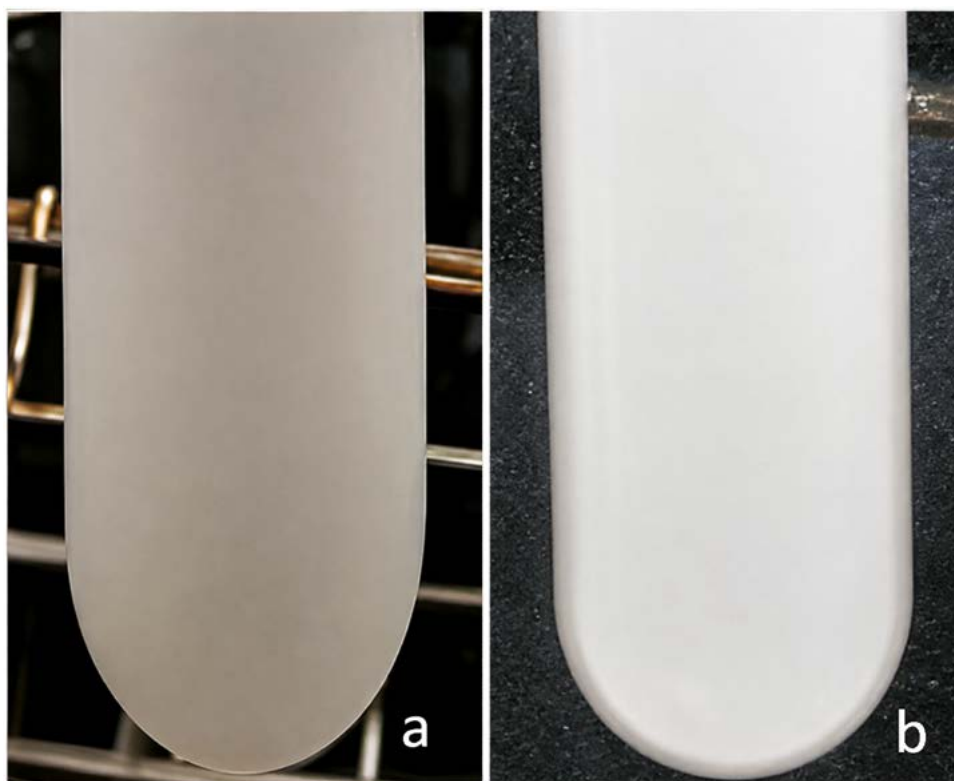


Figure 6. Image of pickering emulsion after 1 day (a) and after 14 days (b).

4. Conclusions

The study prepared XG/Ly NPs with sizes of 200 to 500 nm using the biological cutting method. The result shows that nanoparticles contributed to forming a barrier on the surface of oil resulting in improving their stability. Further research can focus on optimizing the XG circuit-cutting process and investigating factors affecting the pickering emulsification process.

Conflict of interest

The authors have no conflicts of interest to declare.

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A comprehensive review of oregano (*Origanum vulgare* L.) essential oil: Physicochemical properties, antimicrobial activities, and applications in food safety

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ABSTRACT

Oregano (*Origanum vulgare* L.) essential oil (OEO) has attracted growing attention as a natural antimicrobial agent for modern food safety applications. This interest is driven by rising consumer demand for clean-label preservatives and the need to control foodborne pathogens. The OEO exhibits broad-spectrum antibacterial activity, primarily due to its high content of phenolic monoterpenes such as carvacrol and thymol. These compounds disrupt microbial cell membranes and metabolic functions, leading to leakage of cellular contents and cell death, even at relatively low concentrations. The physicochemical properties of OEO, with strong aroma, hydrophobicity, and volatility, enhance its antimicrobial activity but also present challenges in formulation and food compatibility. The OEO has been successfully incorporated into various food preservation strategies, including edible films and coatings, antimicrobial packaging, and vapor-phase treatments, to inhibit microbial growth and extend shelf life. However, its practical application is limited by sensory impacts at effective concentrations and instability caused by evaporation or degradation. To overcome these challenges, recent innovations have focused on controlled-release delivery systems like nano-encapsulation, emulsions, and biodegradable polymers to improve stability and performance. This review summarizes the antimicrobial mechanisms of OEO, evaluates its applications across diverse food systems, and discusses current limitations, innovative formulation strategies, and future directions for its application to enhance food safety.

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1. Introduction

Foodborne microbial contamination remains a serious public health threat and a significant challenge for the food industry. Bacterial pathogens are responsible for the majority of foodborne illness outbreaks and cases (Olsen et al., 2000), leading to substantial health and economic consequences. While conventional chemical preservatives and chlorine-based sanitizers are used to reduce microbial loads, they may produce toxic by-products (e.g., chlorinated organics) and face consumer resistance due to concerns over synthetic additives. Therefore, interest in natural antimicrobials from herbs and spices has increased, offering safer and more acceptable alternatives to enhance food safety (Oussalah et al., 2007). Essential oils, the aromatic and volatile plant extracts, have gained attention for their strong antimicrobial properties and potential use as food preservatives. Among them, OEO is widely studied for its effectiveness against foodborne pathogens.

Oregano (*Origanum vulgare* L.), from the mint family (Lamiaceae), produces essential oil rich in phenolic terpenoids (Leyva-López et al., 2017). The OEO typically contains high levels of carvacrol (60 - 80%) along with thymol, p-cymene, and γ -terpinene (Adebayo et al., 2013; Cattelan et al., 2018). Carvacrol is the major active component and is classified by the U.S. FDA as Generally Recognized as Safe (GRAS) (Fraj et al., 2019). Thymol, a structural isomer of carvacrol, also contributes to the oil's antimicrobial and antioxidant effects. The main antimicrobial mechanisms involve membrane disruption, leakage of cell contents, and enzymatic inhibition (Kolypetri et al., 2023). Numerous studies have confirmed OEO's antibacterial effects (Kim et al., 1995; Oussalah et al., 2007) and demonstrated

its effectiveness in vapor-phase systems (Chang et al., 2017; Kwon et al., 2017). It also inhibits fungi like *Aspergillus*, *Botrytis*, *Penicillium*, and *Z. bailii* (Hernández et al., 2023). However, OEO's volatility, poor water solubility, and strong aroma limit its direct use in foods (Santos et al., 2022). Encapsulation and delivery technologies have been investigated to overcome these issues (Liu et al., 2019; Hernández et al., 2023; Sirati et al., 2024), thereby enhancing OEO's stability, release control, and overall functionality in food preservation. This review distinguishes itself from prior works on oregano essential oil (OEO) by providing a comprehensive synthesis of its physicochemical properties, antimicrobial mechanisms, and practical applications in food safety, with a particular emphasis on recent advancements in delivery systems and their implications for overcoming formulation challenges. While previous reviews have explored OEO's phytochemistry, biological activities, and antimicrobial properties, they often focus on specific aspects, such as chemical composition or in vitro efficacy, with limited integration of practical food safety applications. This manuscript uniquely bridges these domains by critically evaluating OEO's role in diverse food systems, meat, seafood, produce, and active packaging, while addressing current limitations like sensory impact and matrix interactions. Furthermore, it highlights cutting-edge innovations, such as nanoemulsions, biopolymeric nanoparticles, and smart release systems, which enhance OEO's stability and efficacy, offering insights into scalable solutions for clean-label preservation. By incorporating recent studies and providing a holistic perspective on OEO's potential, this review serves as a timely resource for researchers and industry professionals aiming to advance natural antimicrobial strategies in food safety.

2. Physicochemical Properties of Oregano Essential Oil

OEO is a volatile, aromatic, and hydrophobic oil rich in monoterpenoid compounds, particularly phenolic monoterpenes like carvacrol and thymol (Fraj et al., 2019; Santos et al., 2022). Carvacrol is typically the dominant component, comprising 50 - 70% of the oil, while thymol is the second major phenolic (Noshirvani et al., 2017; Radünz et al., 2021). In *O. vulgare*, a composition with 68.7% carvacrol has been reported, along with 12.5% p-cymene, 4.7% γ -terpinene, and minor compounds such as trans-caryophyllene (5%) and β -myrcene (de Almeida et al., 2023). These components contribute to OEO's strong antimicrobial and antioxidant properties. However, other oregano chemotypes may be thymol-dominant, depending on genetics, origin, and environmental factors.

A commercial oil containing 67% carvacrol confirmed this variability (Božik et al., 2017). Due to its volatility and hydrophobicity, OEO requires careful handling to preserve bioactivity. Although it can impart a slight tint to transparent films or gels, its color effect is minimal at food-safe concentrations ($\leq 1\%$) (Pan et al., 2024). Table 1 highlights how OEO's potent natural activity also presents formulation challenges, as even small changes in the carvacrol-to-thymol ratio can influence efficacy, stability, and sensory impact. The discrepancy between the broader 20 - 75% carvacrol range in Table 1 and the 60 - 80% range in some introductions stems from this variability, where the wider range accounts for diverse oregano sources (e.g., wild vs. cultivated, different species like *Poliomintha longiflora*), while the narrower range reflects standardized, high-carvacrol chemotypes used in commercial or therapeutic applications.

Table 1. Physicochemical parameters of oregano essential oil¹

Parameter	Value/Description
Density (20°C)	0.90 - 0.95 g/mL
Color	Colorless to light yellow or brown, depending on fractionation
Odor	Strong, pungent, herbaceous; softer in terpenic fractions
Refractive index (20°C)	1.49 - 1.50
Major constituents	Carvacrol (20 - 75%), thymol (0 - 20%), p-cymene (5 - 15%), γ -terpinene (5 - 30%), β -caryophyllene ($\leq 5\%$)
Boiling points	Carvacrol: 236°C; thymol: 232°C; p-cymene: 177°C; γ -terpinene: 183°C
Solubility in water	Practically insoluble; hydrophobic
Appearance & aroma	Clear pale yellow to amber oil; strong herbaceous, spicy aroma
Stability	Good thermal stability up to 230°C; antioxidant-rich, but prone to volatilization

¹Sources: Kim et al. (1995); Seydim & Sarikus (2006); Liu et al. (2019); Mora-Zúñiga et al. (2022).

3. Antimicrobial Mechanisms of Oregano Essential Oil

The OEO exhibits a multifaceted mode of action against microorganisms, targeting both structural components and physiological processes of cells. Its broad-spectrum efficacy is largely attributed to the presence of phenolic terpenoids (especially carvacrol and thymol) which can penetrate microbial cell envelopes and disrupt vital functions.

3.1. Disruption of microbial cell membrane integrity

The hydrophobic components of OEO can integrate into bacterial lipid membranes, increasing membrane fluidity and permeability, which disrupts integrity and causes leakage of vital intracellular contents (Santos et al., 2022). Exposure to OEO or carvacrol leads to the release of ions, ATP, and nucleic acids, collapsing the proton motive force and resulting in cell death. Carvacrol disrupts the outer membrane of Gram-negative bacteria by releasing lipopolysaccharides and increasing cytoplasmic membrane permeability (Burt & Reinders, 2003; Seydim & Sarikus, 2006). In vitro studies have confirmed carvacrol's potent membrane-disruptive effects, including cell lysis and leakage of cellular material (Kim et al., 1995). These effects are largely due to interference with the phospholipid bilayer by compounds like carvacrol and thymol. A mechanistic study by Oussalah et al. (2007) showed that Spanish oregano oil severely damaged the membranes of *E. coli* O157:H7 and *Listeria monocytogenes*, supporting membrane disruption as the main bactericidal mechanism (Oussalah et al., 2007). Generally, Gram-positive bacteria are more susceptible to OEO than Gram-negatives, due to the latter's outer membrane acting as a barrier

(Oussalah et al., 2007; Delcours, 2009). However, OEO at sufficient concentrations can still permeabilize Gram-negative cells (Seydim & Sarikus, 2006). For example, *E. coli* O157:H7 and *Salmonella typhimurium* showed higher MICs than *Staphylococcus aureus* (Oussalah et al., 2007). In tuna applications, OEO significantly reduced bacterial growth and biogenic amine formation, confirming its preservative potential in seafood products (Wang et al., 2022).

