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CONTENT

Factors affecting the relationship quality between coffee farmers and local traders: A case study in a highland commune of Dak Lak, Vietnam

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ABSTRACT

The study examined factors affecting the relationship quality between coffee farmers and local traders. This study used data collected from 201 coffee farmers. The results showed that there were five factors affecting the relationship quality, including collaboration, perceived price, profit/risk sharing was power asymmetry, and effectiveness communication. Profit/risk sharing was the most important factor positively influencing the relationship quality between coffee farmers and local traders while power asymmetry negatively affected the relationship quality. The study also indicated that relationship quality positively impacted farmers' profit and relationship continuity intention between coffee farmers and local traders. Findings could be considered in making programs to develop the agricultural supply chain, especially to the coffee market in Vietnam.

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1. Introduction

Coffee is one of Vietnam's key export agricultural products with a turnover of over three billion USD, accounting for 15% of the country's total agricultural exports. Coffee production has created employment for thousands of rural laborers and greatly contributed to the economic and social development of Dak Lak province (Nguyen & Bokelmann, 2019). Relationship quality maintains business relationships between farmers and buyers, ensures the sustainable development of coffee production, and contributes to economic development of Dak Lak province. In coffee production and consumption, relationship quality helps to limit the disadvantages of nature, provides safe and high-quality food, and increases the competitiveness of products in the market.

Relationship quality helps maintain long-term relationships. Relationship quality is an important aspect of maintaining and evaluating relationships between buyers and sellers. Relationship quality is the awareness of relationship through three components: trust, satisfaction, and commitment. This relationship enables a competitive advantage for farmers to achieve superior business performance in the marketplace. In the agricultural supply chain, relationship quality enables farmers to bond with their buyers regarding production inputs and outputs (Schulze et al., 2006). There are many reasons for which relationship quality among supply chain's partners can reduce monitoring costs, increase cooperation and help stakeholders to deal with difficulties in coffee production (Bandara et al., 2017). Furthermore, improved relationship quality contributes to business performance for stakeholders (Baihaqi & Sohal, 2013). However, the lack of linkages in coffee production and consumption still remains because relationship quality has not been improved in this industry. The relationships are relatively loose and not legally binding. Therefore, it is necessary to study the determinants of relationship quality to strengthen and enhance the relationship.

Research on relationship quality focuses mainly on advanced economies (Schulze et al., 2006; Schulze & Lees, 2014; Lees & Nuthall, 2015), but has received little attention in transition economies. At the same time, factors affecting relationship quality in the coffee industry have different characteristics compared to those of other industries (Gërdoçi et al., 2017; Nandi et al., 2018). In Vietnam, most studies mainly focus on analyzing factors affecting the linkage between farmers and buyers in the agricultural sector (Nga & Niem, 2017). Some other studies discuss factors influencing small-scale farmers' choice of buyers (Nguyen & Bokelmann, 2019; Pham et al., 2019). The research on relationship quality has different research streams, but there has been no consensus on the conceptualization and construct measurement. Most studies suggest that trust, satisfaction, and commitment are the three dimensions of relationship quality in agricultural supply chains. Studies on factors affecting relationship quality between farmers and local traders have been very limited. Studies have mainly focused on factors that influence relationship quality with little regard to the effectiveness of specific management measures. Therefore, this study is conducted to examine factors affecting the relationship quality between farmers and local traders. The paper also offers some suggestions for better management of the relationship to ensure stable coffee production and consumption, and improve farmers' income.

2. Materials and Methods

2.1. Empirical studies on relationship quality

Relationship quality is a concept of the relationship marketing theory, which originated by Dwyer (1987) and built into the theoretical system of relationship quality by Crosby (1990). Recent studies have determined that relationship quality improves the relationship between buyers and suppliers (Schulze et al., 2006; Schulze & Lees, 2014), maintains the sustainability of relationship, and strengthens cooperative partnership (Fischer, 2013).

To measure and assess the relationship quality, researchers have employed three fundamental aspects on relationship quality, including satisfaction, trust, and commitment (Crosby et al., 1990). Satisfaction describes the situation when the purchasing process meets the needs, expectations, and goals of the parties. Suppliers' satisfaction with other partners helps build stable relationships (Schulze & Lees, 2014). Satisfaction leads to trust and relationship maintenance. Trust creates cooperation in buying and selling relationships, which in turn leads to successful relationship building (Dwyer et al., 1987; Crosby et al., 1990). Trust has widely been discussed in the distribution channel literature (Ebrahim-Khanjari et al., 2011; Capaldo, 2014). Commitment is a measure of the desired relationship and the willingness to maintain and strengthen it. Commitment represents a partner's belief that the alliance with the second partner is important and worth protecting (Nyaga et al., 2010). Thus, commitment is a very crucial measure in a long-term relationship between partners (Chen et al., 2011).

The relationship between farmers and their buyers enables farmers to connect with other stakeholders in the agricultural supply chain (Schulze et al., 2006). Commitment thrives when supply chain partners maintain the relationship for the long term (Chen et al., 2011). Satisfaction also leads to less litigation and relationship termination. Satisfaction among partners leads to the exchange of ideas, thereby allowing them to resolve their issues amicably (Nyaga et al., 2010). Literature has shown various directions for relationship quality research. Previous studies have determined that relationship quality improves the relationship between buyers and suppliers, maintains the sustainability of relationships, and strengthens cooperative partnership.

2.2. Factors affecting relationship quality

Previous studies indicate that factors affecting relationship quality are often mentioned as collaboration, perceived price, profit/risk sharing, power asymmetry, effectiveness communication. Close cooperation helps stakeholders to effectively balance supply and demand, and to enhance mutual benefits, thereby strengthening the relationship quality (Lees & Nuthall, 2015).

Price satisfaction positively affects the development of relationship quality (Jena et al., 2011; Sun et al., 2018). The profit/risk sharing factor is considered as a measure to reinforce the relationship quality (Lages et al., 2005; Sun et al., 2018). In a B2B relationship, power asymmetry implies that stronger partners are more likely to push the weaker partners to make more favorable decisions for them (Lees & Nuthall, 2015; Bandara et al., 2017). This leads to diminished quality of the relationship between farmers and local traders. Effectiveness communication positively affects the relationship quality between the farmers and local traders. Effectiveness communication is to guide and ensure that stakeholders are fully informed in the most responsive manner (Schulze et al., 2006; Kac et al., 2016).

The relationship continuity intention and farmers' profit factor are considered as a direct and positive result from relationship quality (Jena et al., 2011). A quality relationship requires the desire to maintain long-term relationship stability. Relationship continuity intention is considered a positive outcome of a quality relationship (Schulze et al., 2006). Relationship quality helps stabilize production, makes coffee easier to sell in the market, and increases coffee farmers' income. Thus, the relationship between buyers and sellers has become increasingly important in enhancing business performance (Baihaqi & Sohal, 2013).

From the transaction cost economics (TCE) perspective, a lot of literature deals with the various forms of governance structures in supply chains, with an emphasis on vertical integration. This paper intends to develop and empirically test a farmer-buyer relationship in terms of relational governance. In this paper, TCE theory and relational theory are combined to study the relationship quality between coffee farmers and local traders in the coffee supply chain.

Given above findings, seven hypotheses have been defined as follows:

H_1: Collaboration positively affects the relationship quality between farmers and local traders.

H_2: Perceived price positively affects the relationship quality between farmers and local traders.

H_3: Profit/risk sharing positively affects the relationship quality between farmers and local traders.

H_4: Power asymmetry negatively affects the relationship quality between farmers and local traders.

H_5: Effectiveness communication positively affects the relationship quality between farmers and local traders.

H_6: Relationship quality positively affects farmers' profit.

H_7: Relationship quality positively affects the relationship continuity intention between farmers and local traders.

Based on the literature review and theoretical framework, a model of factors affecting the relationship quality between coffee farmers and local traders is proposed:

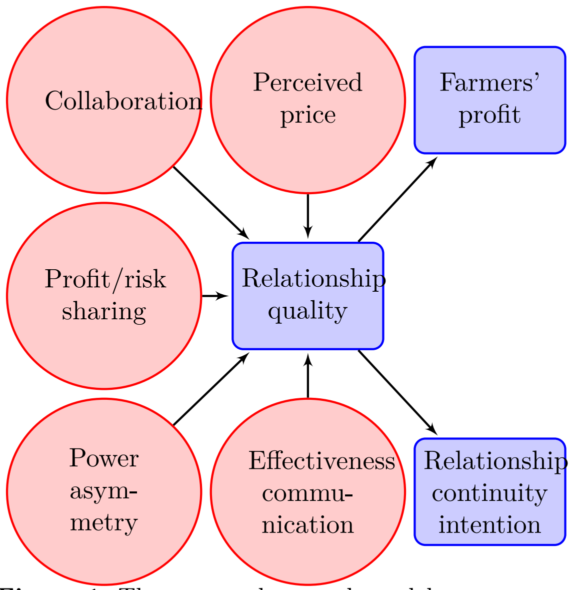

Figure 1. The proposed research model.

In Figure 1, the factors affecting the relationship quality in the proposed research model are mentioned as: (1) Collaboration, (2) Perceived price, (3) Profit/risk sharing, (4) Power asymmetry, (5) Effectiveness communication. At the same time, the relationship continuity intention and farmers' profit factor are considered as a positive result from relationship quality.

2.3. Research methods

2.3.1. Selection of study area

Ea kiet, a highland commune in the Cu M'Gar district of Dak Lak province, is chosen for this study (Figure 2). Due to its unique geographical

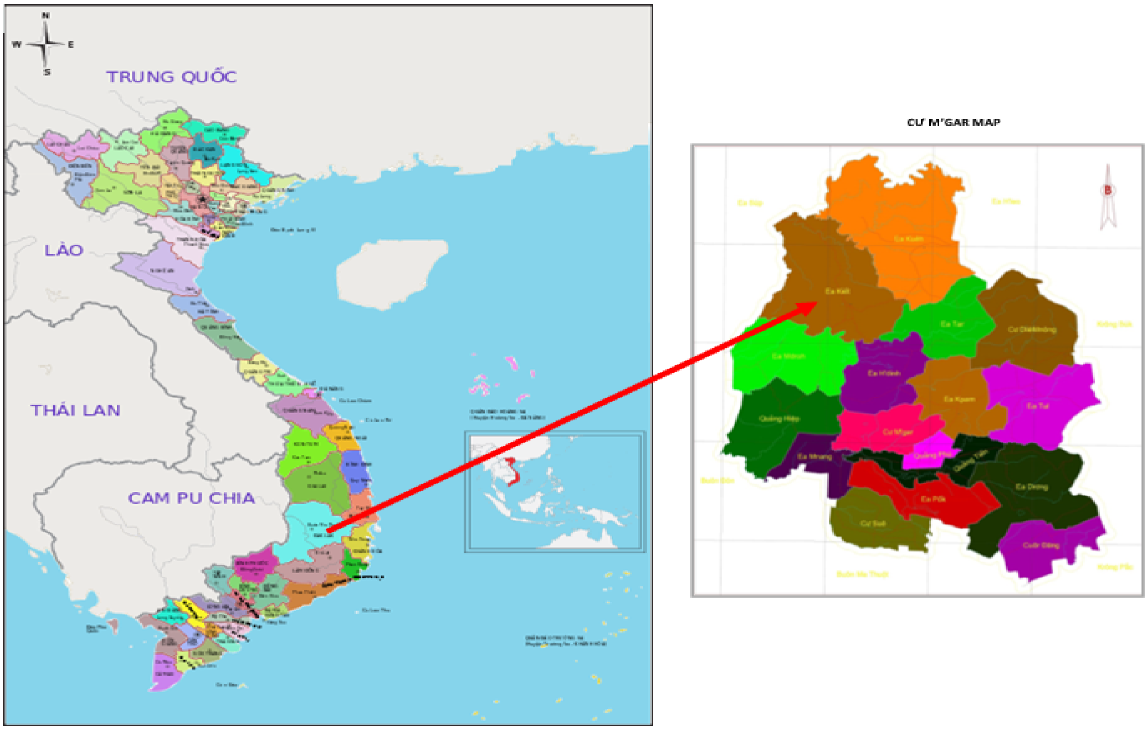

Figure 2. Study area.
Source: Statistical office of Cu M'Gar district, 2020.

location with high altitude and favorable natural conditions with rich basaltic soil, Ea Kiet commune is one of the largest coffee-producing localities in Dak Lak. Coffee production employs rural laborers and greatly contributes to the economic and social development of the region. Local authorities have developed a model that encourages the coordination of production and distribution between smallholder farmers and industrial coffee processors. In addition, transactions between farmers and local traders in Ea Kiet represent the whole Central Highlands region.

2.3.2. Data collection

According to Hair et al. (1998), the ratio between the number of observations and the number of variables should be 5:1. Therefore, the minimum sample size must be 170. We used in-person survey method to approach 201 coffee farmers who have been selling their products to local traders. Most respondents are small-scale farmers (coffee-growing area < 2 ha). The sample was selected using quota sampling. The surveyed households were selected according to the total number of coffee-producing households in each village and the coffee-producing area of the households. The statistical analysis has been conducted using SPSS and AMOS software.

2.3.3. Data analysis

Exploratory factor analysis (EFA) was conducted once the scales meet the reliability requirements. Confirmatory Factor Analysis (CFA) was utilized for evaluating the scale's convergent validity and discriminant validity. Finally, Structural Equation Model (SEM) was applied to estimate the research model and the proposed hypotheses. To assess the factors that might influence the relationship quality, a five-point Likert scale was used, where 1 = total disagreement and 5 = total agreement.

3. Results and Discussions

3.1. Socioeconomic characteristics of coffee farmers

Descriptive statistics show that the average age of coffee farmers is 42 years old with the highest age group of 35 - 45 (32.8%) and 45 - 55 (28.9%).

Table 1. Socioeconomic characteristics of coffee farmers

Variables		Quantity	Percent (%)	Total
1. Gender	Male	181	90	201
	Female	20	10	
2. Age	< 25	11	5.4	201
	25 - 35	47	23.4	
	35 - 45	66	32.8	
	45 - 55	58	28.9	
	> 55	19	9.5	
3. Education	1 - 5	41	20.4	201
	6 - 9	63	31.3	
	10 - 12	78	38.8	
	> 12	19	9.5	
4. Ethnic	Kinh	178	88.6	201
	Other	23	11.4	
5. Farm size	< 0.5 ha	68	33.8	201
	0.5 - 2 ha	125	62.2	
	> 2 ha	8	4.0	

The percentage of males involved in coffee production constitutes 90% of the total number of households. The average education level is 9 in this study (Table 1).

The average farm size is 1.3 ha. The number of farmers with coffee land smaller than 0.5 ha, from 0.5 - 2 ha, and more than 2 ha account for 33.8%, 62.2%, and 4.0% of the total farmers, respectively. The coffee harvest at Ea Kiet always lasts about one month, from late November to early December. Most coffee farmers obtain a gross margin of 60 to 80 million VND/ha/crop year. Coffee farmers achieve an average productivity of 2 - 3 tons/ha. In local coffee bean market, local traders still acquire the largest share of the market supply.

3.2. Scale reliability assessment in the research model

This study uses Cronbach's Alpha to test the strictness and correlation of items in the scale. Four observed variables were deleted because corrected item-total correlation is below 0.3 (Gliem & Gliem, 2003). The results show that the eight factors with 30 variables ensure reliability and can be used for the next step.

The result of EFA has guaranteed tests: (1) Reliability of variables (Factor loading > 0.5); (2) Eigenvalue = 1.098 > 1; (3) Research model's suitability test (0.5 < KMO = 0.836 < 1); (4) Bartlett's test for correlation of variables (Sig. = 0.000 < 0.05); (5) Cumulative variance test = 67.65% > 50% (Gerbing & Anderson, 1988; Cudeck, 2000). EFA results form 8 constructs in the study (Table 2).

The result of CFA reveals that all goodness-of-fit measures exceed the recommended acceptance levels (Chi-square = 675.114; df = 377 (P = 0.000); CMIN/df = 1.791 (< 3). All factor loadings are above 0.5 and statistically significant. Therefore, the observed variables are closely related to their representative factors. Furthermore, other goodness-of-fit indices are also met (TLI = 0.907; CFI = 0.920; GFI = 0.831 and RMSEA = 0.063 (< 0.08)). As a result, it can be concluded that the model well fits the data (Steiger, 1990).

The result of CFA confirms the unidimensionality and convergent validity of eight scales. It demonstrates that the composite reliability of the unidimensional scales is greater than 0.7 and the average variance extracted (AVE) is greater than 0.5 (Table 3). Therefore, all scales meet the requirements of reliability and convergent validity (Fornell & Larcker, 1981).