3.2. Interference with cellular metabolism and homeostasis

Beyond damaging microbial membranes, OEO also disrupts metabolism and cellular homeostasis. Once membrane integrity is lost, OEO can dissipate the proton gradient and membrane potential, reducing ATP synthesis and triggering an energy collapse. Even at sub-lethal concentrations, OEO components may penetrate cells and interfere with intracellular targets. These constituents inhibit enzymes involved in energy production, such as those in the TCA cycle and ATP synthesis, and affect macromolecule biosynthesis (Kim et al., 1995). This enzymatic disruption leads to toxic intermediate accumulation or loss of vital metabolites. Oregano phenolics also impair respiratory chain enzymes and those involved in cell wall synthesis, reducing growth and viability (Kim et al., 1995). OEO further disturbs pH balance and ion homeostasis, causing cytoplasmic acidification and protein dysfunction. Cells exposed to carvacrol often alter membrane fatty acid composition, indicating stress responses and homeostatic disruption (Santos et al., 2022). Some OEO components may even damage DNA or interfere with nucleic acid synthesis (Kim et al., 1995). As shown in Table 2, OEO exerts broad antimicrobial effects on Enterobacteriaceae.

Its multi-targeted actions, ATP depletion, biosynthesis inhibition, oxidative stress, make resistance development difficult (Bharadwaj et al., 2022) and explain its continued efficacy against antibiotic-resistant strains (Selim, 2011). OEO's efficacy stems from its ability to disrupt microbial cell membranes, increasing permeability and causing leakage of cellular contents, with carvacrol's hydrophobic nature enabling deep penetration into lipid bilayers, leading to proton motive force collapse and cell death. Thyme oil, rich in thymol, shares similar membrane-disruptive properties but is slightly less effective against Gram-negative bacteria due to lower carvacrol levels, while cinnamon oil, driven by cinnamaldehyde, targets microbial enzymes and cell wall synthesis, offering consistent inhibition

but less pronounced membrane disruption than OEO, and clove oil, with eugenol as its primary component, disrupts membrane integrity and inhibits energy metabolism but shows moderate activity, less potent than OEO's broad-spectrum effects (Peñalver et al., 2005; Oussalah et al., 2007). OEO's additional mechanisms, such as quorum sensing inhibition and biofilm disruption, particularly against *Pseudomonas* and *Listeria*, further enhance its superiority, as seen in niosome-encapsulated formulations that amplify anti-biofilm effects by 2 - 4 times (Sirati et al., 2024). These comparative insights highlight OEO's multifaceted antimicrobial action, making it a leading candidate for food safety applications compared to other essential oils.

Table 2. Antimicrobial activity of essential oils against *Enterobacteriaceae*

Essential oil	Most effective against	MIC range (μL/mL)	Antimicrobial effectiveness
Oregano	<i>E. coli</i> , <i>Salmonella</i> spp., <i>Proteus</i> spp.	0.25 - 1.0	Most potent; carvacrol-rich; broad-spectrum inhibition
Thyme	<i>E. coli</i> , <i>Enterobacter</i> spp.	0.5 - 1.0	Strong antimicrobial; high thymol content
Cinnamon	<i>Klebsiella</i> spp., <i>Salmonella</i> spp.	0.5 - 1.5	Effective; consistent inhibition
Clove	<i>E. coli</i> , <i>Proteus</i> spp.	1.0 - 2.0	Moderate activity; eugenol as main compound
Rosemary	Limited strains	2.0 - 4.0	Least effective; low activity against most strains

Source: Peñalver et al. (2005). MIC: minimum inhibitory concentration.

3.3. Inhibition of quorum sensing and biofilm formation

A key factor in microbial persistence in food environments is biofilm formation and quorum sensing (QS) communication. Oregano essential

oil has shown strong anti-biofilm activity, partly due to its ability to disrupt QS signaling and inhibit biofilm development. It interferes with the expression of genes involved in bacterial adhesion and exopolysaccharide production.

For example, a niosome-encapsulated OEO formulation enhanced anti-biofilm efficacy by 2 - 4 times and downregulated key biofilm-related genes (Sirati et al., 2024). OEO likely blocks QS receptors or inhibits the synthesis of QS molecules. In practice, OEO vapors reduced bacterial contamination on food-contact surfaces, particularly polypropylene, with decreasing effectiveness on stainless steel and glass (Pinto et al., 2024). Sirati et al. (2024) showed that niosome-delivered OEO not only prevented biofilm formation but also reduced existing biomass, confirming its role in QS

disruption (Sirati et al., 2024). OEO’s membrane-disrupting action may also facilitate antimicrobial penetration into biofilms. Additionally, its phenolics may oxidize or inactivate signaling molecules. Though the exact quorum-quenching mechanisms are still under study, OEO has been effective against *Pseudomonas* spp. and *Listeria* (Selim, 2011; Sirati et al., 2024). Biopolymeric nanoparticles loaded with OEO demonstrated strong antimicrobial activity, supporting its promise in food preservation and medical applications (Table 3) (Romero-Montero et al., 2024).

Table 3. Essential oil-loaded biopolymeric nanoparticles for microbial control

Nanoparticle type	Essential oil	Target pathogens/ biofilms	Results
Chitosan-based NPs	Oregano, Cinnamon	Tea, <i>E. coli</i> , <i>S. aureus</i> , <i>L. monocytogenes</i>	Strong antimicrobial and anti-biofilm activity; biodegradable and mucoadhesive
Alginate-based NPs	Thyme, Clove	<i>Pseudomonas</i> , <i>Candida</i> spp.	Controlled release; enhanced stability; effective against mixed-species biofilms
Lipid-based NPs (Nanoliposomes, Niosomes)	Oregano, Citral	<i>L. monocytogenes</i> , <i>P. aeruginosa</i>	Improved membrane interaction and delivery; reduced toxicity
Hybrid polymeric NPs	Various essential oil combinations	Multidrug-resistant strains	Synergistic antimicrobial effects; potential for food and biomedical use

Source: Romero-Montero et al. (2024).

Importantly, the anti-biofilm property of OEO enhanced food safety by targeting the modes of growth that often protect pathogens from cleaning processes and preservatives. By preventing biofilm formation, OEO makes microbial contaminants more susceptible to removal and inactivation in food processing environments.

4. Applications of Oregano Essential Oil in Food Safety

Oregano essential oil’s broad-spectrum antimicrobial activity has spurred extensive research into its use as a natural preservative in foods. Researchers have evaluated OEO in a variety of food systems and preservation

strategies, including direct incorporation into foods, surface treatments, and active packaging. It has demonstrated effective inhibition of many foodborne microbes, but its practical application must balance microbial efficacy with sensory and regulatory considerations. This section reviews the evidence on OEO's antimicrobial performance in food-relevant contexts, organized by application area.

4.1. Antimicrobial efficacy in model systems and *in vitro* studies

Initial studies demonstrated OEO's strong antimicrobial activity through *in vitro* assays. In a screening of 28 plant essential oils against *E. coli* O157:H7, *Salmonella* typhimurium, *Staphylococcus aureus*, and *Listeria monocytogenes*, oregano oil from *O. heracleoticum* and *O. compactum* ranked the most effective, with MICs $\leq 0.05\%$ (v/v) for all four pathogens (Oussalah et al., 2007). Carvacrol, OEO's main component, showed potent bactericidal effects against *E. coli*, *Salmonella*, *Listeria*, *Vibrio*, and more, outperforming other terpenoids (Kim et al., 1995). It completely killed *Salmonella* and *Vibrio* at 250 $\mu\text{g/mL}$, while other compounds like linalool were far less effective. Both carvacrol and thymol have been shown to inhibit numerous foodborne pathogens, including *B. cereus*, *S. aureus*, *Shigella*, and *V. cholerae* (Adebayo et al., 2013). The action is attributed to membrane disruption and proton gradient collapse. In lab media, even 0.25 - 0.5 mg/mL of OEO compounds can fully eliminate *E. coli* and *S. Typhimurium* (Kim et al., 1995). Though food matrices may reduce efficacy, baseline tests confirm OEO's exceptional antimicrobial potential, often rivaling thyme, clove, and cinnamon oils (Oussalah et al., 2007; Gutierrez et al., 2009).

4.2. Use of oregano essential oil in meat and poultry products

Meat and poultry products are highly prone to spoilage and pathogen contamination, making them ideal targets for oregano essential oil (OEO) as a clean-label antimicrobial. Direct application, such as marination or incorporation into ground meat, has shown promising results. For example, OEO (0.5 - 1.0%) significantly inhibited *E. coli* O157:H7 and Vancomycin-Resistant Enterococcus (VRE) in minced beef stored at 7°C over 14 days, reducing microbial loads compared to controls (Selim, 2011). Similarly, feta cheese treated with OEO showed reduced VRE and *E. coli* levels during storage. In trout fillets, a combination of 0.2% OEO, salt, and vacuum packaging extended shelf life by inhibiting spoilage bacteria like *Photobacterium phosphoreum* (Frangos et al., 2010). On meat surfaces, OEO effectively targets pathogens such as *Listeria monocytogenes* and *Salmonella* (Oussalah et al., 2007). While direct brushing or tumbling with OEO can reduce spoilage, strong flavors may limit doses (Santos et al., 2022). In minced pork, OEO at 0.02 - 0.04% (v/v) reduced *L. monocytogenes* by up to 2.5 log CFU/g (Guo et al., 2024). Encapsulated OEO in coatings or films, like chitosan-oregano wraps on chicken breast, suppressed *Campylobacter* and *Salmonella* during chilled storage (Fraj et al., 2019; Shen et al., 2022). These studies highlight OEO's versatility in meat preservation strategies.

4.3. Applications in seafood and fish products

Frangos et al. (2010) demonstrated that OEO could extend the shelf life of fresh fish, especially when combined with other preservation methods (Frangos et al., 2010). A recent study introduced niosome-encapsulated OEO, enhancing its antimicrobial and anti-biofilm activity against

fish-borne pathogens like *Aeromonas* and *Pseudomonas* spp., with a 2 - 4-fold increase in efficacy (Sirati et al., 2024). These 219 nm vesicles improved OEO's stability, dispersion, and release in aquatic environments. The formulation was also shown to be biocompatible and non-toxic to mammalian cells. Additionally, OEO effectively inhibited *Photobacterium phosphoreum* in fish broth and cold-smoked salmon when applied as a wash or dip (Mejlholm & Dalgaard, 2002).

4.4. Oregano oil in fruits, vegetables, and minimally processed foods

essential oil has gained attention as a natural antimicrobial for fresh produce, particularly minimally processed fruits and vegetables that often harbor *E. coli* O157:H7, *Salmonella*, and *Listeria* (Santos et al., 2022). Application methods include dipping, spraying, or incorporating OEO into edible coatings. In one study, iceberg lettuce dipped in oregano oil (alone or with rosemary oil) showed significant reductions in *L. monocytogenes*, *Y. enterocolitica*, and *A. hydrophila* (Xylia et al., 2021), while oregano-thyme mixtures also suppressed *E. coli* and spoilage flora on lamb's lettuce (Sirati et al., 2024). Combined with organic acids, OEO reduced *Salmonella* on tomatoes without compromising quality. Nanoemulsion washes and modified atmosphere packaging (MAP) with oregano oil and citral helped preserve lettuce and reduce pathogens like *E. coli* and *S. Typhimurium* (Hao et al., 2021). Hao et al. (2021) reported that 3 µL/L OEO vapor in modified atmosphere packaging decreased microbial growth and maintained lettuce quality for 10 days at 5°C (Hao et al., 2021). OEO also disrupts bacterial

biofilms, and its vapor form, released via sachets or coated films, offers sustained antimicrobial action with minimal sensory changes (Cho et al., 2020). This approach has been applied to cherry tomatoes and is suitable for other produce like salads and sprouts (Kwon et al., 2017). Effective concentrations in produce washes range from 0.1 - 0.5% (Frangos et al., 2010).

4.5. Active packaging and edible coating incorporating oregano oil

Oregano essential oil is promising for active food packaging, edible films, coatings, and encapsulation. Embedding 1 - 4% OEO into whey protein isolate films, Seydim & Sarikus (2006) found that ≥ 2% OEO fully inhibited *E. coli* O157:H7, *S. aureus*, *Salmonella enteritidis*, and *L. monocytogenes* in agar assays (Seydim & Sarikus, 2006). Compared to garlic or rosemary oil, the 2% oregano-oil film showed superior inhibition zones. A soybean polysaccharide film with OEO in a Pickering emulsion improved oil retention and extended antimicrobial activity against *E. coli* and *Staphylococcus* (Liu et al., 2019). Another film based on cassava starch-chitosan with OEO and citric acid blocked *Zygosaccharomyces bailii* yeast growth (Hernández et al., 2023). Shu et al. (2024) showed that OEO vapor prolonged kai lan shelf life under ambient and cold conditions (Table 4) (Shu et al., 2025). Guzmán-Pincheira et al. (2025) used alginate-chitosan films with OEO and observed enhanced activity against *L. monocytogenes* and *S. aureus*, with film thickness ranging from 37.7 - 38.2 µm (Guzmán-Pincheira et al., 2025). These findings support OEO-based packaging as an effective strategy for microbial control in vegetables.