To satisfy the discriminant validity requirement, the AVE for two constructs should exceed the squared correlation between them. There is no correlation between any two constructs that is higher than either of the square root of constructs' AVEs. At the same time, maximum shared variance (MSV) is less than average variance extracted (AVE) (MSV < AVE). This pro-

Table 2. The factor loadings

Factors	Sign[1]	Factor loadings							
		1	2	3	4	5	6	7	8
Effectiveness communication	EC1	0.872							
	EC2	0.789							
	EC3	0.795							
	EC4	0.981							
Farmers' profit	FP1		0.629						
	FP2		0.946						
	FP3		0.861						
	FP5		0.854						
Relationship quality	RQ1			0.768					
	RQ2			0.814					
	RQ3			0.749					
	RQ4			0.970					
Power asymmetry	PA1				0.693				
	PA2				0.681				
	PA3				0.710				
	PA4				0.913				
Perceived price	PP1					0.741			
	PP2					0.655			
	PP3					0.706			
	PP4					0.854			
Relationship continuity intention	CI1						0.573		
	CI2						0.814		
	CI3						0.657		
	CI4						0.869		
Collaboration	CN1							0.787	
	CN2							0.868	
	CN3							0.776	
Profit/risk sharing	RS1								0.737
	RS2								0.751
	RS3								0.924
Eigenvalues		8.145	3.725	2.752	2.135	1.953	1.644	1.347	1.098
Cumulative variance = 67.65%		26.072	11.563	8.068	6.165	5.343	4.445	3.384	2.617
Cronbach's Alpha		0.916	0.899	0.907	0.856	0.823	0.848	0.853	0.844

[1]EC: effectiveness communication; FP: farmers' profit; RQ: relationship quality; PA: Power asymmetry; PP: perceived price; CI: relationship continuity intention; CN: collaboration; RS: profit/risk sharing.

Table 3. Results of reliability and convergent

Component scales	Number of observed variables	Composite reliability (CR)	Average variance extracted (AVE)
Collaboration (CN)	3	0.855	0.663
Perceived price (PP)	4	0.825	0.542
Profit/risk sharing (RS)	3	0.846	0.648
Power asymmetry (PA)	4	0.858	0.603
Effectiveness communication (EC)	4	0.919	0.740
Relationship quality (RQ)	4	0.909	0.714
Relationship continuity intention (CI)	4	0.849	0.585
Farmers' profit (FP)	4	0.905	0.706

Table 4. Results of discrimination validity

Component scales	Number of observed variables	Average variance extracted (AVE)	Maximum Shared Variance (MSV)
Collaboration (CN)	3	0.663	0.367
Perceived price (PP)	4	0.542	0.108
Profit/risk sharing (RS)	3	0.648	0.158
Power asymmetry (PA)	4	0.603	0.343
Effectiveness communication (EC)	4	0.740	0.033
Relationship quality (RQ)	4	0.714	0.218
Relationship continuity intention (CI)	4	0.585	0.367
Farmers' profit (FP)	4	0.706	0.215

vides support for discriminant validity among the constructs (Table 4).

3.3. Structural equation modeling analysis and hypothesis test

SEM analysis with indices such as df = 387, Chi-square = 766.685, P = 0.000, CMIN/df = 1.981 < 3 and other goodness-of-fit indices were also achieved. Thus, five factors affecting the relationship quality between farmers and local traders include collaboration, perceived price, profit/risk sharing, power asymmetry, effectiveness communication. The most important contributor to the relationship quality is profit/risk sharing with a regression weight of 0.28. Collaboration (0.20) is the second most important relationship quality determinant, followed by effectiveness communication (0.17) and perceived price (0.16). Finally, power asymmetry factor has a significantly negative impact (-0.19). The results also show that the relationship quality factor positively affects farmers' profit (0.48) and relationship continuity intention (0.50) among coffee farmers and local traders (Figure 3). Those five determinants explain approximately 35% of the variance in the relationship quality score.

In addition, the path coefficients are statistically significant (P-value < 0.05; C.R > 2) and are consistent with the model (Table 5). Therefore, hypotheses H_1, H_2, H_3, H_4, H_5, H_6, and H_7 are accepted at a significant level of 5%. The results of the hypotheses test confirm statistically significant relations between the factors in the model.

The study uses the Bootstrap method with the number of resamples N = 500 to test the reliability of estimates (Schumacker & Lomax, 2004). The bootstrap method involves iteratively resampling a dataset with replacement to test the reliability of the estimates. The results show that the standard errors of Bias are very small (SE-Bias < 0.05), so it can be concluded that the estimates in the model are reliable (Table 6).

3.4. Discussion

These results can be better explained in practice. Clearly, the business relationship also occurs at least in part through positive collaboration. The collaboration covers all aspects that can be shared by stakeholders to achieve an in-depth understanding (Touboulic & Walker, 2015). A positive collaboration contributes to the stability of relationships by reducing the probability of partners switching. Collaboration involves resolving conflicts among supply chain stakeholders so that relationships can remain for a long time. Furthermore, the collaboration in business relationships mostly helps to enhance the relationship quality. Besides, effectiveness communication is also the main determinant of relationship quality, holding an important mediation role. Communication refers to accessing information (prices, market orientation, quality requirements, and promotion plans) to help farmers adapt more quickly to market changes. Thus, communication positively influences relationship quality. From a TCE perspective, information sharing counteracts opportunistic behavior and reduces adverse selection as well as moral hazards.

Profit/risk sharing helps to reduce instability, leading to relationship maintenance. Buyers share risks with farmers in terms of regularly exchanging market information and manufacturing tech-

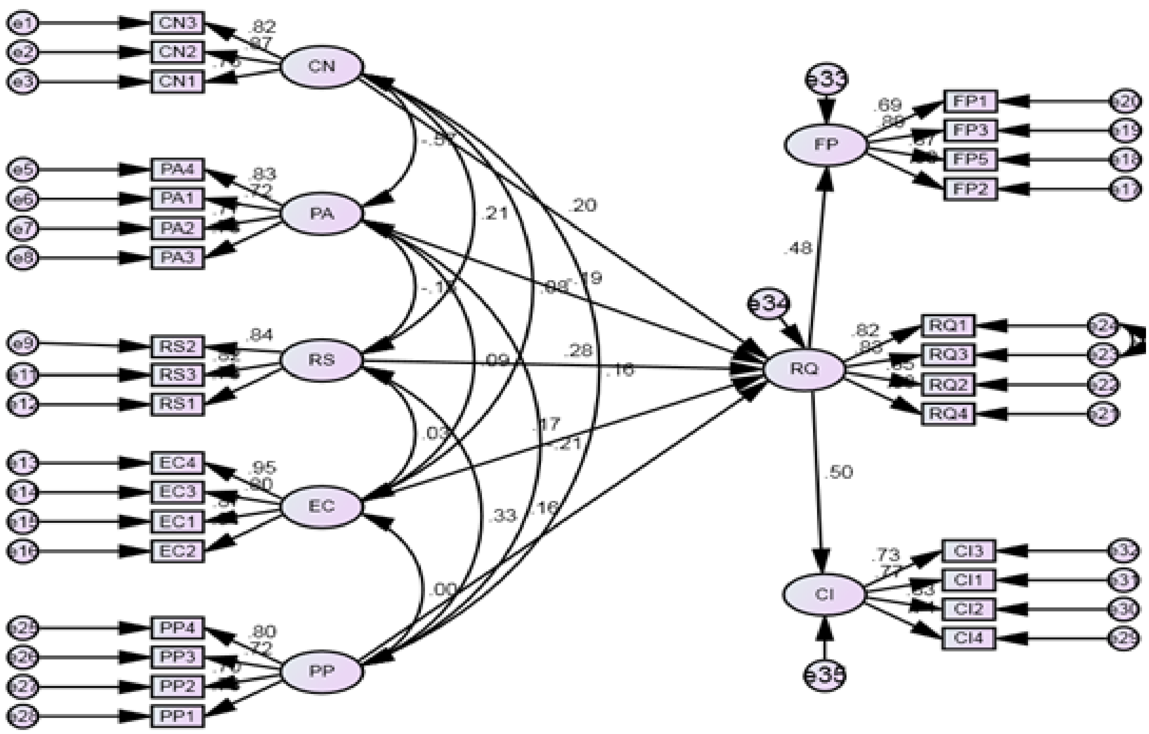

Figure 3. Factors affecting relationship quality.

Table 5. Results of hypotheses test

Hypotheses	Relations[1]	Estimate	S.E.	C.R.	P-value	Conclusion
H_1	CN → RQ	0.201	0.100	2.224	0.026	Accepted
H_2	PP → RQ	0.159	0.071	2.105	0.031	Accepted
H_3	RS → RQ	0.280	0.080	3.690	0.000	Accepted
H_4	PA → RQ	-0.195	0.102	-2.159	0.010	Accepted
H_5	EC → RQ	0.169	0.075	2.566	0.035	Accepted
H_6	RQ → FP	0.484	0.077	6.662	0.000	Accepted
H_7	RQ → CI	0.495	0.051	6.142	0.000	Accepted

[1]EC: effectiveness communication; FP: farmers' profit; RQ: relationship quality; PA: Power asymmetry; PP: perceived price; CI: relationship continuity intention; CN: collaboration; RS: profit/risk sharing.

Table 6. Results of Bootstrap test

Parameter[1]	Estimate	SE	SE-SE	Mean	Bias	SE-Bias
RQ ← CN	0.223	0.112	0.004	0.223	0.001	0.005
RQ ← PA	0.150	0.126	0.004	-0.217	0.003	0.006
RQ ← RS	0.295	0.086	0.003	0.290	-0.005	0.004
RQ ← EC	-0.220	0.084	0.003	0.199	0.005	0.004
RQ ← PP	0.193	0.066	0.002	0.154	0.004	0.003
FP ← RQ	0.512	0.085	0.003	0.512	0.000	0.004
CI ← RQ	0.314	0.058	0.002	0.309	-0.005	0.003

[1]EC: effectiveness communication; FP: farmers' profit; RQ: relationship quality; PA: Power asymmetry; PP: perceived price; CI: relationship continuity intention; CN: collaboration; RS: profit/risk sharing.

niques to help farmers orientate the production direction in the most optimal way. When the price of coffee in the market increases, local traders will be having more profit. Farmers will engage with local traders who are willing to share a part of the profit with them (Lages et al., 2005; Sun et al., 2018). Therefore, profit/risk sharing is essential factor in the relationship between sellers and buyers. Next, if farmers are satisfied with the product price, they will continue to cooperate with buyers. Perceived price satisfaction includes short- and long-term satisfaction when comparing the price received to the price paid. Producers are more likely to be attracted to buyers with a reasonable price. Producers' satisfaction with the received price has the capacity to influence their perception of the relationship quality as well as their willingness to remain loyal to the buyers.

Power asymmetry refers to the ability of one partner to influence or control the behavior of another partner in a manner contrary to the desire of the second partner. Power asymmetry negatively affects the relationship quality between farmers and local traders. The market power asymmetries between business partners can create a feeling of insecurity and vulnerability among small partners in the supply chain. Due to their power, intermediaries follow some practices (e.g. delayed payment, renegotiation of the agreed price, withdrawing from the agreement, etc.) that increase costs and risks for smallholder farmers. Thus, equal power distribution might be a precondition for economic agents to get involved in business relationships and an important determinant of relationship quality (Bandara et al., 2017).

Relationship quality positively affects farmers' profit and relationship continuity intention between farmers and local traders. Relationship continuity intention is considered as a result of a quality relationship. A quality relationship enables farmers to continue selling their coffee to the previous purchasing partners. Farmers will also introduce these partners to other neighboring farmers. Besides, farmers' profit from prior relationships is an indicator of relationship quality. The relationship between buyers and sellers has become increasingly important in the agribusiness sector (Lees & Nuthall, 2015), contributing to the enhancement of farmers' interests in general and enhancing business performance in particular. In this study, building relationships with buyers helps stabilize production and increase coffee farmers' income. Good relationships make coffee easier to sell in the market. The relationship also helps create linkages in coffee production and consumption.

4. Conclusion and policy implication

Relationship quality maintains business relationships with local traders and ensures the sustainable development of coffee production. Farmers are the key contributors to the development of Vietnam's coffee sector. Local traders are the vital players in the local coffee supply chain in Dak Lak Province, enabling farmers to optimally orientate coffee production. The study identifies five elements positively impacting on the relationship quality, including collaboration, perceived price, profit/risk sharing, effectiveness communication, and power asymmetry. Profit/risk sharing is the most important factor affecting relationship quality. Power asymmetry can lead to insecurity and vulnerability for small-scale farmers. The research also indicates that relationship quality positively influences the profit and relationship continuity intention of coffee farmers towards local traders.

At present, the relationship among stakeholders has not been closely built in the agricultural supply chain. It is still relatively loose and not legally binding. It is suggested that policymakers should focus on increasing transparency and information sharing to improve the relationship quality between coffee farmers and local traders. Results of the study could be considered in other agricultural products related to the relationship between farmers and local traders, enhancing the development of the agricultural supply chain. The findings can be reinforced to agricultural products in countries with poor infrastructure, especially in regions where traders are the main purchasing channel.

5. Limitations of the study

The paper has a small sample size (201 farmers) and has not focused on in-depth research on the whole issue. The study only selects some factors affecting the quality relationship between farmers and local traders. In addition, many other factors such as uncertainty, payment conditions, support services, procurement audits, etc. have not been included in this study. Another possible limitation is that it examines the relationship between farmers and their buyers (local traders), but the data were collected from one-side of the dyads. Future studies can consider testing the model using the perspectives of both the partners.

Conflict of interest

The authors declare no conflict of interest.

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Factors influencing the adoption of "One must do, five reductions" in rice production in the Mekong River Delta: A case study in Soc Trang province, Vietnam

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ABSTRACT

After years of experimenting, the "One must do, five reductions" (1M5R) (in Vietnam referred to as 1P5G), is being promoted by Vietnam's Department of Crop Production as an advanced technique in rice production. Nevertheless, a certain proportion of rice farmers in the Mekong Delta are reluctant to implement 1M5R. This study collected data from 116 rice farming households in Soc Trang province to assess factors influencing the decision to adopt the new technique. The results showed that the 1M5R model offered better economic efficiency than the traditional producing model in terms of profit, revenue/cost ratio, and profit/cost ratio. The estimated Binary Logistic model revealed that labor, production experience, and production area significantly contributed to farmers' adoption of 1M5R. These results are the empirical evidence of the potential of 1M5R, supporting its promotion in Vietnam's Mekong River Delta.

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1. Introduction

Sustainable agriculture is a long-term objective of Vietnam since agriculture has always been a vital role in the country's economy. Among primary export agricultural commodities, rice is the most essential product because it significantly contributes to Vietnam's GDP and food security. The Mekong River Delta is called the rice bowl of Vietnam, as it accounts for more than 50% of the country's output (GSOV, 2021). For decades, farming methods have been continuously improved to achieve the efficiency of rice production in the delta, which enabled Vietnam to become one of the most rice exporters in the world. Nevertheless, rice production in the Mekong River Delta is fragmented and vulnerable to external pressures (Nguyen et al., 2015; Hoang et al., 2018; Hoang et al., 2019). The average farm size per household is 1 ha, in which 48% of the rice fields are 0.5 to 2 ha, 38% less than 0.5 ha, and 10% more than 2 ha (Connor et al., 2020). Small scale farming is less likely to achieve economies of scale, and they are less resilient to disturbances, especially natural climate extremes. Moreover, the excessive use of inputs to boost production generated adverse externalities on the environment and human health (Chau et al., 2015) and diminishing marginal returns (GSOV, 2021). Thus, to ensure that rice production is sustainable, advanced farming techniques are continuously researched and developed.

The "One Must Do, Five Reductions" (1M5R) is an integrated technology package that evolved from the "Three Reductions, Three Gains" (3R3G) program. "One Must" means the use of certified seeds, and "Five Reductions" encompasses the reduction of seed rate, fertilizer use, pesticide use, water use, and post-harvest losses (Stuart et al., 2018). 1M5R is developed to minimize negative impacts from excessive input uses as well as to increase rice productivity, raise incomes for farmers, expand economically effective rice cultivation models, ensure human safety and environmental sustainability. After years of experimenting in many southern provinces, Vietnam's Department of Crop Production has acknowledged 1M5R as an advanced technique in rice farming. As a result, 1M5R was certified by a Presidential decree (532 - QD - TT - CLT) as the national program after 3R3G to implement best rice cultivation practices (Stuart et al., 2018). A great amount of effort has been used to promote it through workshops, trainings, focus group discussions and demonstration sites (Connor et al., 2020), but not every rice farmer is willing to adopt and implement it.

Therefore, understanding farmer behaviors and decision making is necessary to promote sustainable agriculture (Feola et al., 2015). Many studies attempted to investigate factors influencing the adoption of new farming technologies, resulting in various factors from economics, environment, and psychology. For example, Dessart et al. (2019), examined the positive effects of behavioral factors and social and cognitive factors in increasing the adoption of environmental practice. Bopp et al. found significant influences of socio-economic characteristics, personal needs, and environmental factors on adopting sustainable agricultural practices in Chile. Besides, farmers' perceptions of easiness, benefit, satisfaction and expectation can affect the willingness to implement advanced farming technologies and models (Ekane et al., 2016; Connor et al., 2020; Wehmeyer et al., 2020).

In the Mekong River Delta, the capability of 1M5R in reducing negative environmental impacts and increasing profitability has already been examined (Truong et al., 2013; Stuart et al., 2018). Its adoption increases together with improved levels of educational, participation in cooperatives, and training attendance (Le et al., 2021). By contrast, factors that hinder adoption include difficulties to apply the desired best practices, the suitability for cropping patterns, and weather conditions (Connor et al., 2020). As previous findings indicate that adoption behaviors are different depending on the agricultural context, it is necessary to have more insights into the technical package so that appropriate policies can be made. In such context, this study was conducted to provide an additional empirical understanding of the economic potential of 1M5R along with factors influencing its adoption.

2. Materials and Methods

2.1. Study site

Soc Trang is an agricultural province where more than 60% of the province's labor concentrates in agricultural production. The total land area of Soc Trang is 322,330 ha, of which the rice-cultivated area is 171,200 ha.

This study was conducted in Nga Nam Town, one of the primary rice producers of Soc Trang province. The local rice production area is 18,176 ha (accounting for 83.47% of the agricultural land area). However, in recent years, local rice farmers repeatedly have to face many risks in production, resulting in precarious income. The most concerning menace in the Mekong River Delta are the increasing impacts of climate change, in which saltwater intrusion is most evident (Hoang-Phi et al., 2021). Besides, market prices of agricultural inputs and outputs have been fluctuating in a detrimental direction to farmers.

2.2. Data collection

This study uses primary data collected from 116 rural households. The survey employed a random sampling method and a semi-structured questionnaire. There were three categories of collected information: (1) household information (including gender, age, educational levels, production experience, and demographic characteristics); (2) information on farming techniques and financial efficiency (including crop types, seed usage, fertilizer, and pesticides, water management, crop care, harvesting and cultivation costs, yield, selling price); and (3) information regarding farmers' knowledge of 1M5R.

2.3. Methods

2.3.1. Participatory rural appraisal (PRA), focus group discussion (FGD), and key informant panel (KIP)

The PRA, FGD, and KIP are common techniques that are utilized to study farmers' perception and adoption of advanced technologies (Ngoan & Howeler, 2007; Pandey et al., 2011; Abakemal et al., 2013). The FGDs was conducted with six groups in three communes of Nga Nam Town. The interviewees encompassed people who either participated or did not participate in the 1M5R program. The participants were those who have experience and understanding of rice production at the study site. The author also employed KIP to interview ten key informants, including farmer collaborators (3 people), representatives of farmers' associations (3 people), and locally knowledgeable elders (4 people). Discussed contents covered the history and current development of rice production in the area; encountered advantages and difficulties in applying 1M5R; factors influencing people's decision to implement 1M5R; and their potential solutions.

2.3.2. Binary logistic regression

Because surveyed households can be categorized into groups of those that implemented and those that did not implement 1M5R, Binary logistic regression was suitable to assess factors affecting the adoption of the new technology package. The formula of the model is:

$$\ln\left[\frac{\mathrm{P(Y{=}1)}}{\mathrm{P(Y{=}0)}}\right] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_i X_i$$

Where in:

Y=0 means the household did not adopt 1M5R

Y=1 means the household adopted 1M5R

X_i are the explanatory variables (Table 1)

Table 1. Explanatory variables in the regression model

Variables	Expected Correlation	Unit	Explanation
X_1	+	Years	The educational level of the household head. The more years of schooling the householder, the more knowledge he/she obtains, leading to greater awareness of 1M5R's benefits and higher participating possibilities.
X_2	+	People	The number of laborers within the family. Families with more laborers have more incentives to apply a new farming model to increase income.
X_3	+	Hectare	Rice production area. Greater production scales have better cost-efficiency when applying advanced technologies
X_4	+	Years	Rice production experience

3. Results and Discussion

3.1. Rice production and the development of 1M5R at the study site

The 1M5R program that is currently applied in Nga Nam town was developed from former farming system programs starting in the early 1990s. These programs were received and appreciated by farmers and exhibited positive outcomes. In 2009, Soc Trang province conducted

Table 2. Results from PRA discussions

Year	Program/Event
1994	Integrated Pest Management (IPM)
1996	Introducing NES: no early spray for leaf-eating insects Occurrence of Yellow snail pandemic
2001	Occurrence of Brown aphids, seedless crops
2009	Introducing the Three Reductions, Three Gains" (3R3G) program
2012	Occurrence of Barley yellow dwarf, ragged stunt virus – RRSV, and hoarfrost The province declared a state of emergency
2013	Introducing the "One must, five reductions" program

Table 3. Rice production area

Production area	Adopting 1M5R		Non-adopting 1M5R		Total sample	
	Count	Percentage	Count	Percentage	Count	Percentage
< 1 ha	10	18.87	18	28.57	28	24.14
1 - 2 ha	11	20.75	29	46.03	40	34.48
2 - 3 ha	16	30.19	9	14.29	25	21.55
3 - 5 ha	11	20.75	7	11.11	18	15.52
5 - 8 ha	2	3.77	0	0.00	2	1.72
≥ 8 ha	3	5.66	0	0.00	3	2.59
Sum	53	100.00	63	100.00	116	100.00

the pilot implementation of the 3R3G program in some selected districts. In order to reduce greenhouse gas emissions, Soc Trang Provincial Agricultural Extension Center, in collaboration with the National Agricultural Extension Center, organized 18 training courses for farmers on applying 3R3G and SRI rice cultivation techniques. In addition, there were training, technical transfer, and demonstration of water-saving irrigation rice farming models in 2 districts of Nga Nam and Long Phu. As a result, the local government and farmers evaluated water-saving irrigation techniques as highly feasible. Currently, rice farmers in My Tu, Tran De and Nga Nam have partly started participating in the 1M5R program (Table 2).

In the study site, the rice planting schedule consists of 2 seasons, of which Winter-Spring is the main farming season in a year. The Winter-Spring rice crop usually begins in November and harvests in February of the following year. The Autumn-Summer crop is from May to August.

There were 53 households adopted 1M5R in the sample and 63 households did not adopt the technique. Households with a 1 - 2 ha production area accounted for the highest proportion of 34.48%, followed by less than 1 ha (24.14%) and 2 - 3 ha (21.55%) (Table 3). Households whose rice fields were larger than 5 hectares or more only accounted for a relatively low proportion. There is a noticeable difference in the production scale between the two groups. Farmers adopted 1M5R had larger average fields and concentrated in larger production scale categories. Also, 54.31% of the households cultivated on slightly alum-contaminated alluvial soil. Other types of soil included mildly salt-contaminated alluvial soil, alluvial soil, and clay.

Water sources for rice farming were similar in both groups. Almost all of the surveyed households obtained water from local rivers and canals by self-invested pumping systems. Only 10% of the sample utilized water provided by cooperatives.

Rice varieties also showed no differences as farmers mainly use highly adapted varieties to alum-contaminated and salt-contaminated fields such as RVT fragrant rice, OM 4900, OM 5451 (Table 4). The RVT fragrant rice was especially favoured in both planting seasons thanks to its high resistance to extreme climate conditions and various pests and diseases such as brown aphids, rice blast, and sheath blight.

Household heads graduated from secondary school accounted for 48.28% of the total sample, high school 22.41%, and elementary school 19.83%. Such educational levels revealed that farmers in Nga Nam town dropped out of school early. Educational standard is comparably low in both 1M5R adopted and non-adopted groups. On average, each family had two to three people engaging in rice farming activity and they had 20 - 30 years of experience.

All households participating in the program were trained in the 1M5R technique. However, the number that was supported to implement the model was limited (12 households) (Table 5). Nevertheless, many of them were self-invested in deploying the model, which indicates that farmers genuinely recognize the benefits of the 1M5R program and are willing to adopt it. Thus, more support from the authorities are required to encourage and attract more farmers to participate in the program. Currently, in the study area, 1M5R is not the sole farming technique applied by farmers as it is combined with other programs to enhance production efficiency. For example, from 2018 to 2019, a project titled "Adaptive livelihoods ensure food security and climate change response for vulnerable communities in Vietnam" was implemented in Nga Nam district by the Bread for the World, Action on Poverty, The Consultative Institute for Socio-Economic Development of Rural and Mountainous Areas. This project helped farmers adapt to salt intrusion in Nga Nam district by combining the five reductions of 1M5R with 5 must, including 1) Record production logs, input origins, and products; 2) Products are not contaminated with banned substances; 3) Have community and environmental responsibility, honesty and transparency in production; 4) Achieving the certificate of registered organic standards (being tested and evaluated); 5) Harmonize socio-economic and environmental efficiency. This technique helped reduce financial vulnerability from climate change, and adaptability is also better both financially and ecologically.

The collected data revealed variances in production costs between the traditional farming model and the 1M5R model (Table 6). For example, for every 1000 m^2, the differences between non-adopting and adopting families were 94.96 thousand VND and 128.88 thousand VND in the Winter-Spring and Summer-Autumn crops, respectively. The divergences can be attributed to advances in planting stages such as line sowing and selected fertilizing and spraying in reasonable periods. Specifically, the seed cost of the 1M5R model was 175.81 thousand VND/1000

Table 4. Popular rice varieties

Varieties	Adopting 1P5G households				Non-adopting 1P5G			
	Winter-Spring	%	Summer-Autumn	%	Winter-Spring	%	Summer-Autumn	%
OM 4900	10	18.87	8	15.09	15	23.81	15	23.81
OM 5451	4	7.55	10	18.87	3	4.76	12	19.05
RVT 33	62.26	30	56.60	36	57.14	30	47.62	
Others	6	11.32	5	9.43	9	14.29	6	9.52
Sum	53	100	53	100	63	100	63	100

Table 5. Implementation of 1M5R

Participation	Count	Percentage
Households participating in the 1M5R program	53	
Trained households	53	100
Of which		
Supported to implement	12	22.64
Self-invested to implement	33	62.26
Unsuccessfully implement	8	15.09

m^2 for both planting seasons, while the traditional farming practice had to pay 198.54 thousand VND and 201.55 thousand VND/1000 m^2 for Winter-Spring seasons and Summer-Autumn seasons, respectively. The total expenses of fertilizer and pesticide showed a similar trend as it cost non-adopting households 40 to 60 thousand VND/1000 m^2 more than adopted households. In addition, farmers who applied 1M5R had lower expenses in hiring laborers for sowing, fertilizing, and spraying.

In general, the 1M5R model resulted in better returns for farmers participating in the program 1M5R adopting families earned 76 to 223 thousand VND/1000 m^2 more than non-adopting families. Ratios of revenue/cost and profit/cost were also higher in the participant group. The above analysis is mainly based on the cost and revenue data of the rice production process. On the other hand, the 1M5R program also helps farmers identify and be aware of the impacts of climate variations, facilitates cooperation and large-scale centralized production.

3.2. Factors affecting the adoption of 1M5R

Among the proposed explanatory variables, educational level had a significant level of 0.366, indicating no correlation between schooling and the possibility of adopting the 1M5R program. According to the survey, most households only reached elementary and secondary school, so this variable has little variation and shows no influence on farmers' decisions.

On the other hand, laborers, experience, and production area all had significant correlations with the dependent variable (Table 7). The labor variable was positively correlated with 1M5R adoption, indicating that households with more laborers are more likely to adopt the model. Phases in the model require human efforts to perform optimally, so it is easier for households with more workers to apply the technology package successfully. Production experience also helps increase the chances of implementing 1M5R. Thus, the more experienced rice producers are, the more likely they will accept new farming models to improve productivity and reduce costs. According to the survey results, households participating in 1M5R whose production experience over 40 years accounted for 23% of the sample. Therefore, local rice farmers had a lot of experience and were well aware of the disadvantages of traditional farming practices, so they were willing to accept new production models. Lastly, the adoption of 1M5R increases together with the production area.

The estimated model implies that the state needs to have policies to retain experienced agricultural workers in rural areas instead of letting them switch to non-agricultural activities or migrate to big cities in search of employment. In fact, the application of 1M5R technology requires labor resources to meet the production stages according to the process. Besides, because many households use a small and fragmented land area, it is necessary to propagate to the people to understand the meaning of "Canh Dong Mau Lon", aiming toward forming and expanding the high-quality rice production region.

4. Conclusions

The area of rice cultivation in the study area is generally stable. From 2009 to the present, there is just a slight increase in the production area. The access and application of scientific and technical advances of the majority of farmers have been enhanced. Moreover, agricultural mechanization was promoted; the canal system was gradually dredged, and there have been constructions of irrigation pumping stations. These improvements created favorable settings for the application of the 1M5R program.

Currently, more than 40% of the rice cultivat-

Table 6. Average costs and economic efficiency per 1000 m^2 of rice field

	Criteria	Adopting 1M5R		Non-adopting 1M5R	
		Winter-Spring	Summer-Autumn	Winter-Spring	Summer-Autumn
Costs (thousand VND)	Seeds	175.81	175.81	198.54	201.55
	Fertilizers	498.69	496.92	520.54	520.92
	a. NPK 16 - 16 - 8	19.50	27.69	17.66	15.84
	b. NPK 20 - 20 - 15	136.26	135.20	131.97	149.24
	c. NPK 24 - 24 - 20	7.92	7.13	7.27	8.89
	d. DAP	86.40	86.04	69.06	71.35
	e. Nitrogenous	51.50	51.38	61.21	62.35
	f. Phosphate	17.57	17.73	8.34	8.44
	g. Potassium	45.29	45.81	31.86	32.61
	h. Organic	0.00	0.00	0.00	0.00
	i. Manure	0.00	0.00	0.00	3.17
	j. NPK 25 - 25 - 5	129.53	116.51	165.74	149.31
	k. Others	4.72	9.43	27.42	19.72
	Pesticides	320.34	299.92	359.44	359.66
	Irrigation	127.50	44.79	124.48	43.39
	Hired Soil preparing	18.12	18.24	17.40	15.94
	Rented tillage machines	117.22	120.87	129.82	141.62
	Hired Sowing	17.86	17.66	33.78	22.96
	Hired Fertilizing	37.75	33.79	23.82	23.69
	Spraying	81.76	74.93	66.51	71.44
	Harvest	240.78	246.53	256.48	257.19
Efficiency	Cost (thousand VND)	1635.84	1529.48	1730.81	1658.35
	Revenue (thousand VND)	4660.80	2597.38	4532.38	2649.96
	Profit (thousand VND)	3024.96	1067.90	2801.57	991.61
	Revenue/Cost	2.85	1.70	2.62	1.60
	Profit/Cost	1.85	0.70	1.62	0.60

Table 7. Estimated regression model

	B	S.E.	Wald	Df	Sig.	Exp(B)
X_1	0.059	0.065	0.818	1	0.366	1.061
X_2	0.944	0.274	11.895	1	0.001	2.569
X_3	0.030	0.018	2.761	1	0.097	1.031
X_4	0.010	0.010	5.164	1	0.023	1.000
Constant	-3.984	0.971	16.838	1	0.000	0.019

ing area applies the 1M5R model, but each household's adoption level is different. The application of 1M5R requires regular monitoring and relatively flat rice fields, but some farmers are still familiar with traditional farming practices. There are sites with rough field conditions, incomplete irrigation systems, and limited training provided to farmers, making it difficult to expand the program.

The comparison proved that 1M5R adopted households need fewer investments but gain better returns than traditional farming practices. In addition, laborers, experience, and production area were shown to contribute to adopting the technology package significantly.

In conclusion, 1M5R is a technique that helps rice farmers produce more effectively. Economically, it reduces investment costs, improves profits and incomes for farmers. In terms of environment, the 1M5R technology lessens environmental pollution by reducing the quantity of chemical fertilizers and pesticides in stages of production. Moreover, the efficiency of water use in rice cultivation has been considerably improved from the application of 1M5R. Finally, 1M5R is socially efficient because it enhances farmers' technical skills as well as reduces labor cost requirements.

Conflict of interest

The authors declare no conflict of interest.

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Effect of three different organic fertilizers on growth, yield, and essential oil content of basil (*Ocimum basilicum* var. *pilosum*)

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ABSTRACT

Basil (*Ocimum basilicum* var. *pilosum*) is an herbaceous plant species exhibiting various economic and medicinal values. This study aimed to determine the effect of different organic fertilizers within creasing application rates on the plant growth, fresh yield, and essential oil content of basil grown in Thu Duc city. The treatments were a factorial combination of three kinds of organic fertilizers (cow manure fertilizer (CMF)–control, worm castings manure fertilizer (WCF) and Komix organic fertilizer (KOF)) and five levels of application (5-control, 10, 15, 20 and 25 tons/ha). The field experiment was arranged in split-plot design with three replications. Generally, when applying WCF and KOF with the amount of 5, 10, 15, 20, and 25 tons/ha, the indexes resulted in higher plant height, stem diameter, number of primary branches, number of leaves and chlorophyll index CMF. Among the three types of fertilizers, WCF gave higher results than KOF and CMF. Specifically, the tree height reached 37.9 cm, the base diameter was 5.5 mm, and the number of leaves was 13.3 branches/plant. There was no significant difference in plant growth when increasing the fertilization rate from 15 to 25 tons/ha. The results showed that the application of 25 tons/ha of WCF gave to best results of fresh weight (93.3 g/plant), theoretical yield (24.9 tons/ha), actual yield (14.7 tons/ha), essential oil content (0.47 mL)/100 g), and yield of essential oil (117 L/ha). The economic efficiency analysis showed that the highest total profit after three harvests (VND 737,570,000 per ha) was obtained at the treatment of 25 tons/ha WCF, while that of 5 tons/ha achieved the highest real benefit-cost ratio (3.26).

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1. Introduction

Basil (*Ocimum basilicum* var. *pilosum*) is an herbaceous species belonging to the group of basil. Basil is a typical spice of the South-Central provinces and the Central Highlands, especially famous in Phu Yen province. Basil contains essential oils and oleoresin that are used as a flavouring agent in food, in cosmetics and ingredients in traditional medicine (Tambun et al., 2017).