Table 4. Effect of oregano essential oil vapor on kai lan shelf-life

Treatment condition	Storage temperature	Shelf-life extension	Results
OEO vapor (active packaging)	22°C (ambient)	Extended from 3 to 7 days	Reduced microbial load; maintained visual quality and texture
OEO vapor (active packaging)	4°C (refrigerated)	Extended from 5 to 14 days	Delayed yellowing and spoilage; suppressed microbial growth
Control (no OEO)	Both conditions	3 days (ambient), 5 days (cold)	Faster spoilage, discoloration, and higher microbial counts

Source: Shu et al. (2025).

Beyond edible films, OEO has been incorporated into synthetic packaging like LDPE and PVA. Active films with OEO microcapsules in a soybean protein isolate/cellulose matrix significantly inhibited *E. coli* and *S. aureus* (Wang et al., 2022). Kwon et al. (2017) embedded OEO into PVA films for passive vapor release and found that storing cherry tomatoes with 2 - 3% OEO films for 7 days at 4 and 22°C suppressed *Salmonella*, molds, and mesophilic bacteria without affecting quality. Encapsulated OEO in polycaprolactone nanocapsules retained carvacrol for 60 days (Fraj et al., 2019). Fan et al. (2024) applied OEO-loaded zein-pectin-chitosan nanoparticles to sausage and saw reduced lipid oxidation and bacterial growth over 20 days (Fan et al., 2024). Halloysite nanotubes exhibited better OEO delivery than diatomaceous earth, showing stronger antibacterial and antioxidant activity (Oun et al., 2022). Nguyen et al. (2025) found that OEO, cinnamon oil blends (ratios 8:2 and 9:1) had the lowest MICs against *S. aureus*, *B. cereus*, and *A. flavus*, while a 6:4 blend ratio was most effective against *A. flavus*, and cassava starch films with the blend fully inhibited fungal

growth in Vietnamese bread for at least 5 days. These applications confirm OEO as a powerful, natural solution for active packaging.

5. Safety, Regulatory Status, and Sensory Considerations

5.1. Toxicological assessment of oregano essential oil

Oregano essential oil, along with its key phenolic compounds carvacrol and thymol, is known for strong antimicrobial and antioxidant properties. Classified as GRAS by the U.S. FDA (Kim et al., 1995), OEO is widely used in food systems with low toxicity concerns at typical flavoring levels. Mansoori et al. (2019) found that carvacrol caused no significant harm even at high doses in animal models (Mansoori et al., 2019), and Santos et al. (2022) confirmed that oregano oil is well tolerated in minimally processed foods (Santos et al., 2022). Sirati et al. (2024) showed that OEO encapsulated in niosomes had no cytotoxicity in HFF cells at concentrations higher than food-use levels (Sirati et al., 2024). Edible films with OEO maintained

safety across various temperatures and humidity conditions (Hernández et al., 2023). OEO also showed strong anti-leishmanial effects *in vitro* and *in vivo*, reducing parasite loads without host toxicity (Tomiotto-Pellissier et al., 2022). Overall, oregano oil is considered safe when used in controlled-release systems at effective antimicrobial doses.

5.2. Sensory impact

A key challenge in using OEO in foods is its strong aroma and flavor, dominated by thymol and carvacrol, which can overpower food even at low levels. Frangos et al. (2010) found that trout fillets treated with 0.2% OEO remained acceptable to sensory panels, but at 0.4%, the aroma became too intense (Frangos et al., 2010). Mejlholm & Dalgaard (2002) also noted that effective EO concentrations for shelf-life extension often produce unwanted flavors (Mejlholm & Dalgaard, 2002). In meats and cheeses, 0.5 - 1% OEO can suppress bacteria, but often introduces strong herbal notes. Selim (2011) reported that while oregano-supplemented beef and feta had microbial benefits, sensory monitoring was essential. Cultural preferences and product types of influence acceptability, what works in sausages may fail in juice or dairy. In fresh-cut fruits, even effective antimicrobial doses may harm sensory appeal. Studies aim to find the highest OEO dose that reduces microbes without compromising taste (Jackson-Davis et al., 2023). Strategies like encapsulation, synergistic hurdles, or flavor masking help minimize OEO's sensory impact (Santos et al., 2022).

6. Challenges and Future Perspectives

6.1. Current limitations and challenges

Although OEO has proven antimicrobial

efficacy, several limitations hinder its full-scale use in food preservation. Sensory impact is a primary concern, concentrations effective against pathogens may exceed flavor acceptability thresholds. Frangos et al. (2010) showed that concentrations above 0.4% led to undesirable sensory effects. Furthermore, OEO's efficacy drops in complex matrices where fats and proteins bind hydrophobic compounds like carvacrol, reducing microbial contact. An OEO level that is bactericidal in lab tests may be ineffective in fatty foods. Another issue is batch variability, OEO's carvacrol and thymol levels can differ depending on subspecies and harvest time, affecting potency. Its volatility also limits stability, as heat, light, or oxygen can degrade active components. Protective delivery methods, such as encapsulation, are thus needed to preserve efficacy. Microbial variability also matters: Gram-negative bacteria may resist OEO, and sub-lethal exposure might trigger tolerance. Economically, OEO is costlier than synthetic preservatives, and large-scale use must balance effectiveness and price. Regulatory uncertainty around using OEO above flavoring levels further complicates adoption. Overall, addressing sensory, matrix, stability, and regulatory challenges is key to its broader application.

6.2. Future perspectives

Ongoing research is addressing the limitations of OEO to unlock its full potential in food safety. A major strategy involves advanced delivery systems like nanoemulsions, biopolymer nanocapsules, and liposomes/niosomes (Pontes-Quero et al., 2021; Sirati et al., 2024), which help stabilize OEO and control its release. Encapsulation protects volatile compounds during processing and allows gradual, sustained antimicrobial action without overwhelming

flavor. Smart release systems, such as pH-sensitive or heat-triggered carriers, can deploy OEO where microbial threats are greatest. Edible films and coatings using Pickering emulsions or microgel carriers are being developed to extend activity while reducing sensory impact (Liu et al., 2019). Synergistic preservation is another avenue: combining OEO with modified atmosphere packaging, high pressure, ultrasound, or UV light allows reduced OEO doses while maintaining microbial control. OEO’s antioxidant benefits further support food stability in modified atmosphere packaging. Pairing OEO with other natural antimicrobials like cinnamon oil, nisin, or thyme oil has shown promising synergy; for example, thyme oil plus oregano oil significantly lowered MICs (de Almeida et al., 2023). Such blends could enhance efficacy at sub-threshold flavor levels. Mechanistic insights, such as how carvacrol disrupts bacterial membranes, are driving innovations in delivery design. OEO’s antifungal and antiviral potential is also under study. Notably, it exhibited strong antifungal action against *Rhizoctonia solani* with an EC50 of 22.89 µg/mL, suggesting broader agricultural use beyond food safety (Wu et al., 2023) (Table 5).

Table 5. Antifungal effects of oregano essential oil against *Rhizoctonia solani*

Aspect	Results	Significance
Antifungal potency	22.89 µg/mL (stronger than carvacrol alone)	Demonstrates high efficacy of whole OEO
Cell morphology	Hyphae deformed, collapsed after OEO treatment	Indicates membrane disruption
Ultrastructure	Plasmolysis and organelle degradation	Confirms internal cell damage
Transcriptomics	887 upregulated, 1138 downregulated	Suggests stress and metabolic disruption
Oxidative stress	Increased ROS and lipid peroxidation	Leads to membrane damage
Membrane permeability	Higher leakage of nucleic acids & proteins	Confirms loss of membrane integrity
Hydrogen peroxide	Dose-dependent increase	Triggers oxidative damage
Antioxidant enzyme activity	Increased 2.3x after OEO treatment	Cell defense mechanism activated
Sclerotia formation	Completely inhibited at 50 µg/mL	Prevents survival structures of <i>R. solani</i>

Source: Wu et al. (2023).

The future for oregano essential oil in food safety lies in innovation at multiple levels: technological (encapsulation and packaging), combinatorial (synergies with other methods), and perceptual (managing sensory and consumer expectations). With these advancements, OEO's robust antimicrobial power can be more fully realized in keeping foods safe naturally. Oregano and other essential oils have steadily been moving from experimental studies into practical, effective tools for the food industry, such as incorporating OEO with gelatin-based edible films to inhibit effectively pathogens (Wu et al., 2023). As these hurdles are addressed, oregano essential oil is poised to play an increasingly important role in the future of food preservation as a sustainable, consumer-friendly solution.

7. Conclusions

Oregano essential oil offers a promising natural substitute for synthetic preservatives in food processing, drawing on its distinctive physicochemical attributes, volatile, hydrophobic, and abundant in phenolic monoterpenes such as carvacrol and thymol, to deliver robust antimicrobial and antioxidant effects. Numerous *in vitro* and *in situ* investigations have confirmed OEO's broad-spectrum activity against foodborne pathogens and spoilage agents, including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and fungi like *Zygosaccharomyces bailii* and *Aspergillus* species. This versatility has enabled its successful integration into various food matrices, from meat and poultry to dairy, fresh produce, seafood, and baked goods. To overcome inherent drawbacks such as volatility, instability, and intense sensory profiles, researchers have turned to innovative delivery systems like edible films, nanoencapsulation, and vapor-phase

packaging, which facilitate controlled release and prolonged antimicrobial performance while preserving the sensory and nutritional integrity of foods. Nevertheless, hurdles persist, including sensory acceptability in certain products, interactions with complex food matrices, unclear regulatory guidelines for preservative dosages, and the need for consistent oil composition across batches. Looking ahead, investigations may prioritize synergistic combinations with other preservation techniques, assessments of consumer preferences, and scalable encapsulation approaches that balance efficacy with flavor masking. Achieving regulatory alignment and fostering public confidence will prove essential for broader adoption. In essence, OEO stands as a multifaceted, clean-label option for contemporary food preservation, with collaborative efforts across disciplines poised to realize its broader promise.

Conflict of interest

The authors declare that there is no conflict of interest.

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Optimizing phenolic extraction and evaluating health benefits of *Morinda citrifolia* L. fruit

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ABSTRACT

The primary objective of this investigation was to determine the optimal conditions for extracting phenolics from *Morinda citrifolia* fruit, while also examining the trace element content and biological properties of the extract. The study scrutinised the impact of various extraction conditions, specifically, ethanol concentration, extraction duration, and temperature, on the total phenolic content within *Morinda citrifolia* fruit. Utilising the single-factor and response surface methods (RSM), the optimal extraction conditions were predicted to be a 60% ethanol concentration, an extraction duration of 5.4 h, and a temperature of 72°C. These conditions yielded the highest phenolic content at 7.38 mg gallic acid equivalent (GAE)/g dried weight (dw). Additionally, the fruit contained 106 and 14 mg/kg dw of zinc and copper, respectively. The extract demonstrated robust DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity with an IC₅₀ value of 137 µg/mL. Moreover, it demonstrated efficacy against various bacteria, including Gram-positive strains such as *Bacillus subtilis* and *Bacillus cereus*, as well as Gram-negative strains like *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*.

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1. Introduction

Morinda citrifolia L., widely known as Noni, belongs to Rubiaceae family and widely grows in many tropical and sub-tropical regions from Southeast Asia to Australia (Supavadee et al., 2020). *M. citrifolia* fruit has many health benefits,

due to the rich in phytochemicals, particularly phenolic compounds, and minerals such as zinc, copper (Basar & Westendorf, 2012; Bramorski, 2020). Phenolic compounds are the second most abundant group of organic compounds in the plant kingdom, after cellulose. They have attracted

significant attention due to their association with a reduced risk of chronic degenerative diseases, including cancer, diabetes, Alzheimer's disease, and cardiovascular conditions (Nardini, 2022). Moreover, zinc and copper function as cofactors for enzymes. Notably, zinc plays a crucial role in the synthesis and action of insulin. The literature reports showed that insulin deficiency makes it impossible for cells to use glucose as an energy source, resulting in high-glucose concentration in the bloodstream and leading to a condition known as hyperglycemia (Barman & Srinivasan, 2022).