Organic fertilizer contains both macro and micronutrients, which helps to reduce dependence on chemical fertilizers. Bufalo et al. (2015) indicated that applying the appropriate amount of organic fertilizer for basil created favorable conditions for photosynthesis in plants and the accumulation of nutrients. Therefore, increasing plant yield and essential oil content. In Vietnam, the organic fertilizer market is diverse with multiple different types, such as cow dung manures, green manures, microbial fertilizers, and mineral organic fertilizer, however, the utilization of them on basil is not well understood in the study area. Therefore, the objectives of this study were to determine the appropriate types and application levels of CMF, WCF, and KOF for maximizing the yield and essential oil content of basil grown on loamy sand soil in Thu Duc City.

2. Materials and Methods

2.1. Experimental design

The experiment was carried out at the Experimental field of the Faculty of Agronomy, Nong Lam University, Ho Chi Minh City from September 2020 to January 2021. The two-factor experiment was laid out in a split-plot design with three replicates. The main plot (H) consisted of three kinds of organic fertilizers (CMF-control, WCF, and KOF). The sub-plot (L) included five levels of application rates (5-control, 10, 15, 20, and 25 tons/ha). The basil was planted at a density of 266,667 plants/ha. The spacing between blocks, plots, rows and plants were 1.0, 0.5, 0.25, and 0.15 m, respectively. The plot area was 6 m^2 (6.0 m $\times$ 1.0 m) and total experimental area was 270 m^2. All treatments were fertilized with the same dose of 500 kg lime, 135 kg N, 75 kg P_2O_5, and 120 kg K_2O per hectare (ha).

The physicochemical properties of soil and organic fertilizers are presented in Table 1. Soil sample in the experiment tested in Southern Forestry Science Institute. The data showed that the soil is clay loam, moderately acidic, low in soil organic matter, total N but high in total P_2O_5 and K_2O. The organic fertilizers used in the experiment had organic matter of 22.0 - 88.68%. Total N, P_2O_5 and K_2O of organic fertilizers ranged from 1.06 - 2.50%, 0.07 - 4.0% and 1.31 - 6.0%, respectively (Table 1).

Table 1. Selected physicochemical properties of soil and organic fertilizer used in the experiment

Properties	Soil[1]	CMF[2]	WCF[3]	KOF[4]
Texture				
Sand (%)	45			
Silt (%)	28			
Clay (%)	28			
pH_{H_2O}	5.58			
MC (%)		22.19	15.0	25.0
OM (%)	1.28	88.68	22.0	22.0
Total N (%)	0.06	1.06	1.57	2.50
Total P_2O_5 (%)	0.15	0.07	1.24	4.0
Total K_2O (%)	2.5	1.31	0.67	6.0

CMF = cow manure, WCF = worm castings fertilizer, KOF = Komix organic fertilizer, OM=organic matter, MC = moisture content.
[1]Forest Science Institute of South Vietnam, 2021; [2]Research Institute for Biotechnology and Environment. [3]SFARM, 2020; [4]TSJSC, 2012.

2.2. Data collection and statistics

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 detail, the plant height, the number of leaves, stem diameter, the number of primary branches before harvest, the number of leaves on the main stem, and the number of shoots was calculated 20 days after planting (DAP). The average fresh weight of stems and leaves (g/plant) was calculated in 90 DAP: Measured the average weight fresh of stems and leaves of 10 target plants, cut with a sickle at 3 cm from the ground (P_1). Theoretical fresh yield (ton/ha) = P_1 (g/plant) x 10^{-6} $\times$ Planting density (plant/ha). Actual harvested yield (ton/ha) = [Weight of fresh stems and leaves of each base plot (kg) x 10^{-3} $\times$ 10,000]/Area of the base plot (m^2).

For the analysis of the essential oil content of the basil, 100 g/pot of branches and leaves of 10 target plants, collected at the time point when the plants on the experimental plot had buds and begun to bloom, were used. The samples after being harvested were kept for 2 - 3 hours at room temperature, then the essential oil was distilled by steam distillation (Khang & Khien, 2001). 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 in 90 DAP. The calculation was based on the formula: Content of essential oil in leaves, stems (%) = (Volume of essential oil)/(Weight of sample) × 100. Theoretical yield of essential oil (L/ha): Calculated as essential oil content of leaves and stems (mL/100 g) × Theoretical fresh yield (ton/ha) × 10.

For the analysis of the economic efficiency, total cost (VND/ha/three harvests) = seeds + fertilizers + pesticides + labor + electricity, water; Total revenue (VND/ha/three harvests) = Actual yield (kg/ha) x selling price (VND/kg); Total profit (VND/ha/three harvests) = Total revenue - Total cost, and benefit - cost ratio = Total profit/Total cost, were measured.

Data were subjected to analysis of variance (ANOVA) using R software. Treatments means were separated using, Duncan's rank test at the 5% level of significance.

3. Results and Discussion

3.1. Effect of types and amounts of organic fertilizers on growth of basil (*Ocimum basilicum* var. *pilosum*)

As shown in Table 2, among three kinds of fertilizer, the treatment using worm castings fertilizer (WCF) produced the highest (39.7 cm) while plant height was highest when basil was fertilized with fertilizer 25 tons/ha (38.9 cm) in five levels of organic fertilizer. The height of basil plants was affected by the interaction between the type and amount of organic fertilizer, ranging from 28.8 to 41.8 cm. The highest plants were seen in the treatment using 25 tons/ha (41.8 cm) of WCF and there was a very significantly different ($P \leq 0.01$) from the plants in the treatment using 5 tons/ha of CMF (28.8 cm).

The basil with the largest stem diameter was found in the treatment using WCF (5.5 mm) and it was statistically significantly different from the other two treatments at Table 2. The use of fertilizer at a rate of 25 tons/ha created the largest stem diameter (5.5 mm) but it was not statistically different from the treatment using 20 tons/ha (5.4 mm). In addition to, the basil plants had a stem diameter ranging from 4.8 - 6.0 mm depending on different types and amounts of organic fertilizers. The basils in the treatment using WCF at a rate of 25 tons/ha had the largest stem diameter (6.0 mm) and there was very significantly different ($P \leq 0.01$) from the basils fertilized with CMF at a rate of 5 tons/ha (4.8 mm).

The study also revealed that the application of different amounts of organic fertilizers generated differences in the number of primary branches on basil. The highest number of primary branches (13.4 branches) were observed on the plants that applied 25 tons/ha of organic fertilizers and gradually reduced when applied the organic fertilizers with the lower rates (Table 2). The number of primary branches in the experiment were lower than that in the experiment of Topalov et al. (1966), which ranged from 24.6 to 25.6 branches/plant. Dinh Hai An noted that at the time of 60 DAP, the number of primary branches fluctuated from 20 to 30 branches/plant when studying the effects of manure and foliar fertilizers on the growth, development and essential oil content of the plant.

The data in Table 2 showed that the basil plants in the treatments using WCF at a rate of 25 tons/ha and 20 tons/ha showed the highest number of leaves (16.8 leaves) in Table 2 and there was significantly different from those observed in the treatment using 5 tons/ha and 10 tons/ha of CMF and KOF (14.8 and 15.0 leaves).

Statistical analysis in Table 2 showed significant differences in the number of shoots, ranging from 14.5 - 20.2 shoots. Basils in the treatment were applied WCF with the amount of 25 tons/ha had the highest number of post-regenerated shoots (20.2 shoots), whereas the basils fertilized with 5 tons/ha of KOF produced the lowest number of shoots (14.5 shoots).

3.2. Effect of types and amounts of organic fertilizers on fresh yield and essential oil yield of basil (*Ocimum basilicum* var. *pilosum*)

In Table 3, the treatment with the highest weight of fresh stems and leaves using earthworm organic fertilizer (80.2 g/plant) was statistically significantly different from the treatment using Komix fertilizer (69.1 g/plant) and cow manure fertilizer (66.5 g/plant). Using 5 tons/ha for the lowest weight of fresh stems, branches, and leaves (62.0 g/plant). Using WCF at 25 tons/ha continued to show the highest effects on the average fresh weight of stems and leaves of basil (93.3 g/plant). Whereas, the lowest average fresh weight of stems and leaves was observed on basils in the treatment with 5 tons/ha of CMF (55.0 g/plant).

Table 2. Effect of sources and application levels of organic fertilizers on plant growth of basil (*Ocimum basilicum* var. *pilosum*)

Parameters (20 DAP)	Organic fertilizers (H)	Application rates (L) (ton/ha)					
		5 (control)	10	15	20	25	Average H
Plant height (cm)	CMF (control)	28.8^{c}	29.7^{bc}	33.5^{abc}	34.2^{abc}	35.9^{abc}	32.4^{b}
	WCF	34.9^{abc}	35.9^{abc}	39^{a}	39.1^{a}	41.8^{a}	37.9^{a}
	KOF	36.3^{abc}	37.3^{ab}	38.4^{ab}	37.3^{ab}	39.0^{a}	37.7^{a}
	Average L	33.3^{c}	34.3^{bc}	36.6^{ab}	36.9^{a}	38.9^{a}	
	CV = 12.8%; FH = 3.5*; FL = 1.7*; FHL = 1.9**						
Stem diameter (mm)	CMF (control)	4.8^{c}	4.9^{bc}	5.1^{bc}	5.1^{bc}	5.1^{bc}	5.0^{b}
	WCF	5.2^{abc}	5.4^{abc}	5.3^{abc}	5.8^{ab}	6.0^{a}	5.5^{a}
	KOF	4.8^{c}	5.1^{bc}	5.2^{abc}	5.4^{abc}	5.5^{abc}	5.2^{b}
	Average L	4.9^{c}	5.1^{c}	5.2^{bc}	5.4^{ab}	5.5^{a}	
	CV = 8.6%; FH = 1.1*; FL = 1.1*; FHL = 1.1**						
Primary branches (branch/plant)	CMF (control)	12.2^{ab}	11.5^{b}	12.2^{ab}	13.0^{ab}	13.4^{a}	12.5^{b}
	WCF	12.7^{ab}	13.3^{a}	13.4^{a}	13.5^{a}	13.5^{a}	13.3^{a}
	KOF	12.9^{ab}	13.0^{ab}	13.2^{a}	13.1^{ab}	13.4^{a}	13.1^{a}
	Average L	12.6^{b}	12.6^{b}	12.9^{ab}	13.2^{ab}	13.4^{a}	
	CV = 6.6%; FH = 2.6*; FL = 4.5*; FHL = 3.0*						
Number of leaves (leave/plant)	CMF (control)	14.8^{d}	14.8^{d}	15.2^{cd}	15.5cd	15.7^{bcd}	15.2^{b}
	WCF	15.5^{cd}	15.9$^{a\text{-}d}$	16.1^{abc}	16.8^{a}	16.8^{a}	16.2^{a}
	KOF	15.0^{d}	15.1^{cd}	15.5^{cd}	15.5^{cd}	16.6^{ab}	15.5^{ab}
	Average L	15.1^{d}	15.3^{cd}	15.6^{bc}	15.9^{ab}	16.4^{a}	
	CV = 3.8%; FH = 1.3*; FL = 0.6**; FHL = 0.5**						
Number shoots (shoot)	CMF (control)	15.4^{bc}	17.0^{abc}	17.1^{abc}	17.5^{abc}	18.5^{ab}	17.1
	WCF	15.9^{bc}	16.7^{bc}	16.9^{abc}	17.5^{abc}	20.2^{a}	17.4
	KOF	14.5^{c}	15.3^{bc}	16.5^{bc}	16.9^{abc}	17.3^{abc}	16.8
	Average L	15.3^{c}	16.3^{bc}	16.8^{abc}	18.4^{ab}	18.7^{a}	
	CV = 10.7%; FH = 0.5^{ns}; FL = 0.7*; FPL = 0.5*						

Values within the same group followed by the same letters are no significant different at $P \leq 0.05$ according to the Duncan's multiple range test; * and ** are significantly different at the 5% and 1% levels, respectively. CMF = cow manure, WCF = worm castings fertilizer. KOF = Komix organic fertilizer.

Data in Table 3 showed that the treatment using 25 tons/ha of WCF generated the highest theoretical fresh yield with 24.9 tons/ha. Applying CMF at a rate of 5 tons/ha produced the lowest theoretical fresh yield (14.7 tons/ha). This result was in accordance with a report by Anwar et al. (2005), who reported that fertilizing 5 tons/ha of vermicompost with 50 kg N + 25 kg P_2O_5 + 25 kg K_2O per hectare gave basil the highest yield of 16.85 tons/ha. The highest dry yield was 4.05 tons/ha and the highest essential oil yield was 121.30 L/ha.

The highest theoretical yield (21.9 tons/ha) was discovered when applied 25 tons/ha of organic fertilizer and it was statistically significantly different from using 5 tons/ha (15.8 tons/ha). As Table 3 revealed using WCF at a rate of 25 tons/ha created the highest actual fresh yield with 14.7 tons/ha. On the contrary, using CMF at a rate of 5 tons/ha produced the lowest actual fresh yield with 8.4 tons/ha.

With the equal amounts of different fertilizers, WCF showed higher essential oil content than the other two fertilizers, specifically the highest in the amount of fertilizer 15 and 25 tons/ha with 0.47% at Table 3.

In short, different types and levels of fertilizer

Table 3. Effect of sources and application levels of organic fertilizer on yield components and yield of basil (*Ocimum basilicum* var. *pilosum*)

Parameters (90 DAP)	Organic fertilizers (H)	Application rates (L) (ton/ha)					
		5 (control)	10	15	20	25	Average H
Fresh weight (g/plant)	CMF (control)	55.0^{c}	57.3^{c}	65.3^{bc}	75.3^{abc}	79.7^{abc}	66.5^{b}
	WCF	66.3^{abc}	70.3^{abc}	87.3^{ab}	77.7^{abc}	93.3^{a}	80.2^{a}
	KOF	64.7^{bc}	65.0^{bc}	67.0^{abc}	73.0^{abc}	75.3^{abc}	69.1^{b}
	Average L	62.0^{c}	64.2^{bc}	70.0^{b}	80.5^{a}	82.8^{a}	
	CV = 12.6%; F_H = 1.1*; F_L = 2.8**; F_{HL} = 1.3*						
Theoretical yield (ton/ha)	CMF (control)	14.7^{c}	17.4^{bc}	18.8^{abc}	20.1^{abc}	20.1^{abc}	18.2^{b}
	WCF	17.4^{bc}	19.5^{abc}	21.3^{abc}	23.3^{ab}	24.9^{a}	21.3^{a}
	KOF	15.3^{c}	17.3^{bc}	17.7^{abc}	17.9^{abc}	20.7^{abc}	17.8^{b}
	Average L	15.8^{d}	18.1^{c}	19.3^{bc}	20.4^{ab}	21.9^{a}	
	CV = 2.6%; F_H = 1.1*; F_L = 2.8**; F_{HL} = 1.3*						
Actual fresh yield (ton/ha)	CMF (control)	8.4^{b}	10.8^{ab}	11.8^{ab}	11.9^{ab}	12.0^{ab}	11.0^{b}
	WCF	10.9^{ab}	11.3^{ab}	12.8^{ab}	13.8^{ab}	14.7^{a}	12.7^{a}
	KOF	9.0^{ab}	10.2^{ab}	11.1^{ab}	12.0^{ab}	13.4^{ab}	11.1^{b}
	Average L	9.4^{d}	10.8^{c}	11.9^{b}	12.6^{ab}	13.4^{a}	
	CV = 5.8%; F_H = 1.7*; F_L = 0.5**; F_{HL} = 1.4*						
Essential oil content (mL/100g)	CMF (control)	0.30^{c}	0.33^{bc}	0.40^{ab}	0.40^{ab}	0.40^{ab}	0.37^{a}
	WCF	0.36^{bc}	0.40^{ab}	0.40^{ab}	0.47^{a}	0.47^{a}	0.42^{a}
	KOF	0.36^{bc}	0.36^{bc}	0.36^{bc}	0.37^{bc}	0.40^{ab}	0.37^{a}
	Average L	0.34^{bc}	0.36^{c}	0.39^{ab}	0.41^{a}	0.42^{a}	
	CV = 13.6%; F_H = 4.5*; F_L = 1.1*; F_{HL} = 0.5*						
Theoretical yield of essential oil (L/ha)	CMF (control)	55.2	62.8	75.5	77.7	80.7	70.4b
	WCF	67.2	77.6	83.7	89.1	95.6	82.6^{a}
	KOF	66.4	60.3	67.4	72.3	82.4	69.8^{b}
	Average L	62.9^{c}	66.9^{c}	75.5^{b}	79.7^{ab}	86.2^{a}	
	CV = 9.5%; F_H = 0.1*; F_L = 0.8*; F_{HL} = 1.1^{ns}						

Values within the same group followed by the same letters are no significant different at $P \leq 0.05$ according to the Duncan's multiple range test; * and ** are significantly different at the 5% and 1% levels, respectively. CMF = cow manure fertilizer, WCF = worm castings fertilizer. KOF = Komix organic fertilizer.

affected the theoretical yield of essential oils. The treatment of 25 tons/ha of fertilizer generated the highest theoretical yield of essential oils 82.6 L/ha. The theoretical yield of essential oil fluctuated from 55.2 to 95.6 L/ha when considering the interaction between the two factors (Table 3.

For the benefits of using WCF, vermicompost contain more nutrients than the other organic product from which it is processed (Buchanan et al., 1988) and thus it acts as a rich source of nutrients for plant growth and promotion (Ismail, 2000). Vermicompost decreases the amount of heavy metal incorporated to soil compare to compost as described by Lim et al. (2016). It has been also stated that vermicompost may have more compounds serve as a plant hormone which enhances plant growth and development compared to compost (Najar et al., 2015; Coulibaly & Bi, 2010). Our study results were in accordance with Shahriari et al. (2015), who reported that application of vermicomposting at doses of 10 tons/ha, 5 tons/ha, and 0 tons/ha to basil, the corresponding values were higher: plant height 54.3 cm versus 52.46 cm and 44.98 cm; dry weight 13.03 g/plant compared with 9.72 g/plant and 7.64 g/plant; the number of leaves/plant is 182.7 - 162.2 and 118.4, respectively; at the same time, the ratio and yield of essential oil also increased correspondingly when the amount of vermicompost was increased.

3.3. Effect types and amounts of organic fertilizers on economic efficiency of basil (*Ocimum basilicum* var. *pilosum*)

Results at Table 4 showed that when selling with price of VND 25.000 per kg basil, applying worm castings manure at the amount of 25 tons/ha gained the highest total revenue (VND 1,002,500,000 per ha/3 harvests) and total profit (VND 737,570,000 per ha/3 harvests); Total cost was highest (VND 439,930,000 per ha/3 harvests) in the treatment using 25 tons/ha of cow manure. However, the benefit-cost ratio when growing basil in the treatment using worm castings manure at a rate of 5 tons/ha was the highest (3.26).

Table 4. Effect sources and application levels of organic fertilizers on economic efficiency of basil

Treatment		Actual yield	Total revenue	Cost	Total profit	Real rate of return
Types of fertilizers	Applied rates (ton/ha)	(ton/ha/3 harvests)	(VND/ha/3 harvests)			
	5	20.4	510,000,000	199,930,000	310,070,000	1.55
	10	23.7	592,500,000	259,930,000	332,570,000	1.28
Cow manure fertilizer	15	28.3	707,500,000	319,930,000	387,570,000	1.21
	20	30.3	757,500,000	379,930,000	377,570,000	0.99
	25	33.5	837,500,000	439,930,000	397,570,000	0.90
	5	28.1	702,500,000	164,930,000	537,570,000	3.26
	10	29.7	742,500,000	189,930,000	552,570,000	2.91
Worm castings fertilizer	15	32.8	820,000,000	214,930,000	605,070,000	2.82
	20	36.6	915,000,000	239,930,000	675,070,000	2.81
	25	40.1	1,002,500,000	264,930,000	737,570,000	2.78
	5	24.9	622,500,000	168,430,000	454,070,000	2.70
	10	26.7	667,500,000	196,930,000	470,570,000	2.39
Komix organic fertilizer	15	27.7	692,500,000	225,430,000	467,070,000	2.07
	20	32.4	810,000,000	253,930,000	556,070,000	2.19
	25	33.7	842,500,000	282,430,000	560,070,000	1.98

4. Conclusions

The field experiment examined the effects of three types of organic fertilizers with increasing application rates on the plant growth, yield, and essential oil content of basil grown in Thu Duc City. Generally, the application of WCF and KOF resulted in better plant height, stem diameter, number of primary branches, number of leaves, and chlorophyll index than CMF. The results showed that the application of 25 tons/ha of WCF yielded the best results in fresh weight (93.3 g/plant), theoretical yield (24.9 tons/ha), actual yield (14.7 tons/ha), essential oil content (0.47 mL)/(100 g), and yield of essential oil (117 L/ha). The economic efficiency analysis showed the highest total profit after three harvests per hectare (VND 737,570,000 per ha/3 harvests). However, the highest real benefit-cost ratio (3.26) was obtained at the treatment of 5 ton/ha WCF.

Through the implementation of the experiment and the obtained results, the use of worm castings fertilizer with the amount of 5 tons/ha created the total revenue and benefit-cost ratio. However, the effect of these organic fertilizers on basil (*Ocimum basilicum* var. *pilosum*) should be continued on a larger area and with different crops for more comprehensive view of each type of fertilizer.

Conflict of interest

The authors declare no conflict of interest.

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Detection of Newcastle disease virus and H5-subtype Influenza virus in swiftlet houses by Multiplex reverse transcription PCR assay

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ABSTRACT

The purpose of this study was to investigate the presence of Avian paramyxovirus 1 (APMV-1), also known as Newcastle disease virus (NDV), and Avian influenza type A, especially H5 subtype (AIV-H5) in swiftlet houses and swiftlet nest by multiplex RT-PCR (mRT-PCR). The assay used two specific primer pairs designed to detect the conserved sequence of the F gene of NDV and the HA gene of the AIV-H5, with product sizes of 282 bp and 420 bp, respectively. The mRT-PCR was established with the detection limit of 25 copies/reaction for each target virus. The thermal cycle was optimized as follows: cDNA synthesis at 45°C for 20 min, an initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification encompassing denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec, ending by a final extension step at 72°C for 7 min. Eighty-eight field samples including feces and swabs of the nest surface were examined and all samples were confirmed to be negative for these two viruses. The results of this study indicated that the swiftlet nests and the environment of swiftlet houses were not contaminated with NDV or AIV-H5 viruses. Moreover, the established mRT-PCR protocol had good specificity and detection limit, and can be used for routine veterinary diagnosis.

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1. Introduction

In recent years, the swiftlet farming and swiftlet products have become a fast-growing industry in Vietnam. The products related to swiftlets on the market have high economic values due to its traditionally considered values in nutrition and health supports. Thus, understanding infectious pathogens is important to protect this new and prosperous profession. Among the diseases that can cause huge losses in poultry and/or wild birds, Newcastle disease and AIV-H5 influenza A are in the list of big concerns.

Newcastle disease virus (NDV) a member of the genus *Avulavirus* within the *Paramyxoviridae* family, is a negative sense, single-stranded, non-segmented, enveloped RNA virus (Alexander & Senne, 2008). Chambers et al. (1986) stated that the NDV genome is composed of six genes that code for six structural proteins: nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and the RNA polymerase (L). F and HN proteins are considered as central immunogenic proteins of the virion (Meulemans et al., 1968).

Avian influenza virus is enveloped, single-stranded RNA virus with eight segmented genes, belong to the genera influenza virus A in the family Orthomyxoviridae (Lamb, 2001). Avian influenza A virus contains two surface antigens, the hemagglutinin (HA) and neuraminidase (NA) proteins, classified into 18 hemagglutinin (H1-H18) and 11 neuraminidase (N1-N11) subtypes. Classification according to pathogenicity, influenza A virus has two types of low pathogenic avian influenza (LPAI) causing no signs of disease or only exhibit mild illness in birds and high pathogenic avian influenza (HPAI) that can cause infection and death in wild birds, domestic poultry, and other mammals (Alexander, 2007). In particular, highly pathogenic AIV-H5 virus strains cause high mortality rates in both poultry and humans (Claas et al., 1998). In 1996, the H5N1 virus was first detected in geese and was the major cause of avian viral respiratory diseases outbreaks in China. In 2003, the first outbreak of avian influenza H5N1 in Vietnam, millions of poultry were culled only in a short time.

Several investigations found the presence of NDV and AIV-H5 AIV in wild birds (Schelling et al., 1999; Hoye et al., 2010; Hoque et al., 2012). Birds infected with NDV or highly pathogenic AIV-H5 virus may manifest similar symptoms, including acute respiratory disorders, decrease in egg production and high mortality. Conventional diagnostic methods such as clinical examination or gross lesions may not be confirmative enough to differentiate these two viruses.

Currently, diseases in swiftlets have not been carefully examined in the world as well as in Vietnam. Many swiftlet houses were built within or near poultry farms which poses a potential risk of the transmission of pathogenic viruses. Thus, understanding the presence of some viral agents in swiftlet houses is important for a good disease control strategy in the future. Some studies have shown possibility to detect AIV and NDV by multiplex reverse transcription-polymerase chain reaction using throat wash, oral swab, choanal swab samples (Xie et al., 2006; Chen et al., 2008; Tao et al., 2009). In this study, we developed a multiplex RT-PCR method and used for investigation and differentiation of NDV and AIV-H5 subtype in samples collected in swiftlet houses.

2. Materials and Methods

2.1. Controls and field samples

Positive controls for AIV and NDV: H5 gene sequence of 1745 bp of AIV was synthesized by IDT (Integrated DNA Technologies - USA); for NDV, a vaccine containing NDV and Infectious-Bronchitis Virus (containing B1 Type, B1 Strain, Connecticut Type 1,000 doses, Zoetic, USA) was used.

Negative controls: for specificity evaluation, DNA/RNA of bacteria or viruses that are popularly circulating in poultry farm and potentially contaminate the samples such as IBDV, IBV, *Staphylococcus* spp., *Clostridium perfringens*, *Streptococcus* spp., Salmonella spp., *Escherichia coli* were used. These controls were provided by Biomin Veterinary Diagnostic Laboratory in Vietnam (DSM group).

Field samples: Eighty-eight samples were collected from swiftlet houses in Vietnam including feces and swab samples of the nest-surface. Feces were collected at five positions for each floor (one sample for each corner and 1 sample at the center of the floor) and pooled into one sample per floor. For swab, sterile cotton swab was moisturized with physiological saline and used to wipe on the surface of the nest. The nests chosen for sampling were distributed at five spots as described for fecal samples. Samples were stored at 4°C and transported to the laboratory within 24 h. At the laboratory, the samples were stored at -20°C and nucleic acid extraction was performed within 3 days since their arrival.

2.2. DNA/RNA extraction

DNA/RNA from the samples (feces, swab, and vaccine) was extracted using WizPrep^{TM} Viral DNA/RNA Mini Kit, according to instructions of the manufacturer. RNA/ DNA was stored at -20°C until being used.

2.3. Primers

Primer information for NDV and AIV-H5 were obtained from Tang et al. (2012) and were listed in Table 1. For NDV, primers were specific for the fusion (F) gene with a product size of 282 bp. For AIV-H5, specific primers were designed on hemagglutinin (HA) gene with a product size of 420 bp.

2.4. Multiplex RT-PCR (mRT-PCR)

MyTaq^{TM} One-Step RT-PCR Kit was used to optimize primer concentrations and annealing temperature for the mRT-PCR. After a dozen rounds of optimization, the best ratio of primers concentration was determined to be 0.4 µM : 0.2 µM for NDV : AIV-H5 primers, which equivalent to the ratio of 2/1 respectively. mRT-PCR was performed in 20 µL reaction volume con-

Table 1. Primer sequences and estimated product sizes

Virus	Target gene	Primer sequences (5'-3')	Product size (bp)	Reference
NDV	F	F: TCACTCCTCTTGGCGACTC R: CAAACTGCTGCATCTTCC	282	Tang et al., 2012
AIV-H5	HA	F: ACCCAGCCAATGACCTCT R: CACTTTGCCCGTTTACTT	420	

taining 10 µL of MyTaq One-step mix (2X), 2 µL of DEPC-treated water, 2.4 µL of the primer mix, 5 µL of RNA templates, 0.4 µL of RiboSafe RNase Inhibitor, 0.2 µL of Reverse transcriptase enzyme. The target genes were amplified using the following conditions: a reverse transcription at 45°C for 20 min, an initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification encompassing denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec, ending with a final extension step at 72°C for 7 min. The amplified products were analyzed by 1.2% (w/v) agarose gel in 0.5X Tris-Acetic-EDTA (TAE) and stained with Midori Green Advance DNA (Cat#MG04, Nippon Genetics), electrophoresis at 70 volts for 45 min, and visualized under UV light.

2.5. Examination of primer specificity

In reality, environmental samples can contain all types of microorganism existing in an area. Thus, beside the target viruses, contamination with other pathogens from the environment of swiftlet houses into the collected samples can be inevitable. To assure that the mRT-PCR specifically detect NDV and AIV-H5, but not interact with unrelated DNA/RNA, some viruses and bacteria such as IBDV, IBV, *Staphylococcus* spp., *Clostridium perfringens*, *Streptococcus* spp., *Salmonella* spp., *Escherichia coli* were used as negative control samples in the reactions.

2.6. Determination of detection limit in mRT-PCR

To further determine the detection limit of the mRT-PCR assay, the positive control was diluted 10 times continuously from 10^6 to 10^0 copies/µL and used in the reactions. The minimum number of template copies that could give a positive result on gel electrophoresis was considered as the limit of detection of the mRT-PCR.

3. Results

3.1. Optimal annealing temperature for the mRT-PCR

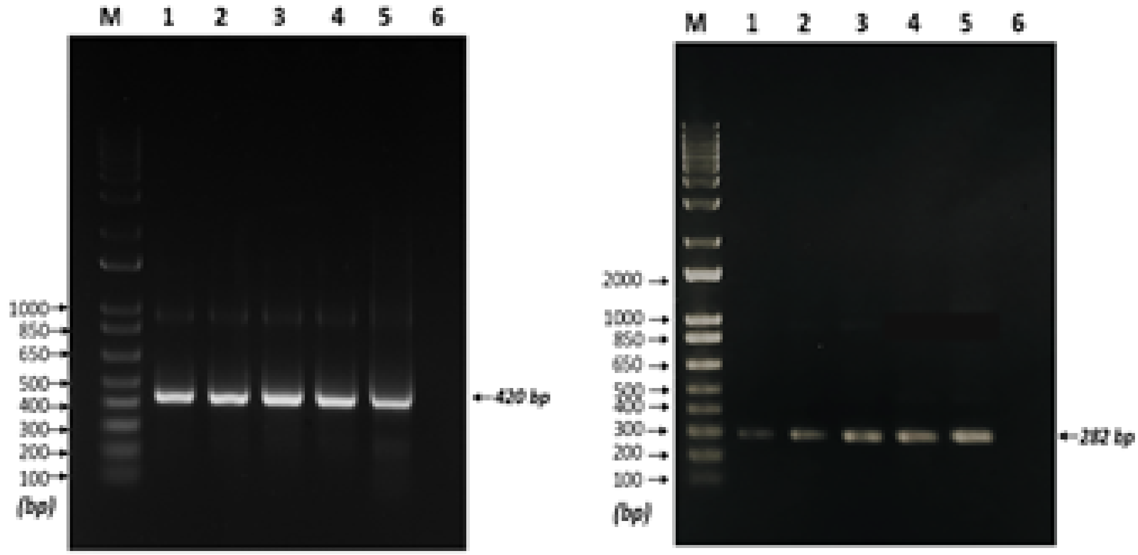
In this study, we established a PCR protocol for laboratory diagnosis of NDV and AIV-H5 in field samples of swiftlet houses and optimized the annealing temperature for the best amplification efficacy. Each primer pair was tested in single RT-PCR (sRT-PCR) at different annealing temperatures: 52°C, 54°C, 56°C, 58°C, 60°C. Results of gel electrophoresis in Figure 1 showed that sRT-PCR reactions successfully amplified the F gene of NDV with product size 282 bp and 420 bp for the HA gene of the AIV-H5. Both primer pairs worked well at the temperature range of 52 - 60°C. The temperature of 58°C was selected for subsequent experiments. In mRT-PCR reaction, the primer pairs generated precisely two amplicons of the expected sizes observed in gel electrophoresis (Figure 2).

3.2. Optimization of the primer concentration for the mRT-PCR

Based on the amplification efficacy when the two primer pairs used equally, the optimization of primer concentration used in mRT-PCR was performed with adjusted ratios in which the primer concentrations of NDV - AIV-H5 were: 1:1 (0.2 µM: 0.2 µM); 2:1 (0.4 µM: 0.2 µM); 2.5:1 (0.5 µM: 0.2 µM); 3:2 (0.6 µM: 0.4 µM). Figure 3 showed the corresponding products, indicating that the ratio 2:1 for NDV - AIV-H5 to be the best and was selected for further examinations.

3.3. Specificity and detection limit of the mRT-PCR

Swiftlet houses are often built in the same areas with other domestic animals, posing a risk of pathogen transmission from poultry to swiftlet and vice versa. Therefore, the specificity of the



(A) (B)

Figure 1. Optimization of annealing temperature in s-PCR. (A) AIV-H5 subtype, (B) NDV. (Lane M): DNA ladder 1 Kb plus, (Lane 1): 52°C, (Lane 2): 54°C, (Lane 3): 56°C, (Lane 4): 58°C, (Lane 5): 60°C, (Lane 6): negative control with pure water. The thermal cycling conditions were cDNA synthesis with 45°C/20 min and 95°C/5 min; 35 cycles of 95°C/30 sec, 52-60°C/30 sec and 72°C/45 sec, a final extension at 72°C/7 min.