Morinda citrifolia products developed from leaves and fruits are being commercialised as capsules, teas, and juices, with the fruit juice being the principal form. Juices may be pasteurised or fermented to produce a probiotic drink (Wang et al., 2009). In current reports, dried *M. citrifolia* fruit powder has been used as a kind of tea with health-promoting properties, including anti-diabetic effect, anti-oxidation, anti-bacterial, anti-ageing effect and other biological activities (Manavalan et al., 2021).

The extraction of phenolic compounds from plant materials is one of the most crucial steps in their utilisation for food, pharmaceutical, and cosmetic applications. Several factors influence extraction, including solvent type and concentration, solid-to-solvent ratio, extraction time, and temperature (Anuar et al., 2013). Among the above-mentioned factors, the solvent is the most important due to its dependence on the polarity of the target compound. Moreover, optimization of the extraction process for phenolic compounds should be conducted in combination with multiple factors (Pereira et al., 2018; Demircan et al., 2022). Optimization by the single-factor method is conventional

but ineffective in providing information on the relationship between extraction factors (Zhang et al., 2007). However, this method could offer essential information for the significant influence regions of factors. Response surface methodology provides a more accurate assessment in the optimization of the extraction factors, which can allow for assessing the effects of all the factors (Pérez-Jiménez et al., 2008; Erol et al., 2022).

Furthermore, the quantity and quality of chemical and nutritional components in plants are influenced by their distribution, which is affected by various soil compositions, climate conditions, and geographical location (Luján et al., 2014).

Thus, the objective of the present investigation was to analyse the influence of solvent types on the extraction efficiency of phenolic compounds and to optimise extraction conditions using the single-factor method and response surface methodology. Moreover, the extract obtained under optimal extraction conditions was found to possess both antioxidant and antibacterial activity. The study also revealed the presence of trace elements in *M. citrifolia* fruit powder, a product from Danang, Vietnam, providing basic information for consumer safety.

2. Material and Methods

2.1. Materials and chemicals

Pure dried *Morinda citrifolia* L. (noni) fruit powder (100%) was a product from Bestone Co., Da Nang, Vietnam. Ethanol, ethyl acetate, n-hexane were supplied from Xilong Co., Ltd (China), Folin-Ciocalteu reagent was purchased from Sigma-Aldrich Co., Ltd, and twice-distilled water were obtained from the laboratory.

The dried noni fruit powder was firstly determined the moisture content. Approximately 35 g of the sample was dried in an oven at 105°C until a constant weight was achieved. After drying, the sample was re-weighed, and the moisture content was calculated using the following equation:

$$\overline{W}_M(\%) = \frac{\overline{m}_1 - \overline{m}_2}{\overline{m}_1} \times 100\%$$

Where, ($\overline{W}_M$) is the average moisture content (%), $\overline{m}_1$ and $\overline{m}_2$ are the average weights of the sample before and after drying at 105°C overnight, respectively. Based on the measurements, the moisture content of the dried noni fruit was found to be 6.7%.

2.2. Determination of metal content

35 g sample was kept in a muffle furnace and incinerated at 550 - 600°C about 13 h. The ash sample was cooled and dissolved in 3 mL 65% HNO₃ acid solution, filtered into a 25 mL volumetric flask and diluted to volume. The concentrations of Zn and Cu were detected with a Shimadzu AA 7000 AAS Spectrometer.

2.3. Extraction of phenolic compounds

2.3.1. Extraction of phenolic compound

To assess the effect of extraction factors on the total phenolic content (TPC), the dried noni fruit powder (5 g) and 100 mL solvent were added into a 250 mL round-bottom flask. The mixture was heated to t°C and kept for hours. After extraction, the sample was cooled to r.t., filtered, and the solvent was evaporated from the extract.

Extraction with Various Solvents: To assess the effect of solvent on the TPC, various solvents (ethanol, ethyl acetate, n-hexane, and water)

were surveyed. The mixture of noni fruit powder and solvent was heated to 60°C and kept for one hour. The TPC of the extract was then estimated.

Single-factor method: To evaluate the effect of different factors on the extraction of phenolic compounds, a series of experiments at different solvent concentrations (10 - 90%), extraction times (1 - 13 h), and extraction temperatures (30 - 90°C) were performed. After extraction, the TPC of the extract was measured.

2.3.2. Determination of total phenolic content

The extract was then diluted adequately with water, and TPC was estimated using the Folin-Ciocalteu assay (Andres et al., 2020). The UV absorbance of the sample was obtained at 765 nm using a UV-Vis Labomed UVD-3200 (LABOMED, Los Angeles, CA). The standard curve for phenolic content in water was obtained, with the equation $Y = 0.0107X + 0.0167$, $R^2 = 0.995$, indicating a strong linear relationship. The TPC was expressed as the amount of gallic acid equivalent (GAE) per g of dried noni fruit powder. It was calculated and reported in units of mg GAE/g dw (mg of gallic acid equivalent per g of dry weight). Each experiment was performed three times in parallel, and the average value was obtained.

2.3.3. Optimization of the phenolic compound extraction using RSM

A box-Behnken design (BBD) with three levels and three factors (17 runs, including five central points) was conducted to evaluate the effect of extraction with various solvent concentration, extraction times, and extraction temperatures on TPC. Then the relationship between TPC and three extraction factors was equated as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3$$

Where Y is TPC (mg GAE/g dw); X_1 is the extraction time (h), X_2 is the extraction temperature ($^{\circ}\text{C}$), and X_3 is ethanol concentration (%). a_0 is the intercept term; $a_1 - a_3$ are linear coefficients; a_{12}, a_{23}, a_{13} are interaction coefficients; and a_{11}, a_{22}, a_{33} are quadratic coefficients.

2.4. Biological activity

2.4.1. Antioxidant potential assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay was measured following the protocols as described by (Shahinuzzaman et al., 2021) with some modifications. In brief, ethanol extract (100 μL) was added to 3.9 mL of a freshly prepared 0.1 mmol/L DPPH solution dissolved in 70% ethanol. The mixture was vigorously shaken and left to stand at room temperature for 30 min in the dark. The absorbance was read using a UV-Vis spectrophotometer at 517 nm with 70% ethanol as the control. The inhibition percentage was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{control} is the absorbance of control; A_{sample} is the absorbance of test sample/standard. Vitamin C was taken as reference standard. Mean values were obtained from triplicate experiments. The inhibition percentage versus concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} ($\mu\text{g/mL}$) value.

2.4.2. Antimicrobial activity

The microorganisms used in this test were obtained from the Microbiology Laboratory, Faculty of Biology Technology, The University of Da Nang of Technology and Education. These

were *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica*. The antibacterial activity of the extract was determined using the agar well diffusion technique as reported by (Magaldi et al., 2004). The agar plate surface of 25 mL medium inoculated by spreading the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer, and a 50 μL volume of a solution of the extract was introduced into the well. Then, agar plates were incubated under suitable conditions. Standard antibiotic, cephalexin was used in order to control the sensitivity of the tested bacteria. The results were expressed as mm of zone of inhibition.

2.5. Statistical analysis

All experimental data were the mean of triplicate $\pm$ standard error (SE). The experimental design and statistical analysis were determined using a Design-Expert 13.00 software (Stat-Ease, Inc., Minneapolis, Minnesota, USA). The model accuracy was checked in terms of the values of R^2 and adjusted R^2 . The significance of the models was calculated by analysis of variance (ANOVA).

3. Results and Discussion

3.1. Trace element content

The contents of Zn and Cu were 106 and 14 mg/kg dw, respectively. The significant high concentration of Zn in *M. citrifolia* fruit was an advantage in treatment of diabetic gangrene and mitigation diabetic complications. Moreover, Zn exhibited in the islet cells of the pancreas was vital for the synthesis, storage, and secretion of insulin; however, diabetic patients excreted a large amount of Zn every day. Cu is not an integral part of enzymes; however, it can enter

the active site of enzymes via carrier substances such as transferrin, ceruloplasmin, albumin, α -macroglobulin, and nickeloplasmin, and can activate enzymes (Narendhirakannan et al., 2005). Moreover, according to National Institutes of Health (NIH), the US recommended daily dietary allowance (RDA) of Zn for male and female are 11 and 8 mg (NIH, 2022a); and that of Cu for adults is under 0.9 mg (NIH, 2022b). Furthermore, it had been reported that a daily intake of 15 - 30 mg of Zn could prevent and treat of diabetes (Niewoehner et al., 1986). Therefore, daily consumption of this fruit was strongly recommended.

3.2. Optimization of phenolic compound extraction

3.2.1. Extraction with various solvents

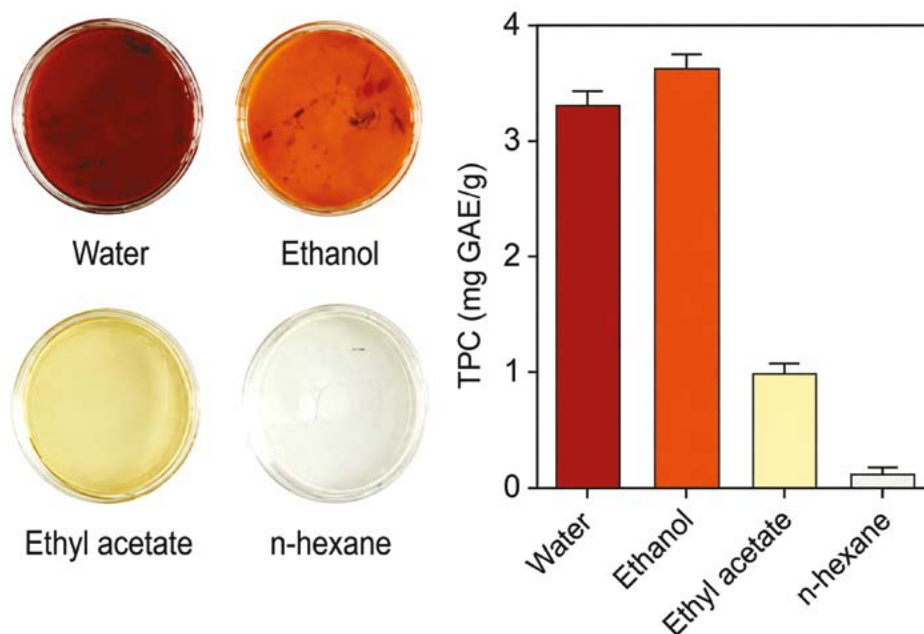


Figure 1. Effect of solvents on extraction efficiency of phenolic compounds.

The effect of water, ethanol, ethyl acetate, and n-hexane solvent on the extraction of phenolic compounds was presented in Figure 1. The results showed that water and ethanol resulted in higher yield than the ethyl acetate and n-hexane. Though the color of the sample extracted by water is darker than that by ethanol, TPC extracted by ethanol is higher than that by water (3.6 and 3.2 mgGAE/g dw, respectively). Total phenolic contents extracted by ethyl acetate or n-hexane were significantly low, with TPC of 1.0 and 0.1 mg GAE/g dw, respectively. The result might explain by the proper polar between solvents and phenolic compounds, and the stability of phenolic compounds in water being affected easier than that in ethanol (Chen et al., 2013). Therefore, the ethanol was chosen as an extracting solvent for further experiments.

3.2.2. Single factor method

Figure 2 presented effects of ethanol concentration, extraction time, and extraction temperature on extraction efficiency of phenolic compounds. The extraction of TPC increased with the increase of ethanol concentration but at the higher ethanol concentration of 50%, the TPC decreased (Figure 2a), which could be attributed to the reduced solubility of phenolic compounds in the 1:1 ethanol-water solvent. Thus, ethanol concentration of 50% was chosen as an optimal concentration for subsequent experiments.