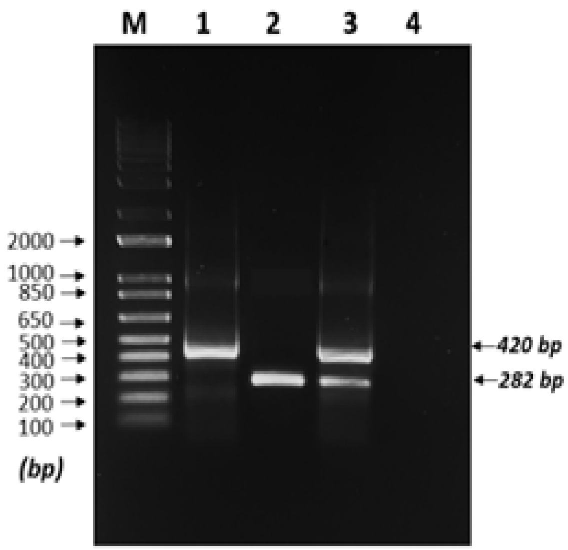


Figure 2. Products of sRT-PCRs and mRT-PCR. (Lane M): DNA ladder, (Lane 1): AIV-H5 420 bp, (Lane 2): NDV 282 bp, (Lane 3): mRT-PCR of all two targets, (Lane 4): negative control with pure water. The thermal cycling conditions were cDNA synthesis with 45°C/20 min and 95°C/5 min; 35 cycles of 95°C/30 sec, 58°C/30 sec and 72°C/45 sec, a final extension at 72°C/7 min.

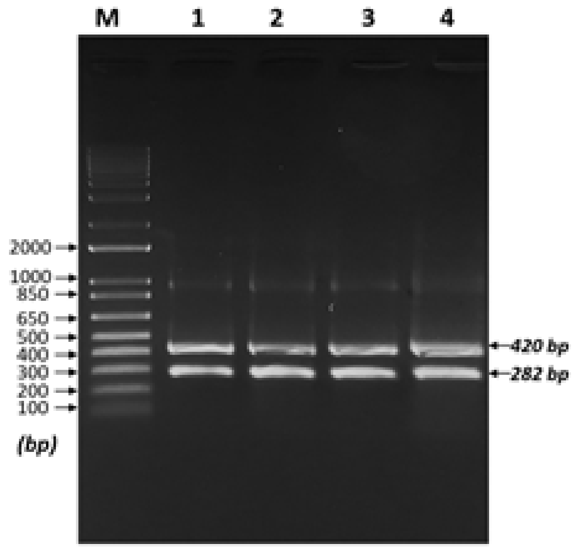
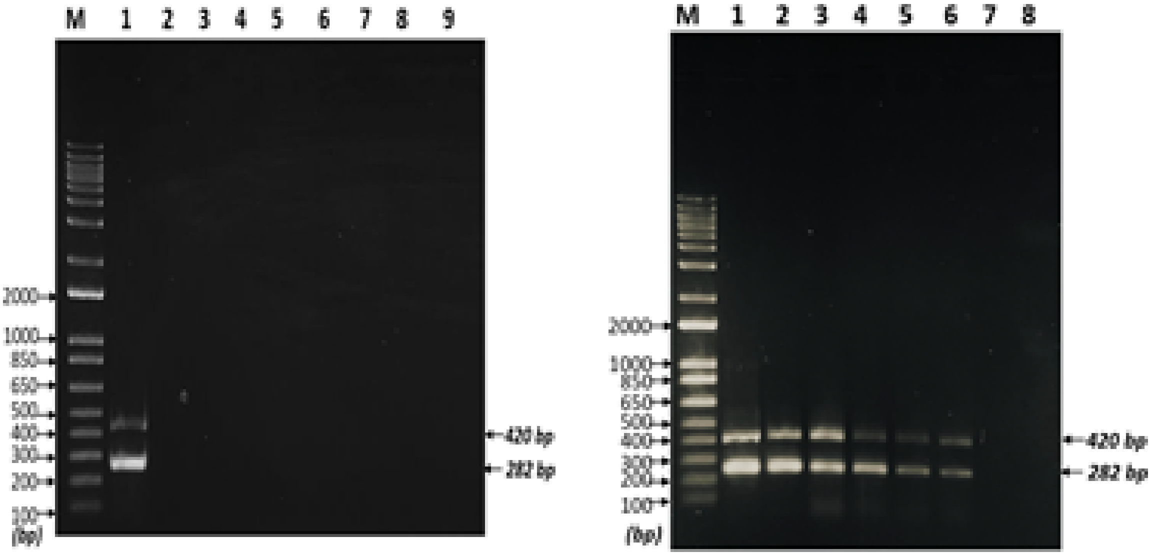


Figure 3. Electrophoresis results of the mRT-PCR with different NDV - AIV-H5 primers ratio. (Lane M): DNA ladder, (Lane 1): 1:1 (0.2 µM: 0.2 µM), (Lane 2): 2:1 (0.4 µM: 0.2 µM), (Lane 3): 2.5:1 (0.5 µM: 0.2 µM), (Lane 4): 3:2 (0.6 µM: 0.4 µM).

mRT-PCR is important to avoid false positive results due to misbinding of the primers to unrelated pathogens. To evaluate the specificity of this mRT-PCR, DNA/RNA extracted from some other pathogenic bacteria and viruses that potentially contaminate in samples such as IBDV, IBV, *Staphylococcus* spp., *Clostridium perfrin-*



(A) (B)

Figure 4. Specificity and detection limit of the mRT-PCR. (A) Result of specificity primers test. (Lane M): DNA ladder, (Lane 1): positive control, (Lane 2): E. coli, (Lane 3): Streptococcus spp., (Lane 4): Staphylococcus spp., (Lane 5): C. perfringens, (Lane 6): IBDV, (Lane 7): IBV, (Lane 8): Salmonella spp., (Lane 9): negative control. (B) The detection limit of the mRT-PCR. (Lane M): DNA ladder, (Lane 1-7): DNA template of AIV-H5 and NDV at $2.5*10^6$ - $2.5*10^0$ copies/reaction of each pathogen, (Lane 8): negative control with pure water.

gens, *Streptococcus* spp., *Salmonella* spp., *Escherichia coli* were used. Results showed that none of these templates were amplified by the primers specific for NDV and AIV-H5 (Fig 4A) confirming the specificity of primers for NDV and AIV-H5.

To check the detection limit of the mRT-PCR, templates of the positive controls were diluted 10-fold serially within the range of 10^6 to 10^0 copies/µL. Afterwards, mRT-PCR reactions were performed with the number of templates of each virus decreasing from $2.5*10^6$ to $2.5*10^1$ copies/reaction. As shown in Figure 4B, the two expected bands of NDV and AIV-H5 appeared clearly in lane 1-6. Nevertheless, lane 7 with the amount of $2.5*10^0$ copies/virus/reaction did not present any products. The experiments were repeated three times with similar results. Therefore, the detection limit of mRT-PCR was $2.5*10^1$ copies per reaction for each pathogen.

3.4. Examination of NDV and AIV-H5 virus in clinical samples

The purpose of this study was to use mRT-PCR to investigate the presence NDV and AIV-H5 subtype in the environment of swiftlet houses.

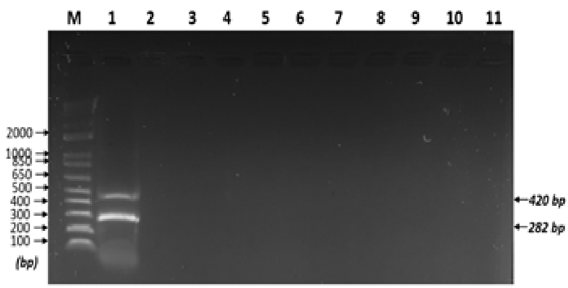
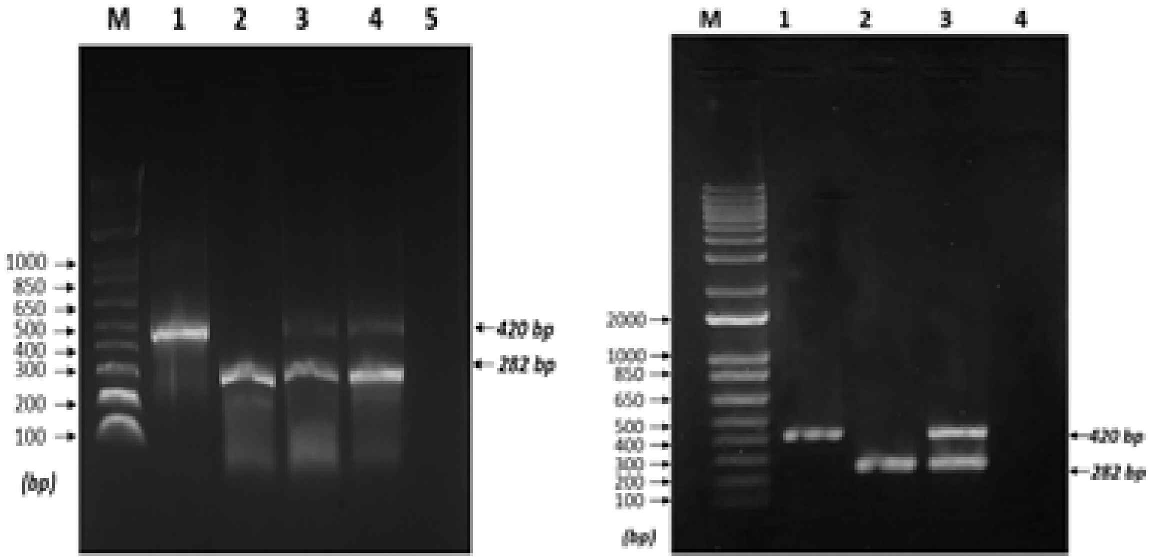


Figure 5. Electrophoresis results of PCR products of some clinical samples. (Lane M): DNA ladder, (Lane 1): positive control, (Lane 2-10): clinical samples, (Lane 11): negative control with pure water.

After the mRT-PCR has been established successfully. It was applied for 88 samples consisting of 44 samples of feces and 44 swab samples of the nest collected from 11 swiftlet houses located in Dong Nai and Lam Dong provinces. Surprisingly, neither NDV nor AIV-H5 was detected in the tested samples as shown partially in Figure 5. The assay was done twice, and negative results were very consistent. To further confirm the negativity as well as to remove all doubts that the viral nucleic acid may be existing, but the mRT-PCR protocol was unable to detect pre-



(A) (B)

Figure 6. Electrophoresis results of PCR products of field samples. (A) Field samples supplemented with positive control samples. (Lane M): DNA ladder, (Lane 1): AIV-H5 (420 bp), (Lane 2): NDV (282 bp), (Lane 3-4): control positive with field samples, (Lane 5): negative control with pure water; (B) Field samples of poultry infected with AIV-H5 and NDV. (Lane M): DNA ladder, (Lane 1): AIV-H5 (420 bp), (Lane 2): NDV (282 bp), (Lane 3): positive control (Lane 4): negative control with pure water.

cisely, some field samples were selected randomly, and the positive control were mixed into the samples before extraction step at the level of 10^{10} copies/extraction and $5*10^{10}$ copies/extraction for NDV and AIV-H5, respectively. Then the extract was used for mRT-PCR following the same protocol performed in Figure 5. Figure 6A demonstrated some of the assays and the results were positive when either one or both viral templates were incorporated into the samples. Additionally, when the mRT-PCR was applied for fecal samples of poultry infected with NDV or AIV-H5, it could function well in detection of the viral target (Figure 6B). The experiments in Figure 6 further confirmed that the negative results of the swiftlet samples were true and there was not any inhibitory effect caused by the sample platform.

4. Discussion

mRT-PCR methods for the identification of AIV-H5 subtype and NDV have been established worldwide (Malik et al., 2004; Xie et al., 2006; Chen et al., 2008). Hoye et al., (2010) showed that AIV-H5 could be transmitted between domestic poultry and wild birds, especially for H5 subtype. Newcastle disease virus is one of the most important viral diseases in domestic birds as well as wild birds (Snoeck et al., 2013; Hirschinger et al., 2021). Even though hundreds of reports about AIV-H5 and NDV surveillance in wild birds are available but there is limited or no information of AIV-H5 in swiflet bird.

El Zowalaty et al. (2021) found 4.1% of cloacal and oropharyngeal swab samples positive for NDV but negative for AIV-H5 in wild birds using RT-PCR. Investigating 380 cloacal swab samples of chickens and ducks for AIV and NDV, Tang et al. (2012) discovered 0.51% (2/380) positive with NDV and 1.02% (4/380) positive with AIV-H5. Some other research could detect AIV-H5 at 4.61% (25/542 samples) (Verhagen et al., 2017); 1.3% (63/4279 samples) (Bevins et al., 2016). While NDV could be detected by RT-PCR at 3.06 - 3.5% for live bird samples (N = 1461) and 0.4% for feces samples (N = 1157) respectively in Africa and in North Queensland of Australia, (Hoque et al., 2012; Cappelle et al., 2015). These studies indicated that there are opportunities to detect NDV or AIV in fecal samples, but the investigation may require a large amount of sample.

The detection limit of our mRT-PCR was 25 copies per reaction for each RNA of pathogen, and specificity primer for reaction. Thus, our mRT-PCR procedure established can be useful for detecting pathogens presence and investigation for pathogen occurrence before further viral analysis of field samples.

5. Conclusions

In summary, we successfully developed an mRT-PCR procedure for detecting the presence of NDV and AIV-H5 virus in field samples. The mRT-PCR was specific and functioned well at the detection limit of 25 copies/target virus per reaction. Application of this mRT-PCR in diagnosis of NDV and AIV-H5 viruses in the environment of swiftlet houses and the surface of bird nests temporarily confirmed the absence of these viruses in the samples tested. The result of this study demonstrated that the environment of swiftlet houses and the bird nests were quite safe from NDV and subtype H5 of AIV.

Conflict of interest

All authors have read the journal's policy on disclosures of potential conflicts of interest, and we declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Effects of different loading densities during transport on survival rates of Asian seabass (*Lates calcarifer* Bloch, 1790) juvenile

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ABSTRACT

This study was carried out to evaluate effects of loading density during transport on water quality and survival rate of Asian seabass (Lates calcarifer) juvenile. The experiment included four treatments of different loading densities: 50 kg/m^3 (T1), 70 kg/m^3 (T2), 90 kg/m^3 (T3) and 110 kg/m^3 (T4) with three replicates for each treatment. The fish with an average weight of 20.50 ± 0.25 g was transported in an aerated and oxygenated heat-insulated tanks. The water temperature in transport was set at 22°C and the concentration of isoeugenol-50% was 6 ppm. Water quality, blood glucose and survival rate of the fish were recorded at the beginning, after transport 6 h and 12 h, and 3 and 7 days after the end of transporting. The results showed that the water quality was declined expressed by the decrease of DO and pH, and the increase of CO_2, TAN and NO_2 during transport but still in suitable ranges for seabass. The blood glucose content of fish increased during transportation due to stress. The survival rates of the fish of all treatments were reduced following transport duration. The fish was well recovered after the transport. At the end of the study, the survival rate of the fish of T1 was highest (96.00%), followed by T2 (95.33%), T3 (90.00%), and T4 (87.63%). Based on the accumulated mortality, loading densities of 70 to 90 kg/m^3 and 50 to 70 kg/m^3 were recommended for transport of seabass juvenile in cooling water (22°C) and isoeugenol-50% (6 ppm) during 6 and 12 h, respectively.

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1. Introduction

Asian seabass or barramundi (*Lates calcarifer* Bloch, 1790) is an euryhaline fish widely distributed in the Indo-West Pacific, from the Arabian Gulf to China, Taiwan, and Northern Australia (FAO, 2020). According to GOAL, seabass production for selected survey countries was about 108,000 MT in 2019, up by 20% compared to 2018, and the forecast for 2019 was at an increase of 6% to around 115,000 MT (Tveteras et al., 2019). In Vietnam, *L. calcarifer* species is distributed in the Eastern of Northern Gulf and the Central Coast. It has been successfully farmed in many coastal provinces in the countries such as Quang Ninh, Hai Phong, Thua Thien - Hue, Quang Nam, Da Nang, Binh Dinh, Khanh Hoa, Binh Thuan, Ba Ria - Vung Tau, Ben Tre, and Soc Trang (Nguyen, 2009; Ly et al., 2016; Nguyen & Nguyen, 2018).

Seabass farming systems in Vietnam are include brackish water ponds and cages suspended in coastal water bodies. However, the major system in the Mekong river delta is seabass farming in ponds (Ly et al., 2016). Currently, seabass hatcheries are mainly located in South Central provinces (Khanh Hoa, Ninh Thuan, Binh Thuan) and Eastern (Ba Ria - Vung Tau) (Tran et al., 2019). Live fish transportation usually produces negative effects on fish due to the degradation of water quality (Rimmer et al., 1997a). One of them is the stressful state of transported fish. Stress can lead to high incidence of disease, decrease in feeding and growth, changes of behavior, and mortality in critical cases (Rucinque et al., 2017). Methods to make the fish sedative, movement, and stress reduction are important in live fish transportation. Cooling water and using anesthetics are the most common methods applied in live fish transport (Yoshikawa et al., 1989; Coyle et al., 2004; Lili et al., 2020). There are many anesthetics used in the laboratory as well as in aquaculture such as tricaine methanesulfonate (MS-222), benzocaine, clove oil, eugenol, and isoeugenol (Rucinque et al., 2017; Priborsky & Velisek, 2018; Schroeder et al., 2021).

Recently, AQUI-S was proposed as an immediate or reduced withdrawal time sedative and used in fish propagation and transportation (Javahery & Moradlu, 2012; Cupp et al., 2017). The common method of anesthetic used in live fish transportaion was immersion (Neiffer & Stamper, 2009). Safely transporting higher loading densities of fish would benefit seed producers by increasing efficiency and reducing costs, but research evaluating transport for individual species is generally lacking (Cupp et al., 2017). This study aimed to evaluate effects of loading densities on water quality and survival rate of seabass juvenile during and post live fish transport.