Then the extraction was performed at 70°C, ethanol concentration of 50% and various extraction time (Figure 2b). A sharp increase of TPC was observed at initial stage (1 - 5 h) and there was a constant decrease after 7 h in TPC of extraction process. Therefore, extraction time

of 5 h was selected for investigating effects of extraction temperature on TPC. The curve of the effect of extract temperature (Figure 2c) showed that the TPC reached the peak at 70°C. When extraction temperature was higher than 70°C, the TPC decreased constantly. This decline might be explained due to the degradation of phenolic compounds at high temperature for long extraction time (Zhong et al., 2019). Thus, based on the results of the single-factor experiments, the optimal conditions for achieving the highest TPC were 50% ethanol concentration, an extraction time of 5 h, and an extraction temperature of 70°C. Furthermore, based on the above results, the ethanol concentration, extraction time, and extraction temperature were set in the range of 30 - 70%, 1 - 9 h, and 50 - 90°C, respectively, to analyze in the response surface method (RSM) design.

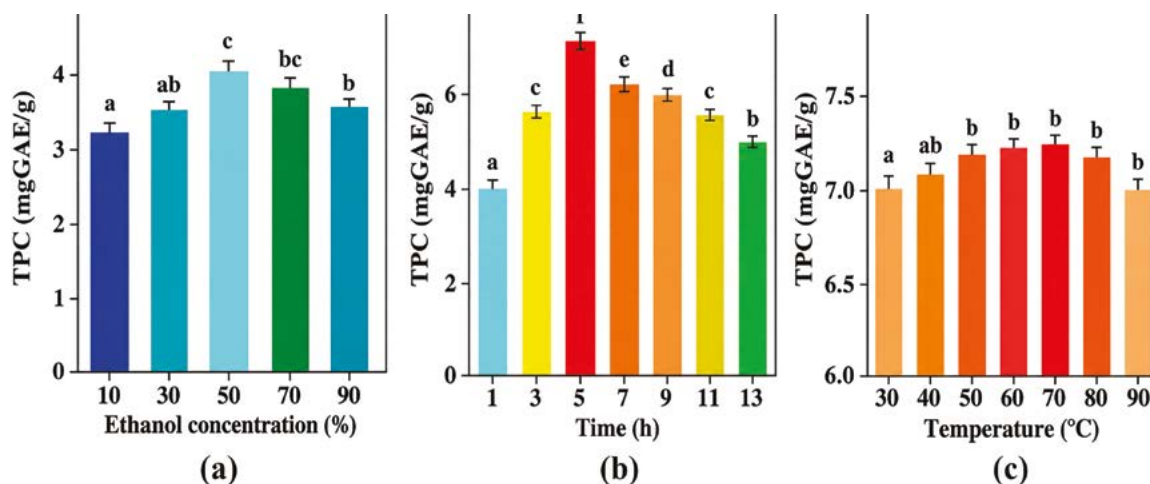


Figure 2. Effect of (a) ethanol concentration, (b) extraction time, and (c) extraction temperature on the extraction of phenolic compounds. Columns with the same letter are not significantly different, as determined by the LSD (least significant difference) test at $P \leq 0.05$.

3.2.3. Optimization of extraction factors using RSM

A box-Behnken design (BBD) was used for response surface optimization with three

process variables (extraction time, extraction temperature, and ethanol concentration) at three levels as shown in Table 1. Values of independent variables and the actual and predicted total phenolic contents are given in Table 2.

Table 1. Experimental values and coded levels of the independent variables used for box-Behnken design

Variables	Factor		Level	
	X	-1	0	1
Ethanol concentration (%)	X1	30	50	70
Extraction time (h)	X2	1	5	9
Extraction temperature (°C)	X3	50	70	90

The relationship of responses and independent variables was obtained:

$$Y = 7.24 + 0.48X_1 + 1.13X_2 - 0.20X_3 - 0.93X_1^2 - 1.71X_2^2 - 0.51X_3^2 + 0.69X_1X_3$$

Where X_1 , X_2 , and X_1X_3 possess a positive effect on responses, whereas other factors showed an adverse influence.

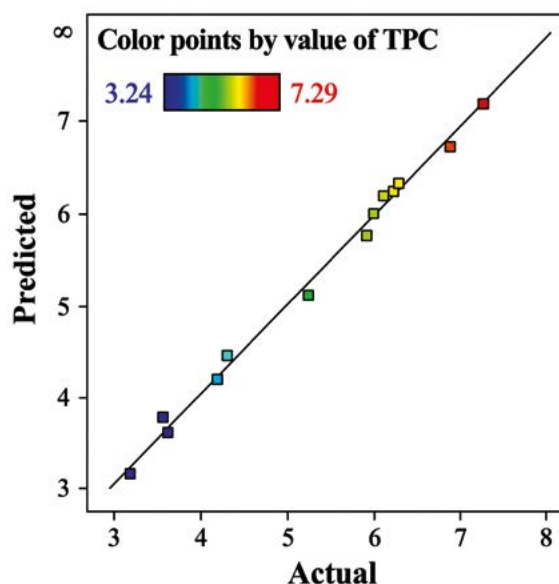


Figure 3. The actual and predicted responses.

Table 2. Plan and results for response surface methodology of total phenolic content

Run	Independent variable			Response (mg GAE/g dw)	
	X1	X2	X3	Actual value	Predicted value
1	50 (0)	1 (-1)	90 (+1)	3.66 ± 0.2	3.61
2	50 (0)	9 (+1)	50 (-1)	6.21 ± 0.4	6.26
3	30 (-1)	5 (0)	50 (-1)	6.09 ± 0.3	6.2
4	50 (0)	5 (0)	70 (0)	7.27 ± 0.4	7.24
5	50 (0)	9 (+1)	90 (+1)	5.99 ± 0.2	6.02
6	30 (-1)	5 (0)	90 (+1)	4.29 ± 0.3	4.43
7	50 (0)	5 (0)	70 (0)	7.21 ± 0.3	7.24
8	30 (-1)	9 (+1)	70 (0)	5.24 ± 0.2	5.08
9	70 (+1)	1 (-1)	70 (0)	3.61 ± 0.2	3.77
10	30 (-1)	1 (-1)	70 (0)	3.24 ± 0.3	3.15
11	50 (0)	5 (0)	70 (0)	7.23 ± 0.4	7.24
12	70 (+1)	9 (+1)	70 (0)	6.27 ± 0.3	6.36
13	70 (+1)	5 (0)	90 (+1)	6.87 ± 0.3	6.76
14	70 (+1)	5 (0)	50 (-1)	5.91 ± 0.3	5.77
15	50 (0)	5 (0)	70 (0)	7.29 ± 0.4	7.24
16	50 (0)	5 (0)	70 (0)	7.18 ± 0.3	7.24
17	50 (0)	1 (-1)	50 (-1)	4.18 ± 0.2	4.15

X1: ethanol concentration; X2: extraction time; X3: extraction temperature. The coded forms of the variables are shown in parentheses.

Table 3. ANOVA for quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Model	32.94	9	3.66	176.2	< 0.001
X ₁	1.81	1	1.8	86.89	< 0.001
X ₂	10.17	1	10.17	489.55	< 0.001
X ₃	0.312	1	0.312	15.02	0.006
X ₁ ²	3.67	1	3.67	176.43	< 0.001
X ₁ X ₂	0.109	1	0.109	5.24	0.056
X ₁ X ₃	1.9	1	1.9	91.67	< 0.001
X ₂ ²	12.36	1	12.36	594.74	< 0.001
X ₂ X ₃	0.023	1	0.023	1.08	0.333
X ₃ ²	1.11	1	1.11	53.34	< 0.001
Residual	0.145	7	0.021	-	-
Lack of Fit	0.138	3	0.046	23.15	0.006
Pure Error	0.008	4	0.002	-	-
Total (corr.)	33.09	16	-	-	-

The ANOVA of quadratic model (Table 3) was significant with a low *P* value (< 0.001), the low lack of fit 0.005, and a high coefficient of determination (*R*²) ascertained the quality of fit. The *F*-value also indicated the influence of the factors on the TPC; the higher the *F* value, the stronger the influence. In this study, the *F*-value of the extraction time was the highest, with a value of 489.55, and the extraction time square was 594.74, indicating that this factor had the most significant influence on the response variable, followed by ethanol concentration and extraction temperature, which had a lesser influence. Therefore, the RSM model with box-behnken design (BBD) was suitable for optimizing the technical parameters in phenolic compound extraction. The value of *R*² was 0.93

and the adjusted *R*² was 0.99 which indicated a good correlation between actual and predicted data (Figure 3).

The effects of combined extraction factors on TPC are presented in Figure 4. The effects with combination of the extraction time and ethanol concentration on TPC were shown in Figure 4a. The phenolic content was simultaneously influenced by both factors at extraction temperature of 72°C. In ethanol concentration regimes given, the TPC enhanced with increase of the extraction time. However, when the extraction time exceeded 6 h, the TPC declined. The results could be explained because the phenolic compounds might be oxidated and/or polymerized at 72°C in extended extraction time over 6 h.

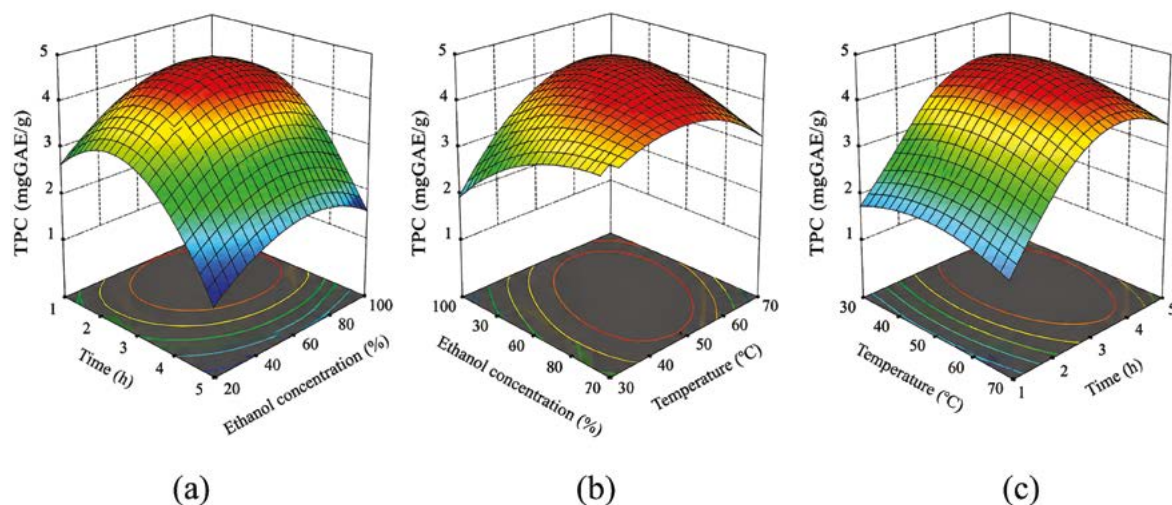


Figure 4. a) Effects of both extraction time and ethanol concentration on the total phenolic content (TPC) at 72°C, (b) Effects of both extraction temperature and ethanol concentration on the TPC after 5.4 h of extraction (c) Effects of both extraction temperature and extraction time on the TPC at ethanol concentration of 60%.

Figure 4b shows the effects of extraction temperature and ethanol concentration on the TPC after 5.4 h of extraction. Effect of extraction temperature on TPC at various ethanol concentration were different. The curve of TPC at 30% ethanol concentration presented a slightly declining trend with increase of extraction temperature. While at a higher ethanol concentration of 70%, the TPC increased with increase of the temperature. This could be explained that the high temperature decreases the viscosity of solvent (viscosity of ethanol is higher than that of water with values of 1.2 and 1.0 cP, respectively), thus the extraction efficiency of solvent with high ethanol concentration could be enhanced (Zardo et al., 2019). The effects of combination of both extraction temperature and extraction time on the TPC at ethanol concentration of 60% (Figure 4c) were significantly dependent on extraction time. In the

temperature range of 50 - 90°C, the curve of TPC reached a peak at about 7 h and then decreased with increase of extraction time. One possible explanation is that extraction time plays a critical role in determining energy consumption and process feasibility (Osorio-Tobon, 2020). Thus, with the increase in extraction time, the TPC increased; however, within this temperature range, when the extraction time was extended, the stability of phenolic compounds was affected.

By applying the desirable function approach, the maximal TPC was predicted to be 7.38 mg GAE/g dw at an ethanol concentration of 60%, an extraction time of 5.4 h, and an extraction temperature of 72°C. This result differed slightly from that of Thoos (2013), which reported 8.81 ± 0.17 mg GAE/g dw of TPC at 75% ethanol, 40 min, and 57°C. In another study, Bramorski (2010) showed a TPC of 91.9 mg GAE/100 mL noni juice. Moreover, the experiments were conducted

under the optimal conditions mentioned above to verify the prediction. The TPC obtained was 7.44 ± 0.15 mg GAE/g dw, which agreed closely with the predicted model. The results indicated that the RSM is more accurate than the single-factor method in extraction optimization for phenolic compounds.