2. Materials and Methods

2.1. Experimental animals

Asian seabass (*Lates calcarifer*) juvenile with an average weight of 20.50 ± 0.25 g and length of 11.52 ± 0.22 cm was used in this experiment. The fish used in the live transporting experiment were relatively uniform size, good appearance, no signs of disease, and scratch. The fish was fasted for 24 h prior to transporting.

2.2. Water and anesthetic

Water used in fish transport was taken from a treated reservoir with quality parameters as following: salinity = 18‰, pH = 8.2, dissolved oxygen (DO) > 4.5 ppm, total ammonia nitrogen (TAN) < 0.2 ppm, nitrite (NO_2) < 0.01 ppm and transparency > 150 cm.

Insulated tanks (30 cm × 40 cm × 38 cm) for transporting fish, containing 40 L of water, were equipped with tubes connected to an air compressor and a liquid oxygen cylinder for aeration and oxygenation (Berka, 1986). All the tubes were installed valves to control air and oxygen volume injection to ensure the even distribution and saturation of DO in each tank. A small hole was created on tank lids to let excess air and oxygen escape without water leaking. The air compressor, oxygen cylinder, and fish tanks were loaded in an insulated truck which air temperature was adjusted around 20 to 22°C during the transport.

Before the experiment, two trials imitated a live fish transportation was carried out to evaluate effects of cooling temperatures and anesthetic of isoeugenol-50% (AQUI-S®) concentrations on seabass juvenile. Based on survival rates of the fish at the end of the trials and on the seventh-day post transporting, appropriate values of 22°C for cooling temperatures and of 6 ppm for isoeugenol-50% concentrations were identified.

2.3. Experiment design

This experiment comprised of four treatments of different loading biomass of fish for transporting: 50 kg/m^3 (T1), 70 kg/m^3 (T2), 90 kg/m^3 (T3) and 110 kg/m^3 (T4), with three replicates for each treatment. The water temperature in all transport tanks was set at 22°C and the concentration of isoeugenol-50% was 6 ppm. The fish was acclimatized to the experiment temperature in a holding container by gradually cooling water with ice from room temperature to 22°C for 30 min and immediately transferred to the experiment tanks and closed with lids. Then all the tanks were loaded into the truck for transporting. In fact, the time to transport fingerlings usually took from 6 h to 12 h.

At the end of the transport (12 h), the water temperature of all tanks was gradually raised to pond water temperature by adding pond water for 30 min to protect the fish from heat stress caused by quick temperature change. After transporting, the fish in each treatment was stocked in 2 m^2 hapas suspended in pond for routine management and fed ad libitum with Uni President's floating seabass pelleted feed of 43% crude protein at 6:00 AM and 17:00 PM.

2.4. Recorded data

Environmental parameters including temperature, DO and pH were measured with an AZ8602 (AZ Instruments); of which, the degree of accuracy of temperature, DO and pH measurement was of 0.1°C, 0.1 ppm, and 0.1, respectively. CO_2 was measured with an EA80 (EXTECH-USA) meter, TAN with a HI97700 (HANNA) meter and NO_2 with a NO_2-30 meter (China); of which the degree of accuracy of CO_2, TAN and NO_2 was of 1 ppm, 0.01 ppm and 0.01 ppm, respectively.

Fish blood glucose was measured with the Medismart Sapphire Plus test kit (Switzerland), using an automatic test strip to take a very small amount of blood 0.6 µL from the fish's tail, measuring range from 20 - 630 mg/dL. Results are displayed for 5 sec. Blood samples were collected at initial, after 6 and 12 h of transport, 3 and 7 days after transport to assess the stress level of fish before, during, and after transport. At each time of data collection, 9 samples were collected for each treatment. After collecting the samples, the fish were returned to the treatment.

The water parameters in the tanks were recorded at 0, 6, and 12 h of the transport, and in the pond on the days of 1, 3, and 7 post transporting. The survival rate (%) of the fish was also recorded at 6 and 12 h of the transport, and on the days of 3 and 7 post transporting.

2.5. Data analysis

Statistical analysis was performed with Microsoft Excel 2010 and SPSS 20.0 for Window software. Data were analyzed with one-way analysis of variance (ANOVA) at the significance level of $P = 0.05$, and when effects were found to be significant, LSD was used to determine differences for each paired treatment. Percentage values were converted to arsin$\sqrt{}$ prior to analyzing. The data in tables were presented as mean ± standard deviation.

3. Results

3.1. Water quality

Water parameters of the tanks recorded in the 6 and 12 h transport times were presented in Table 1.

The water quality of fish tanks was reduced following transport time expressed by the significant decrease of pH and DO, and the increase of CO_2, TAN and NO_2 ($P < 0.05$) compared to initial time, except for NO_2 after transported 6 h (Table 1). The water quality was also reduced following increased stocking densities. In the 6 h transport time, pH means between T1 and T2 treatments, T2 and T3 treatments, T3 and T4 treatments were not significantly different. No significant difference of DO means was found between T1 and T2, T3 and T4 treatments; and of CO_2 means between T2 and T3 treatments, T3 and T4 treatments. TAN means between all treatments were significantly different. The significant difference of NO_2 means was also found between all treatments, except for T1 and T2 treatments (Table 1).

At the end of the transport, no significant difference ($P > 0.05$) was found for pH means between T1 and T2 treatments, T2 and T3 treatments; for DO means between T1 and T2 treatments, and T2, T3, and T4; for CO_2 means between T1 and T2 treatments, T2 and T3 treatments, and T3 and T4; and for NO_2 means between T1 and T2, and T2 and T3 treatments. TAN means between all treatments were significantly different ($P < 0.05$), except for T1 and T2 treatments (Table 1).

In general, there was no considerable fluctuation of the water quality parameters in the pond between different times posts transporting. Temperature, pH and DO values in the afternoon were higher than those in the morning (Table 2).

The glucose content (mg/dL) in fish blood was investigated at the time before transport, after 6 and 12 h of transport, 3 and 7 days post transporting (PT) in the experiments. The data were presented in Figures 1 and 2.

3.2. Glucose indicator

At the time before transport, the blood glucose content of fish fluctuated in the range of 68.3-73.3 mg/dL. The glucose content tends to increase during transportation. After 6 h of transportation, the highest concentration of glucose in the blood of fish in the treatment 110 kg/m^3 (100.8 ± 7.9 mg/dL) was significantly higher ($P < 0.05$) compared with the other treatments. After 12 h of transportation, the glucose content continued to increase, the treatment of 110 kg/m^3 was highest (122.2 ± 10.9 mg/dL), followed by the treatment of 90 kg/m^3 (98.8 ± 8.0 mg/dL) was different from the other 2 treatments. At the time of 3 days post transporting, the glucose content decreased and by the time of 7 days post transporting, the glucose content almost returned to the

Table 1. Water quality parameters in the 6 and 12 h transport time

Time	Treatment	Parameters				
		pH	DO (ppm)	CO_2 (ppm)	TAN (ppm)	NO_2 (ppm)
6 h	Initia	8.20 ± 0.0^d	7.55 ± 0.13^c	0.00 ± 0.0^a	0.50 ± 0.0^a	0.01 ± 0.0^a
	T1	7.60 ± 0.20^c	6.37 ± 0.15^b	11.67 ± 1.53^b	1.13 ± 0.12^b	0.03 ± 0.01^{ab}
	T2	7.47 ± 0.06^{bc}	6.17 ± 0.15^b	14.17 ± 0.58^c	1.43 ± 0.12^c	0.04 ± 0.01^b
	T3	7.33 ± 0.12^{ab}	5.80 ± 0.20^a	15.80 ± 1.00^{cd}	1.73 ± 0.12^d	0.07 ± 0.02^c
	T4	7.13 ± 0.12^a	5.50 ± 0.30^a	16.67 ± 1.53^d	2.20 ± 0.20^e	0.09 ± 0.0^d
12 h	Initia	8.20 ± 0.0^d	7.55 ± 0.0^c	0.00 ± 0.0^a	0.50 ± 0.0^a	0.01 ± 0.0^a
	T1	7.27 ± 0.25^c	6.20 ± 0.26^b	15.83 ± 2.52^b	1.27 ± 0.25^b	0.05 ± 0.01^b
	T2	7.03 ± 0.25^{bc}	5.80 ± 0.20^{ab}	19.83 ± 2.52^{bc}	1.53 ± 0.31^b	0.07 ± 0.02^{bc}
	T3	6.77 ± 0.25^b	5.63 ± 0.15^a	22.47 ± 2.08^{cd}	2.20 ± 0.20^c	0.09 ± 0.01^c
	T4	6.23 ± 0.25^a	5.33 ± 0.42^a	25.33 ± 3.25^d	3.00 ± 0.20^d	0.13 ± 0.03^d

Means within the same column with different superscript letters are significantly different at $P < 0.05$ where a < b < c < d.

Table 2. Water quality parameters in the pond at different times post transporting

Parameters	Time	Day post transporting			Mean ± SD
		1	3	7	
Temperature (°C)	8:00	28.50	28.30	28.50	28.43 ± 0.09
	16:00	29.50	29.40	29.30	29.40 ± 0.07
pH	8:00	8.00	7.90	8.00	7.97 ± 0.04
	16:00	8.50	8.40	8.60	8.50 ± 0.07
DO (ppm)	8:00	5.20	5.40	5.50	5.37 ± 0.11
	16:00	6.70	6.50	6.70	6.63 ± 0.09
CO_2 (ppm)	8:00	9.50	10.50	10.00	10.00 ± 0.33
	16:00	1.60	1.20	0.50	1.10 ± 0.40
TAN (ppm)	8:00	1.30	1.50	1.20	1.33 ± 0.11
NO_2 (ppm)	8:00	0.01	0.02	0.05	0.03 ± 0.02

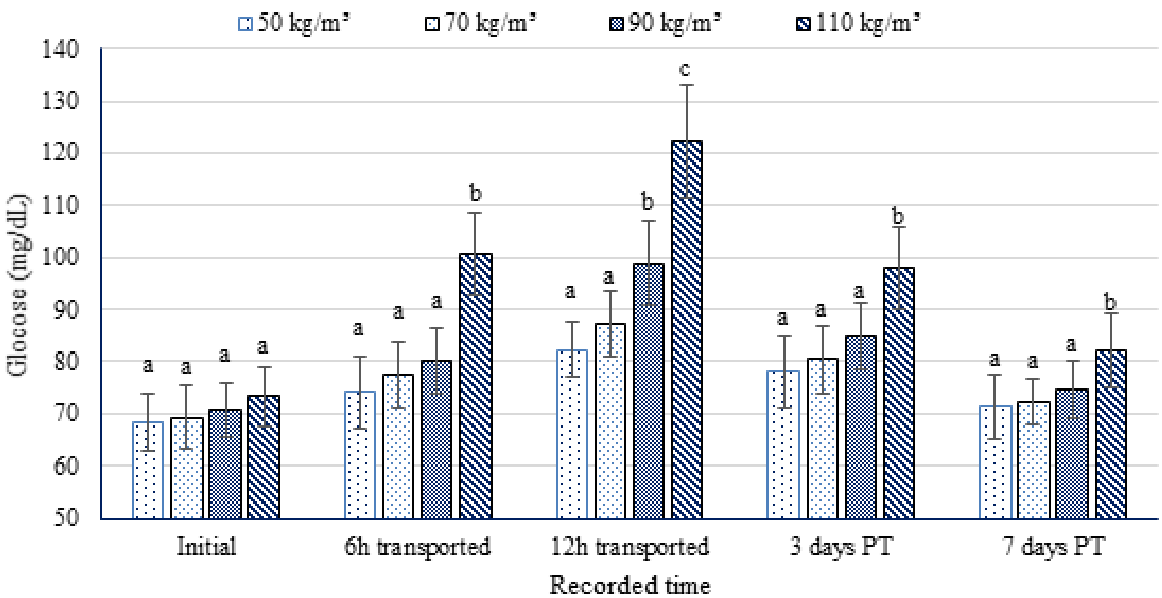

Figure 1. Variation of glucose content (mg/dL) in fish blood at the time of survey.
Columns containing the same letters present no significant difference of mean blood glucose at $P < 0.05$ where a < b < c.

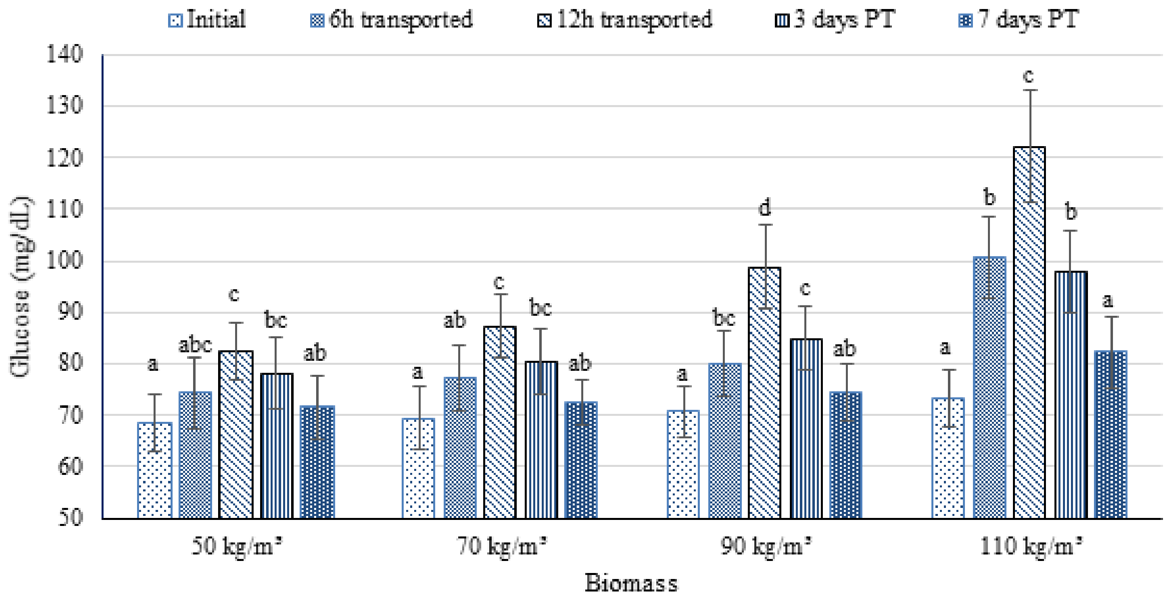

Figure 2. Variation of glucose content (mg/dL) in fish blood in different transport biomass. Columns containing the same letters present no significant difference of mean blood glucose at $P < 0.05$ where a < b < c < d.

original value before transportation. However, in the treatment of 110 kg/m^3, the glucose content was still significantly higher ($P < 0.05$) compared with the other treatments (Figure 1).

Examining the variation of glucose content in each treatment, it showed that the glucose content tended to increase during transport and reached the highest value at the end of the transport duration (12 h). Then the glucose content tended to decreasing during post-transportation. In the treatment with higher biomass, the change in blood glucose concentration was stronger (Figure 2).

3.3. Survival rate

Survival rate of the fish in the 6 and 12 h transport, 3 and 7 days post transporting was presented in Figure 3.

In general, the survival rate (SR) of the fish reduced following transport and post transporting times. In the 6 h transport time, SR means of the fish in T4 treatment (92.67%) was lowest and significantly different ($P < 0.05$) from the others but there was no significant difference ($P > 0.05$) of the SR means between T3 (97.33%), T2 (98.67%) and T1 (98.67%) treatments. At the end of the transport (12 h), SR means of the fish T1 treatment (98,00%) was highest and followed by T2 (97,33%), T3 (92,00%), and T4 (84,00%) treatments. There was a significant difference of the SR means between the treatments, except for T1 and T2 treatments. A same trend of the SR of the fish was found for 3 days post transporting time (with T1 = 96.67%), T2 = 95.33%, T3 = 90.00% and T4 = 80.00%), as well as for 7 days post transporting (with T1 = 96.00%, T2 = 95.33%, T3 = 90.00% and T4 = 78.67%). At these times, the SR means of the fish between the treatments were significantly different, except for T1 and T2 treatments (Figure 3).