3.3. Biological activity

3.3.1. Antioxidant potential assay

The DPPH radical scavenging activity of the noni fruit extract obtained under optimized extraction conditions was $86.9 \pm 0.3\%$, indicating considerable antioxidant potential. The corresponding IC_{50} (half maximal inhibitory concentration) value of $137.00 \mu\text{g/mL}$ suggests moderate radical scavenging activity compared to the standard antioxidant vitamin C ($IC_{50} = 22.61 \mu\text{g/mL}$). Although the extract is less potent than pure vitamin C, its antioxidant performance is noteworthy, especially considering that it is a crude extract containing a complex mixture of phenolic compounds and other bioactive constituents.

In comparison, previous studies have reported a wide range of IC_{50} values for noni extracts. For instance, hydro-methanolic extracts of noni fruit from Sri Lanka exhibited

an IC_{50} of $50.70 \pm 0.20 \mu\text{g/mL}$ (Samarasinghe et al., 2023). Cai et al. (2023) reported IC_{50} values ranging from 1.23 to 2.03 mg/mL for noni polysaccharides at different maturity stages, while Wasina et al. (2010) found that Thai noni leaf extracts had IC_{50} values between 0.20 and 0.35 mg/mL . These variations can be attributed to differences in extraction solvents, techniques, plant parts used, and the chemical composition of the extracts.

3.3.2. Antibacterial activity

Morinda citrifolia fruit powder extract under optimal conditions showed antimicrobial activity against five tested bacterial strains as shown in Table 4. Results of the study indicated that the extract was slightly active against all the studied microorganisms with zone of inhibition of 9 - 15 mm. In general, *P. aeruginosa* appeared as the least resistant strain and *Salmonella enterica* showed the highest resistance. The average dimension of zone of inhibition resulting from *M. citrifolia* extract application against both Gram-positive bacteria, *B. subtilis* and *B. cereus* was 13 and 14 mm, respectively. The findings is also consistent with the report of Kang & Song (2019) that low antibacterial activity of 70% ethanol extract from dried *M. citrifolia* fruit powder was observed against *E. coli* O157:H7 and *S. enterica*.

Table 4. Antimicrobial activity (mm zone of inhibition) of *M. citrifolia* extract

Sample	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Salmonella enterica</i>
<i>M. citrifolia</i> fruit power extract	15 ± 0.2	13 ± 0.3	14 ± 0.2	10 ± 0.2	9 ± 0.2
Cephalexin	40 ± 0.3	35 ± 0.3	32 ± 0.3	34 ± 0.2	28 ± 0.3

Each value in the table was obtained by calculating the average of three experiments ($n = 3$), $\pm$ standard deviation.

4. Conclusions

The *M. citrifolia* fruit found a high level of zinc with 106 mg/kg, thus daily supplementation with this fruit for diabetic patients is strongly recommended. The extraction process was analyzed and optimized by the single factor method and RSM. The optimal extraction conditions predicted by a response surface model were ethanol concentration of 60%, extraction temperature of 72°C, and extraction time of 5.4 h. Under these conditions, the phenolic compound extraction obtained was 7.38 mg GAE/g dw. The DPPH radical scavenging activity of obtained extract at the optimal extraction parameters exhibited $86.9 \pm 0.3\%$. The antimicrobial assay also confirmed that the extract had slightly inhibitory effect on five tested strains regarding both Gram-positive and Gram-negative bacteria.

Conflict of interest

Authors have declared that no competing interests exist.

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Production of squid sausage from purpleback flying squid (*Sthenoteuthis oualaniensis*)

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ARTICLE INFO	ABSTRACT
Research Paper Received: October 09, 2025 Revised: December 01, 2025 Accepted: December 17, 2025 Keywords Gelation Purpleback flying squid Sausage Seaweed Texture *Corresponding author Truong Quang Binh Email: tqbinh@hcmuaf.edu.vn	In recent years, the production of purpleback flying squid (<i>Sthenoteuthis oualaniensis</i>) has increased and plays an important role in Vietnam’s fisheries. This squid species is widely distributed in Vietnamese waters. Although its production is high, the cooked squid is generally considered less palatable than other squid species. When stored for an extended period, it develops an acrid taste, and dried squid often turns black, reducing the product’s commercial value. Therefore, utilizing purpleback flying squid for the production of value-added products, such as squid sausage, is desirable to enhance its utilization and market value. In this study, the effects of different concentrations of NaCl (0.5, 1, 1.5, and 2%) and seaweed (0.5, 1, and 2%) on the color, drip loss, protein solubility, texture, seasoning ratio, and gel strength of squid sausage were investigated. The results showed that the addition of salt and seaweed improved the texture of squid sausage compared to samples prepared without these ingredients. The best texture was observed in sausages formulated with 2% salt. The highest sensory score was achieved with a seasoning formulation consisting of 2% salt, 4% sugar, 0.5% monosodium glutamate (MSG), 0.7% garlic powder, 1% seaweed, 0.5% chili powder, and 1% chili sauce. These findings demonstrate that purpleback flying squid has strong potential for developing value-added products, and the optimized squid sausage formulation provides a promising application to enhance its commercial value and industrial utilization.

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1. Introduction

Processed seafood products are valuable sources of essential nutrients for humans. The purpleback flying squid (*Sthenoteuthis oualaniensis*) is a cephalopod mollusk belonging to the phylum Mollusca and is widely distributed

in tropical and subtropical seas and oceans. *Sthenoteuthis* is found extensively in Vietnamese waters. In recent years, the annual production of purpleback flying squid in Vietnam has been high, estimated at around 1,000 tons. Although *Sthenoteuthis* has higher nutritional value

compared to other squid species (Tran, 2003), its economic value remains relatively low. Despite the high production volume, cooked purpleback flying squid is considered less palatable than other squid species, and when stored for a long time, it tends to develop an acid taste. Additionally, dried squid often turns black, reducing its market value. Nevertheless, the stable supply of purpleback flying squid presents an opportunity to develop value-added products.

Seafood is a rich source of high-quality proteins valued for their functional properties and nutritional benefits (Tahergorabi et al., 2011). The gelling capacity of food proteins is an important functional attribute in food manufacturing (Zayas, 1997). During the production of squid sausage mixed with seaweed, the texture of the raw material after grinding is crucial for gel (paste) formation, which plays a key role in developing the desired product texture. Gel formation, or gelation, is an important characteristic of proteins that affects the microstructure and palatability of meat products (Sun & Holley, 2011).

Protein gel structures form under conditions that disrupt native protein structures, provided that the protein concentration, thermodynamic conditions, and other factors are optimal for forming a stable tertiary matrix (Schmidt, 1981). Myofibrillar proteins largely determine the textural properties of processed protein products (Yasui et al., 1980; Asghar et al., 1985). Gelation is a two-step process consisting of denaturation and aggregation (Kinsella et al., 1994; Matsumura & Mori, 1996). Gelling properties are also influenced by several factors, including the type of myofibrillar proteins, muscle type, species, and processing conditions such as protein concentration, pH, ionic strength, pressure,

heating rate, and temperature (Sun & Holley, 2011).

A gel is an intermediate phase between a solid and a liquid, technically defined as a substantially diluted system that exhibits no steady flow. Protein gelation refers to the transformation of proteins from a sol state (viscous material) to a gel-like state (elastic material). When seafood muscle tissue is comminuted in the presence of NaCl, a viscous soul initially forms, which, upon heating, transforms into a viscoelastic gel. Although the terms gelation and coagulation are often used interchangeably, gelation can be theoretically defined as a protein aggregation phenomenon in which polymer-polymer and polymer-solvent interactions are balanced by attractive and repulsive forces, resulting in a well-ordered tertiary network or matrix capable of trapping large amounts of water (Schmidt, 1981).

Lipids in seaweeds are present in relatively low amounts (1 - 5% of dry matter), yet they are beneficial to human health, as nearly half of these lipids are polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and arachidonic acid (AA). Seaweeds also contain significant amounts of essential minerals (Na, K, Ca, and Mg), trace elements (Fe, Zn, Mn, and Cu), and various vitamins such as L-ascorbic acid (vitamin C), thiamine (B1), riboflavin (B2), cobalamin (B12), folic acid and its derivatives (5-methyltetrahydrofolate, 5-formyltetrahydrofolate, and tetrahydrofolate), and tocopherols (vitamin E) (Peng et al., 2015). Additionally, seaweed is rich in dietary fiber, accounting for 32 - 50% of its dry matter. The soluble fiber fraction represents 51 - 56% of total fibers in green (ulvans) and red algae (agars, carrageenans, and xylans), and 67 - 87%

in brown algae (laminaria, fucus, and others). Soluble fibers are generally associated with cholesterol-lowering and hypoglycemic effects (Dharmananda, 2002).

There have been few studies on products made from purpleback flying squid, as well as limited research on the gelation properties of squid proteins. Therefore, this study aimed to develop a new value-added product from purpleback flying squid and seaweed.

2. Materials and Methods

2.1. Materials and sample preparation

Squid (*Sthenoteuthis oualaniensis*) were purchased at BRAV company (ward 12, district 4, Ho Chi Minh City, Vietnam). Pangasius fish were purchased from a local market. Squid and pangasius were cleaned and rinsed with water to remove the impurities. Seaweed (*Undaria pinnatifida*) is used in grinded and dried form. Squid and pangasius was cut into pieces (3 x 4 cm) and frozen at -18°C for 4 h. After freezing, squid (60%) and pangasius (40%) were grinded with salt (0.5 - 2%). Following the first experiment on suitable salt concentration, the seaweed proportion was surveyed by adding seaweed at 0, 0.5, 1, and 2% during the grinding process. Lastly, the spice and additive ratio for the product was investigated.

Minced squid and pangasius was stuff into collagen case (diameter 24 cm x length 15 cm). The sausages were cooked at 90°C for 30 min in a water bath. Six to eight sausages were prepared for each treatment group.

2.2. Color analysis

The color of gels was measured with a colorimeter (CR-400/410, Konica Minolta).

Including L* (white), a* (+a, red; -a, green) and b* (+b, yellow; -b, blue). The whiteness of gels was then calculated from the formula: whiteness = $100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$ (Ramirez-Suarez & Morrissey, 2006).

2.3. Drip loss

About 2 g sample was wrapped in filter paper then put into a tube and centrifuged at 6000 r/min in 20 min by a centrifuge (Universal 320R centrifuge, Hettich, USA). Samples were weighed before and after centrifugation to calculate drip loss as following:

$$\text{Drip loss (\%)} = \frac{m_1 - m_2}{m_1}$$

With:

m_1 : mass of the sample before centrifugation (g),

m_2 : mass of the sample after centrifugation (g).

2.4. Gel strength

The method of Truong et al. (2017) was employed to determine gel forming ability. A folding test was performed by measuring the resistance of slices (3 mm thickness and 3.5 cm width) folded according to Suzuki (1981) with a 5 point grade system: 5 = no crack showing when folded twice; 4 = no crack showing when folded in half; 3 = cracks develops gradually when folded in half; 2 = crack occurs immediately when folded; 1 = breaks when pressed by fingers.

2.5. Protein solubility

The method of Truong et al. (2017) was employed to measure protein solubility of gels: About 4 g of sample were homogenised for 1 min with 40 mL of a 30 g L⁻¹ NaCl solution. The resulting solution was centrifuged at 4500 r/min

in 15 min. Take 1 mL supernatant aliquot was mixed with 10 mL of a 30 g L⁻¹ NaCl solution and homogenised again. The protein concentration was determined by the Lowry method at 550 nm (Lowry et al., 1951).

2.6. Texture profile analysis (TPA)

In the first experiment samples (24 mm diameter and 15 mm height) were tempered at 20°C for about 1 h and removed from their casings. Brookfield food texture analyzer (CT3, USA) was used to test for TPA. TPA was measured by a cylindrical-shaped probe, 31.1 mm in diameter and 20 mm L height. Samples were compressed at 3 mm/sec to 8 mm of height. In the second experiment, samples (27 mm diameter and 15 mm height) were tempered at 20°C for about 1 h and removed from their casings. Brookfield food texture analyzer (CT3, USA) was used to test for TPA. TPA was measured by a cylindrical-shaped probe, 31.1 mm in diameter and 20 mm L height. Samples were compressed at 3 mm/sec to 5 mm of height.

2.7. Sensory analysis

The sensory evaluation of the final products was conducted using affective test methods (Lawless & Heymann, 2010). One common approach to assess consumer preference for food products is to employ a rating scale that measures the degree of liking or disliking. In this study, the traditional nine-point hedonic scale, which is widely used for sensory evaluation (Lawless & Heymann, 2010), was applied. Each test sample was presented on a white plate and labeled with a three-digit random code. During the evaluation, each panelist received the coded samples, a cup of water for palate cleansing, and an answer sheet. Panelists rinsed their mouths with water after each sample and rated the color, flavor,

and texture of the products. The experiment was arranged into four treatments (NT), with the spice ratios added to 1 kg of raw material as follows:

- NT1: 4% sugar; 0.5% MSG (monosodium glutamate); 0.7% garlic powder; 1% seaweed; 0.25% pepper powder.

- NT2: 4% sugar; 0.5% MSG; 0.7% garlic powder; 2% seaweed; 0.25% pepper powder.

- NT3: 4% sugar; 0.5% MSG; 0.7% garlic powder; 2% seaweed; 0.5% chili powder; 1% chili sauce.

- NT4: 4% sugar; 0.5% MSG; 0.7% garlic powder; 1% seaweed; 0.5% chili powder; 1% chili sauce.

2.8. Statistical analysis

All analytical measurements were performed in triplicates and the results are reported as mean value $\pm$ standard deviation. Data were analysed by one-way analysis of variance (ANOVA) and least significant difference (LSD) at the significance level of $P < 0.05$ using IBM SPSS software version 22.

3. Results and Discussion

3.1. Proximate composition

The proximate composition analysis of the red-eye squid, *Sthenoteuthis oualaniensis*, revealed that the primary constituents of its fresh mass were water (80.75%) and protein (17.57%), with minor fractions of lipid (1.19%) and ash (1.07%). A comparative evaluation with the common cuttlefish, *Sepia officinalis*, highlights key species-specific variations in biochemical profile. While both species exhibited comparable moisture content (*S. officinalis*: 80.85%), the most

notable difference was observed in the major energy components. *S. officinalis* contained a significantly higher Protein content (21%) than *S. oualaniensis* (17.57%). Conversely, *S. oualaniensis* possessed a markedly higher concentration of lipid (1.19%), which was approximately 4.4 times greater than the 0.27% recorded for *S. officinalis*.

The remaining ash content was similar between the two species (1.07% for *S. oualaniensis* vs. 1.2% for *S. officinalis*). These findings underscore that while both species are high-protein, low-fat marine resources, *S. oualaniensis* represents a relatively richer source of lipid compared to *S. officinalis*.

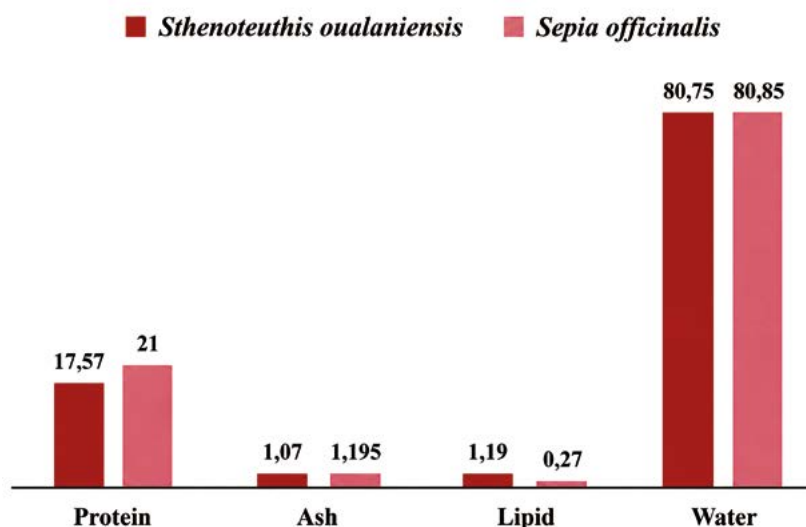


Figure 1. Proximate analysis of *Sthenoteuthis oualaniensis* in comparison to *Sepia officinalis*.

3.2. Effects of salt on quality of sausage

3.2.1. Color of squid sausage

The whiteness of gels prepared with different salt concentrations is presented in Table 1. The whiteness values of treatments NT1 (0.5% salt) and NT2 (1% salt) were not significantly different from each other ($P > 0.05$). However, both NT1 and NT2 exhibited significantly lower whiteness compared to NT3 (1.5% salt) and NT4 (2% salt) ($P < 0.05$). This indicates that increasing the salt concentration above 1% led to a noticeable improvement in the whiteness of the gels. The higher whiteness observed in NT3 and NT4 may be associated with enhanced protein solubilization and gel network formation

at higher ionic strengths, which promote light scattering and thus increase the perceived whiteness.

Whiteness has been reported to be influenced by the degree of protein denaturation and water-holding capacity (WHC) of the gel (Shie & Park, 1999). Although the addition of salt altered the whiteness of the samples, the difference was difficult to distinguish by visual observation. This trend is consistent with findings by Montero et al. (1997), who suggested that the highest whiteness values occur in heat-induced gels due to protein denaturation and aggregation. Overall, salt concentration up to 2% slightly enhanced the whiteness of squid gels, but the effect was not pronounced beyond 1.5%.

Table 1. Results of whiteness of squid gels at different salt concentrations

Treatment	NT1	NT2	NT3	NT4
	0.5% salt	1% salt	1.5% salt	2% salt
Whiteness	67.93 ± 0.27 ^A	68.8 ± 0.37 ^A	71.36 ± 0.95 ^B	71.47 ± 0.6 ^B

Different letters (A, B, C) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

3.2.2. Gel forming ability of squid sausage

Table 2. Folding score of squid gels at different salt concentrations

Treatment	NT1	NT2	NT3	NT4
	0.5% salt	1% salt	1.5% salt	2% salt
Score	2.33 ± 0.58 ^A	3.33 ± 0.58 ^A	4.33 ± 0.58 ^{BA}	4.67 ± 0.58 ^{CA}

Different letters (A, B, C) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

The folding score of squid gels at different salt concentrations is presented in Table 2. The gel prepared with 2% salt (NT4) exhibited the highest folding score among all treatments, while the lowest score was observed in the sample containing 0.5% salt (NT1). The folding scores of NT3 (1.5% salt) and NT4 (2% salt) were significantly higher than those of NT1 and NT2 ($P < 0.05$), indicating that increasing salt concentration markedly enhanced the gel-forming ability of the product. The improvement in gel strength with higher salt concentrations can be attributed to the increased solubilization of myofibrillar proteins, particularly myosin, under elevated ionic strength. The presence of Cl⁻ ions disrupt electrostatic interactions among muscle proteins, promoting protein unfolding and enabling greater exposure of reactive sites for intermolecular bonding during heating. These interactions facilitate the formation of a more cohesive and elastic gel matrix. Furthermore, salt enhances protein-protein interactions by

stabilizing ionic bonds between myosin filaments and improving water-holding capacity (Offer & Knight, 1988).

These results indicated that salt addition up to 2% significantly improved the folding score, reflecting better gel elasticity and structural integrity of squid gels prepared under the same gelling conditions.

3.2.3. Drip loss of squid sausage

The drip loss of squid gels prepared with different salt concentrations is shown in Table 3. There was no significant difference in drip loss among NT1 (0.5% salt), NT2 (1% salt), and NT3 (1.5% salt) ($P > 0.05$). However, NT4 (2% salt) exhibited a significantly lower drip loss compared with the other treatments ($P < 0.05$). This finding indicates that increasing salt concentration to 2% markedly improved the water-holding capacity (WHC) of the squid gels.

Table 3. Drip loss of squid gels at different salt concentrations

Treatment	NT1	NT2	NT3	NT4
	0.5% salt	1% salt	1.5% salt	2% salt
Drip loss	40.17 ± 3.82 ^A	38.17 ± 0.76 ^A	40.33 ± 2.52 ^A	26.33 ± 1.04 ^B

Different letters (A, B) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

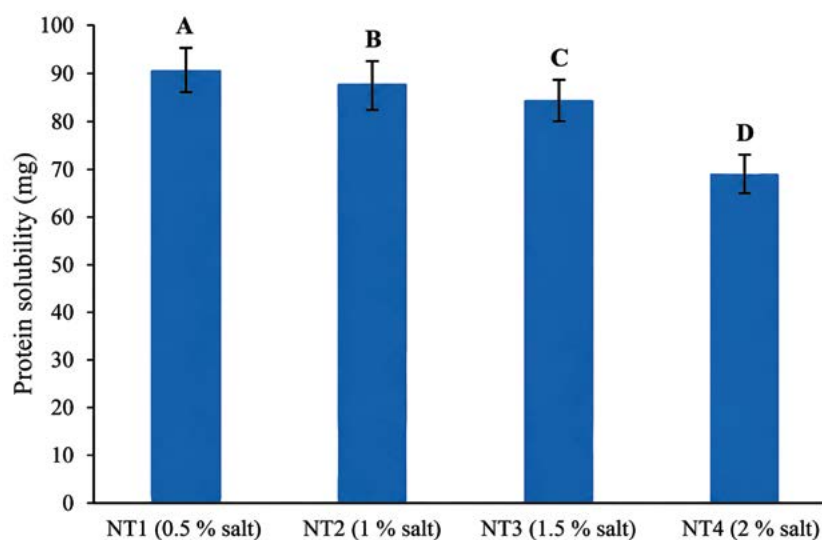
The reduction in drip loss at higher salt levels can be attributed to enhanced solubilization and swelling of myofibrillar proteins, particularly myosin, under increased ionic strength (Gordon & Barbut, 1992). Salt promotes protein unfolding and exposes reactive sites for intermolecular cross-linking during the heating process. These structural changes strengthen the gel network, allowing it to better retain water within the protein matrix. Consequently, higher salt concentrations contribute to reduced exudate formation and improved gel stability during processing and storage.

3.2.4. Protein solubility of squid sausage

The protein solubility of squid gels at different salt concentrations is presented in Figure 2. Protein solubility decreased significantly as the

salt concentration increased ($P < 0.05$). The NT1 (0.5% salt) exhibited the highest solubility (89.87%), whereas NT4 (2% salt) showed the lowest value (70.1%). The reduction in protein solubility with increasing salt content is indicative of protein denaturation and aggregation, particularly of myofibrillar proteins such as myosin, which tend to precipitate following denaturation (Ramírez et al., 2000). The elevated ionic strength at higher salt concentrations likely promotes protein-protein interactions and reduces protein solubility, contributing to the formation of a stronger and more cohesive gel network.

The results suggest that increasing the salt concentration decreases the solubility of squid proteins, reflecting enhanced protein aggregation and gel formation.

**Figure 2.** Protein solubility of squid sausage at different salt concentrations.

Different letters (A, B, C, D) indicate significant differences ($P < 0.05$).

3.2.5. Texture profile analysis (TPA) of squid sausage

The texture profile analysis (TPA) of squid gels at different salt concentrations was presented in Figure 3. The hardness of gels increased significantly with increasing salt concentration, with NT4 (2% salt) exhibiting the highest hardness among all treatments ($P < 0.05$). Gels containing 0.5, 1, and 1.5% salt showed lower and statistically similar hardness values, indicating that higher salt levels enhance gel firmness.

The springiness of gels was not significantly affected by salt concentration ($P > 0.05$), although a slight increase was observed in NT3 (1.5%) and NT4 (2%), suggesting that elasticity was largely maintained across treatments. Cohesiveness, however, increased progressively with salt concentration, with NT3 and NT4 showing significantly higher values than NT1 and NT2 ($P < 0.05$), reflecting a more compact and stable gel network at higher ionic strengths.

Gumminess, calculated as the product of hardness and cohesiveness, decreased with increasing salt concentration. NT1 (0.5% salt) exhibited the highest gumminess (10.06 N), while NT4 had the lowest value (34.8 N), indicating that higher salt levels produced gels that were firmer but less sticky. Chewiness, representing the energy required to masticate the gels,

increased significantly with salt addition. NT4 (2% salt) exhibited the highest chewiness (221.18 mJ), whereas NT1 had the lowest (60.8 mJ), highlighting that increased salt concentration enhances the structural integrity and solidity of the gels ($P < 0.05$).

The observed changes in textural properties can be attributed to the effect of salt on myofibrillar proteins. During emulsification, salt facilitates solubilization of myosin and promotes the interaction between actin and myosin filaments, enhancing gel network formation and water retention (Nguyen, 2006). Consequently, higher salt concentrations result in gels that are harder, more cohesive, and chewier, while maintaining elasticity. In general, the addition of salt markedly altered the texture of squid gels, producing samples with improved gel strength and structural integrity as the salt concentration increased.

The 2% salt concentration (NT4) was determined to be the optimal treatment, as it resulted in the highest whiteness, the maximum folding score (reflecting superior gel-forming ability and elasticity), and the lowest drip loss (indicating significantly enhanced water-holding capacity), thus promoting the formation of the most stable, cohesive, and texturally firm gel structure.

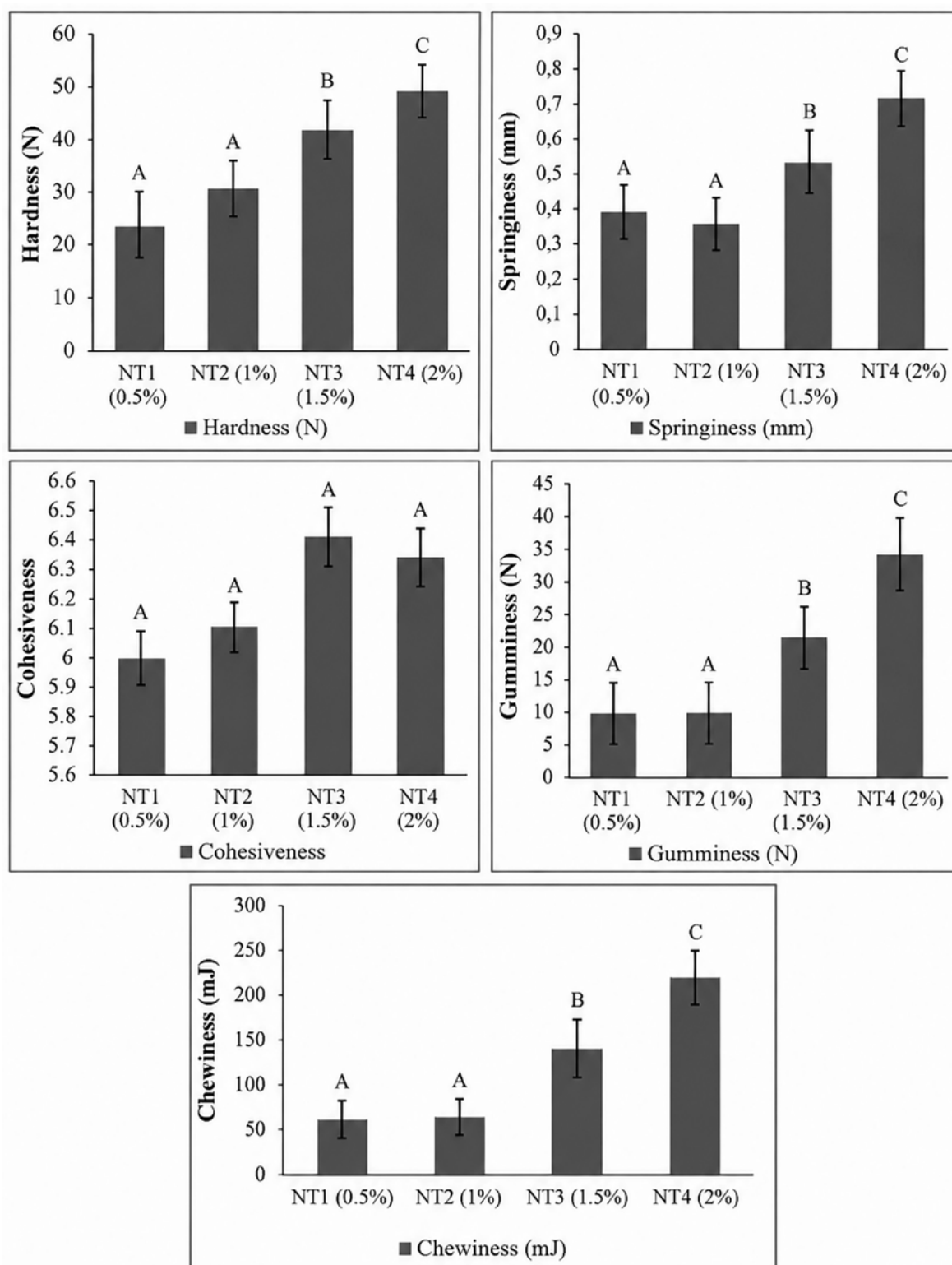


Figure 3. Changes in hardness, springiness, cohesiveness and chewiness of squid gels. Different letters (A, B, C) indicate significant differences ($P < 0.05$).

3.3. Effects of seaweed on quality of sausage

3.3.1. Effect of seaweed addition on the color of squid sausage

Table 4. Results of whiteness of squid gels

Treatment	NT1	NT2	NT3	NT4
	0% seaweed	1% seaweed	1.5% seaweed	2% seaweed
Whiteness	71.9 ± 0.74 ^A	70.2 ± 0.01 ^B	65.63 ± 0.13 ^C	54.22 ± 0.12 ^D

Different letters (A, B, C, D) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

The effect of seaweed addition on the color of squid gels was presented in Table 4. Whiteness decreased significantly as the proportion of seaweed increased ($P < 0.05$). NT1 (0% seaweed) exhibited the highest whiteness (71.9), whereas NT4 (2% seaweed) showed the lowest value (54.22), indicating a clear inverse relationship between seaweed content and gel whiteness.

The reduction in whiteness can be attributed to the natural green pigments present in seaweed, which impart color to the gels and reduce light reflectance. As a result, higher seaweed levels progressively darken the gel and decrease its perceived whiteness. These changes are noticeable by visual observation and are likely to influence the sensory perception and overall appearance of the final product.

These results indicate that increasing seaweed concentration significantly decreases the whiteness of squid gels, highlighting the impact of added

ingredients on the visual quality of the product.

3.3.2. Effect of seaweed addition on gel strength

The folding score of squid gels at different seaweed concentrations is presented in Table 5. No significant differences were observed in folding point among the treatments (NT1 - NT4) ($P > 0.05$), indicating that the addition of seaweed up to 2% did not markedly affect this textural parameter.

Despite the similarity in folding score, the experimental results suggest that the incorporation of seaweed contributes to a firmer and more resilient gel network. This effect may be attributed to the interaction between seaweed polysaccharides and myofibrillar proteins, which can enhance gel cohesion and water retention, improving the overall structural stability of the squid gels.

Table 5. Results of point of squid gels

Treatment	NT1	NT2	NT3	NT4
	0% seaweed	1% seaweed	1.5% seaweed	2% seaweed
Point	4.67 ± 0.58	4.67 ± 0.58	5.00 ± 0.00	5.00 ± 0.00

NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

3.3.3. Effect of seaweed addition on texture profile

Table 6. Texture profile analysis of squid gels at different levels of seaweed

Treatment	NT1	NT2	NT3	NT4
	0% seaweed	1% seaweed	1.5% seaweed	2% seaweed
Hardness (N)	22.67 ± 1.08 ^A	22.3 ± 01.26 ^A	35.17 ± 0.87 ^B	45.39 ± 1.22 ^C
Springiness (mm)	4.66 ± 0.05 ^A	4.98 ± 01.49 ^A	4.42 ± 0.04 ^{BA}	4.41 ± 0.06 ^A
Cohesiveness	0.8 ± 0.02 ^A	0.58 ± 0.1 ^{BA}	0.72 ± 0.01 ^A	0.7 ± 0.02 ^A
Gumminess (N)	17.73 ± 0.81 ^A	13.6 ± 2.92 ^A	25.21 ± 0.4 ^B	31.71 ± 1.44 ^C
Chewiness (mJ)	82.58 ± 4.61 ^A	64.99 ± 3.94 ^B	111.5 ± 2.28 ^C	139.72 ± 6.36 ^D

Different letters (A, B, C) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

The results of the texture profile analysis (TPA) of squid gels with different levels of seaweed were presented in Table 6. The addition of 0.5% seaweed (NT2) did not significantly affect the hardness of the squid sausage compared to the control (NT1) ($P > 0.05$), while higher concentrations of 1.5% (NT3) and 2% (NT4) significantly increased hardness ($P < 0.05$), with NT4 exhibiting the highest hardness of 45.39 N. These results indicate that the incorporation of seaweed contributes to a firmer gel structure. Springiness and cohesiveness showed no significant differences among treatments ($P > 0.05$), although NT2 had the highest springiness (4.98 mm) and NT4 the lowest (4.41 mm), suggesting that low levels of seaweed slightly improve elasticity, whereas higher levels may reduce it. Cohesiveness was highest in NT3 (0.72), but the overall change due to seaweed addition was minimal. Gumminess increased with seaweed concentration, with NT4 showing the highest value (31.71 N), indicating that seaweed enhances the firmness and chewable quality of the product. Chewiness differed significantly

among treatments ($P < 0.05$); it decreased in NT2 compared to the control but increased markedly in NT3 and NT4, reaching a maximum of 139.72 mJ in NT4. Overall, seaweed addition had a significant effect on hardness, gumminess, and chewiness, while springiness and cohesiveness remained largely unchanged, demonstrating that seaweed can improve the textural properties of squid sausages, resulting in a firmer and more resilient product.

The 1% seaweed concentration (NT2) was selected as the optimal formulation, as it maintained high gel strength (Folding Score equivalent to other treatments) while yielding the most visually appealing color score (6.41) among all treatments.

The results of the sensory evaluation of the sausage products were presented in Table 7. There were no significant differences in flavor and color scores among the treatments ($P > 0.05$), although NT2 received the highest average color score of 6.41, while NT4 had the lowest at 5.96. The NT2 was noted by many panelists

for having the most visually appealing color. In terms of flavor, all treatments were similarly rated, with NT3 slightly higher at 6.00, indicating that seaweed addition did not markedly affect the overall flavor perception. However, significant differences were observed in the structure scores among treatments ($P < 0.05$), with NT4 achieving the highest rating of 6.55, reflecting a firmer and more acceptable texture.

3.4. Effect of seaweed addition on sensory quality

Table 7. Results on sensory test of sausage product

Treatment	NT1	NT2	NT3	NT4
Color	5.98 ± 1.25 ^A	6.41 ± 1.46 ^A	6.13 ± 1.28 ^A	5.96 ± 1.17 ^A
Flavour	5.96 ± 1.5 ^A	5.87 ± 1.23 ^A	6.00 ± 1.31 ^A	5.95 ± 1.29 ^A
Structure	5.47 ± 1.16 ^A	5.98 ± 1.22 ^{BC}	6.27 ± 1.05 ^C	6.55 ± 1.04 ^{DC}

Different letters (A, B, C, D) in the same row indicate significant differences ($P < 0.05$). NT1: treatment 1; NT2: treatment 2; NT3: treatment 3; NT4: treatment 4.

4. Conclusions

This study shows that the functional quality of squid sausage made from *Sthenoteuthis oualaniensis* is influenced by the combined effects of salt concentration and seaweed content. Increasing salt levels improved the structural properties of the gel matrix, with the 2% salt treatment providing the most desirable characteristics, including markedly enhanced gel stability and reduced moisture loss. Higher level of seaweed increased gel hardness, however, they also reduced product whiteness. The 1% seaweed level was identified as the most appropriate balance, providing high textural strength while maintaining a more desirable appearance, as supported by the sensory evaluations. The optimized seasoning formulation with 2% salt, 4% sugar, 0.5% MSG, 0.7% garlic powder, 1% seaweed, 0.5% chili powder, and 1% chili sauce,

In general, NT4 demonstrated the best average performance on structure, flavor, and color. Although it did not always achieve the highest individual score for every attribute, the differences were minimal and not statistically significant for flavor and color. These results suggest that NT4 represents the most balanced formulation with an optimal combination of seaweed and seasonings for sensory acceptance.

contributed to enhancing the sensory perception of the product.

Overall, the study demonstrates that optimizing salt concentration, seaweed content and seasoning components can significantly enhance the quality and acceptability of squid sausage produced from *Sthenoteuthis oualaniensis*, thereby supporting its potential for developing value added products.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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