4. Discussion

Fish health state during live transportation is affected by several combined factors including dissolved oxygen (DO), pH, carbon dioxide (CO_2), ammonia (NH_3), and temperature. Respiration by the fish and bacteria causes depletion of DO and production of the toxic metabolite CO_2 and NH_3 accumulated in the transport water. The increase in CO_2 causes water pH to decrease and low pH increases the proportion of the toxic form of CO_2, but decreases the proportion of the toxic form of NH_3 (Berka, 1986; Rimmer et al., 1997a). Rimmer et al. (1997a) found that in closed seabass transport without water cooling and fish anesthesia, dissolved oxygen lev-

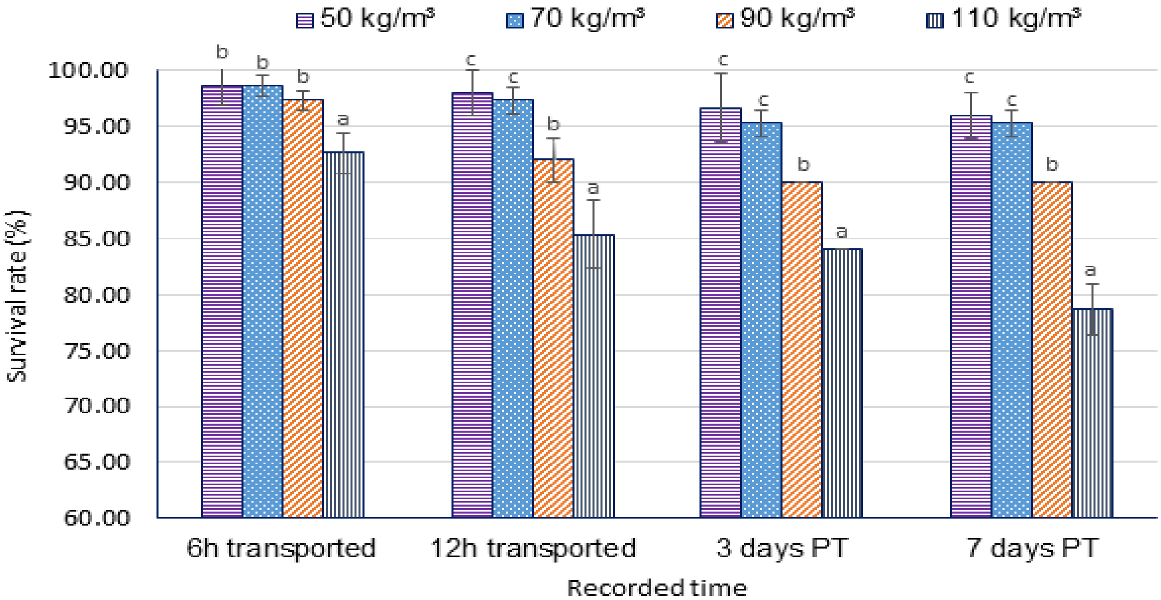

Figure 3. Survival rate of the fish at different time during transport and post transporting (PT). Columns containing the same letters present no significant difference of mean survival rates at $P < 0.05$ where a < b < c.

els at packing were 7.2 mg/L and dropped rapidly to only 3.5 mg/L; CO_2 also built up rapidly, from 13 to 38 mg/L; pH dropped rapidly from an initial value of 8.1 to 6.8 within 1.2 h after packing; in contrast to these variables, ammonia accumulated at a relatively constant rate throughout the experiment. In this study, DO levels of the treatments at packing were high (7.55 mg/L) and dropped consecutively (6.37 - 5.50 mg/L and 6.20 - 5.33 mg/L in the 6 and 12 h transporting times, respectively); in the same recorded times, CO_2 concentrations were low (0 mg/L) and increased (11.67 - 16.67 mg/L and 15.83 - 25.33 mg/L); pH values were high (8.2) and decreased (7.60 - 7.13.67 mg/L and 7.27 - 6.23 mg/L); and TAN concentrations were also low (0.5 mg/L) and increased (1.13 - 2.20 mg/L and 1.27 - 3.00 mg/L) (Table 1 and 2). However, the increase of CO_2 and TAN concentrations and decrease of DO levels and pH values were lower and slower compared to those in the study of Rimmer et al. (1997a). The lower and slower changes of these water parameters in this study could be explained by the cooling water and fish anesthesia which resulted in reduction of the metabolic rate of the fish thereby reducing oxygen consumption and the production of NH_3 and CO_2. High DO levels of the treatments at the end of the transport were due to the continuous oxygenation by the pure oxygen. The fluctuation of the water quality parameters in this study was also similar to that of studies of Simões et al. (2011) and of Gil et al. (2016) in live fish transportation of Nile tilapia (*Oreochromis niloticus*) and olive flounder (*Paralichthys olivaceus*) with the anesthetic of clove oil. The values of pH, DO, NH_3, and NO_2 of the transport water in this study were in suitable ranges for seabass recommended by Tookwinas & Charearnrid (1988). Thereby, the fish had high survival rates (92.67 - 98.67%) across all loading biomass in the 6 h transporting time (Figure 3).

In general, cooling water is only to make the fish sedated - reducing movement and maintaining equilibrium (Yoshikawa et al., 1989). An ideal anesthetic stage in live fish transportation is perfect sedation expressed by only opercular movement which can be achieved with anesthetic use (Simões et al., 2011; Gil et al., 2016). Cupp et al. (2017) found that at high loading densities of yellow perch (Perca flavescens) (240 g/L) and Nile tilapia (*O. niloticus*) (480 g/L), AQUI-S 20E (10% eugenol) concentrations (100 and 200 mg/L) decreased rapidly in transport tank water regardless anesthetic levels, and fish showed no signs of sedation by the end of the transport (6 h). According to Park et al. (2018), lowered temperature was effective in reducing stress measured by plasma cortisol in juvenile and adult red spotted grouper (*Epinephelus akaara*) after exposure to 50 ppm clove oil for 48 h in various temperatures. However, for longer transport the mortality of the fish was increased, particularly at high loading densities of the T3 and T4 treatments. Elevated carbon dioxide concentrations are detrimental to fish and can be a limiting factor in fish transport (Berka, 1986). The survival of fish transported live is directly influenced by carbon dioxide and dissolved oxygen levels in the transport medium, either singly, or in combination (Rimmer et al., 1997b). The increase in the mortality of the fish by the end of the transport (12 h) in this study may be due to the stress of the fish caused by the increase of CO_2 (Table 1). Berka (1986) also noted that aeration of the water will reduce concentrations of dissolved CO_2, if there is adequate ventilation. Moreover, Alabaster et al. (1979) found that high levels of DO decreased the toxicity of ammonia in transport tanks. In the present study, applying aeration and oxygenation in the transport tanks could not prevent fish death but may mitigate the problem of over-accumulation of carbon dioxide and reduce the toxicity of NH_3, particularly at high loading biomass.

The change in blood glucose content of animals is considered as a hematological indicator to assess the stress level of animals. When animals are stressed, the adrenal glands are activated to release glucose to provide more energy to fight stressors, which often leads to increased blood glucose levels (Nguyen, 2005). Nguyen & Do (2014) showed that glucose content of pangasius fingerlings fluctuated and increased during transportation. When transporting for a long time, the glucose content increases due to stress. Dang (2019) when stressing pangasius fingerlings by changing temperature and salinity also showed an increase in blood glucose of fingerlings.

In this experiment, the blood glucose concentration of fish increased during the transport and reached the highest value at the end of the transport. When transporting fingerlings with higher biomass (90 to 110 kg/m^3), the fish was more stressed and the recovery was slower than the treatments with lower biomass (50 to 70 kg/m^3).

In general, the fish well recovered expressed by low mortality, particularly in the T1, T2, and T3 treatments, after the transport. Based on survival rate, loading biomass of 70 to 90 kg/m^3 were proper for live transport for 6 h and 50 to 70 kg/m^3 were proper for live transport for 12 h of seabass juvenile in cooling water of 22°C and sedated with AQUI-S® (containing 50% isoeugenol) at the concentration of 6 ppm.

5. Conclusions

The quality of tank water was declined following transport duration presented by the decrease of DO and pH, and the increase of CO_2, TAN and NO_2. Fish blood glucose levels increase and change with respect to transit time and fish biomass contained in the transport equipment.The survival rates of the fish decreased following times in transport duration and post transport, and following increased loading densities. The suitable loading biomass for live transport of seabass juvenile were 70 to 90 kg/m^3 and 50 to 70 kg/m^3 with a water temperature of 22°C, concentration isoeugenol-50% of 6 ppm and for 6 h and 12 h respectively. This study suggests for carrying out trials on live fish transport of marketable size of seabass.

Conflict of interest

The authors declare that they have no any conflict of interest in this paper.

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Two-step pretreatment for improving enzymatic hydrolysis of spent coffee grounds

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ABSTRACT

Spent coffee ground (SCG) has attracted increasing attention since it contains many useful components such as polysaccharides, protein, lipid, and bioactive compounds. The aim of this research was to enhance the enzymatic hydrolysis to release important sugars in the SCG using different pretreatment methods. Spent coffee grounds were pretreated by alkali pretreatment, organosolv pretreatment, and the combined process. The pretreated material was hydrolyzed by different commercial enzymes including Cellulast, Pectinex, Ultraflomax, and Viscozyme. Monosaccharides, total phenolic content, and antioxidant activity in the hydrolysate were measured and evaluated. The use of Viscozyme achieved the highest reducing sugar yield and showed a significant difference from other enzymes. Alkali and organosolv pretreatment were demonstrated to improve the production of sugars. The alkali pretreatment followed by organosolv treatment effectively removed lignin, resulting in only 14% lignin in the pretreated sample. The maximum reducing sugar concentration reached 6120 mg/L through two-step pretreatment and subsequent enzymatic hydrolysis, corresponding to a yield of 161 mg sugar/g substrate. The SCG hydrolysate contained 2917 mg/L mannose, 1633 mg/L glucose, and 957 mg/L galactose. Phenolic compounds were observed to be released during the enzymatic hydrolysis, giving a total phenolic content of 174.4 mg GAE/L and the SCG hydrolysate also showed an antioxidant capacity equivalent to 263.2 mg/L ascorbic acids after 120 h hydrolysis. This study demonstrated a scalable two-step pretreatment process to obtain important sugars including mannose, glucose, and galactose along with phenolic compounds for further industrial uses.

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1. Introduction

Coffee is one of the most popular beverages in the world and the second largest traded commodity after petroleum. Coffee production generates an enormous amount of solid residues namely spent coffee grounds (SCGs). About nine million tons of SCGs are released into the environment every year, which may cause serious environmental problems (Karmee, 2018). SCG has recently attracted increasing interest since it is a valuable resource of sugars, oils, antioxidants, proteins, and other high-value compounds (Peshev et al., 2018).

SCG is a lignocellulosic material and essentially consists of polysaccharide polymers and lignin. The major polysaccharides in SCG include galactomannan, arabinogalactan, and cellulose. Among the monosaccharides in SCG, mannose constitutes the largest portion (20 - 30%) of its total carbohydrate content, which make it become a promising source for mannose production (Nguyen et al., 2017). Mannose has been widely used in the food, pharmaceutical, cosmetic and poultry industries and acts as starting material for the synthesis of drugs (Hu et al., 2016). Although carbohydrate is the most abundant fraction in SCG (up to 50%), the extraction of sugars from SCG is not simple. Like other lignocellulosic biomass, SCG structure is rigid, dense, and recalcitrant. Without any pretreatment, the bioconversion yield of polysaccharides into monosaccharides is limited.

Pretreatment methods are applied to increase the efficiency of lignocellulose hydrolysis by improving enzyme accessibility to polysaccharides. An efficient pretreatment strategy is generally simple to perform and produces high fermentable sugar yields with the minimal formation of degradation products (Ravindran et al., 2017). High lignin content restricts the efficiency of enzymatic hydrolysis of lignocellulosic biomass. Therefore, removal of lignin is a key strategy for achieving effective pretreatment and hydrolysis. Ranvindran et al. (2017) performed eight different pretreatment methods in SCG but a single process didn't give desirable results. Then, the sequential combined process using concentrated acid, and acetone pretreatment followed up with the ammonia fiber explosion pretreatment showed to achieve the maximum sugar yield. A combined process is a recent strategy since single pretreatment couldn't overcome the recalcitrance of complex biomass.

In this study, SCGs were pretreated using alkali and organosolv pretreatment. Both two methods aim to dissolve lignin by cleaving the ester linkages between polysaccharides and lignin. However, the pretreatments can cause partial degradation of hemicellulose and cellulose at severe conditions of temperatures or alkaline solution concentrations. Therefore, we performed SCG pretreatment at mild conditions and applied two-step pretreatment combining alkali and organosolv pretreatment. This approach is potential to accelerate the enzymatic hydrolysis via promoting delignification but minimizing polysaccharide degradation and expected to be feasible in large scale.

2. Material and Methods

2.1. Material

Spent coffee grounds were collected from several coffee shops in Ho Chi Minh city, Vietnam. The samples were mixed and dried at 50°C to a moisture content of below 10% before use.

Cellulast 1.5 L, Pectinex Ultra SP-L, Ultraflomax and Viscozyme (Novozyme) were supplied by Brenntag (Vietnam). Standard chemicals including glucose, galactose, mannose were purchased from Sigma Aldrich. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3,5-Dinitrosalicylic acid (DNS), Folin-Ciocalteu, gallic acid, albumin and ascorbic acid were purchased from Merck. Other chemicals were purchased from Xilong (China).

2.2. Methods

2.2.1. Defatting

SCG was defatted using hexane (ratio of hexane:biomass is 5:1) by sonication for 30 min. The defatted biomass was dried in an oven at 60°C, and its moisture content was measured before further analysis.

2.2.2. Chemical compositions of SCG

Crude protein and ash content of SCG were quantified according to TCVN 10791:2015 and TCVN 8124:2009/ISO 2171:2007, respectively.

The qualitative analysis of the monosaccharide compositions and lignin content of SCG samples was performed according to Sluiter et al. (2008) and Trinh et al. (2018).

2.2.3. Pretreatment of SCG

Defatted SCG was pretreated using several methods. Alkali pretreatments were carried out using NaOH 1% in an autoclave at 120°C for 15 min at a ratio of biomass to the alkaline solution of 1:5. Organosolv pretreatments were conducted by mixing SCG with acetone at a ratio of biomass to solvent of 1:5 in a sonicator apparatus for 1 h. The solid residue was separated and dried for further use. The two-step pretreatment was initiated using alkali pretreatment followed by organosolv pretreatment.

2.2.4. Enzymatic hydrolysis of SCG

Pretreated SCG was hydrolyzed using several commercial polysaccharide-degrading enzymes including Cellulase, Pectinex, Ultrafomax, and Viscozyme. Enzymatic hydrolysis experiments were carried out in 50 mM citrate buffer (pH 5.0) with 4% biomass (w/v) in a shaking incubator. The ratio of enzyme to SCG is 5%. The hydrolysate was collected by centrifugation for 10 min and filtered through a nylon membrane 0.22 µM before a measurement of reducing sugars and monosaccharides.

2.2.5. Determination of reducing sugars

Reducing sugar content was quantified using 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959). 1 mL of sample was mixed with 1 mL of DNS for 5 min in a boiling bath, then the mixture was kept in a cold water bath for 10 min prior to adding 3 mL of water. Glucose was used as the standard with a range of 50 - 300 mg/L.

2.2.6. Analysis of monosaccharides

Mannose, galactose and glucose were quantified by high-performance liquid chromatography (HPLC Agilent 1200 Infinity II) using a refractive index detector. The Rezex RPM- Monosaccharide Pb+2 (8%) (Phenomenex) column (100 mm × 7.8 mm) was operated at 85°C. The mobile phase is deionized water at a flow rate of 0.2 mL/min.

2.2.7. Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) in the hydrolysate was determined using the Folin-Ciocalteu colorimetric method described previously (Trinh et al., 2018). Antioxidant activity of the hydrolysate was estimated by DPPH assay according to the reported procedure (Trinh et al., 2018). Briefly, 1 mL of sample was added to 1 mL of 0.16 mM ethanolic DPPH solution. The mixture was incubated in darkness for 30 min at room temperature. Ascorbic acid was used as the standard. All results were expressed as mg ascorbic acid equivalent/L of hydrolysate (mg AAE/L).

2.3. Data analysis

All experiments were performed in triplicate. Means and standard deviations (SD) are given for three independent experiments. Statistical analysis was performed using Minitab 16. All analytical results are reported on the dry matter mass of the samples.

3. Results and Discussion

3.1. Chemical compositions of SCG

The chemical composition of SCG is highly variable depending on the type of coffee, its growing conditions and the brewing method. The largest component of SCG is polysaccharides including cellulose and hemicellulose, which make up more than 50% of the dry mass of the SCG (McNutt et al., 2018). In this study, we collected many samples from coffee shops, then mixed them up and dried before use. Mannose and galactose were identified as the main components of the hemicellulose sugars, while glucose is the major composition of cellulose (Figure 1). Total polysaccharides accounted for about 50.1% of the dry SCG. Mannose was the most abundant sugar (28.6%), followed by galactose (12.3%) and glucose (9.2%). Mannose, an important sugar, is used widely in food, medicine, cosmetic, and food-additive industries. Mannose was demonstrated to improve the immune system and give many benefits to health. However, mannose production using chemical synthesis and plant extraction cannot meet the requirements of the industry (Wu et al., 2019). Since SCG is rich in mannose, it is considered as a potential source for mannose production. Lignin is the second most abundant component in SCG, which made up 23.5% (dw). Besides, SCG also contains a significant amount of oil (9.7%), crude protein (14.5%), and small portion of phenolic compounds, ash and caffeine. Similar results have been reported in the literature (McNutt et al., 2018; Nguyen et al., 2019).

3.2. Effect of enzyme on the hydrolysis performance

Enzymatic hydrolysis of SCGs was performed using four types of commercial enzymes including Cellulast, Pectinex, Ultraflomax, and Viscozyme. Temperature is one of the most important factors affecting hydrolysis performance. The hydrolysis experiments were conducted from 35 - 55°C to optimize the working temperature for each enzyme type. Figure 2 showed that Viscozyme and Ultraflomax efficiently worked at 35 - 45°C, while Cellulast and Pectinex displayed the best performance at a wider temperature range of 35 - 50°C. Similar results were mentioned previously (Gama et al., 2015; Andlar et al., 2016). The

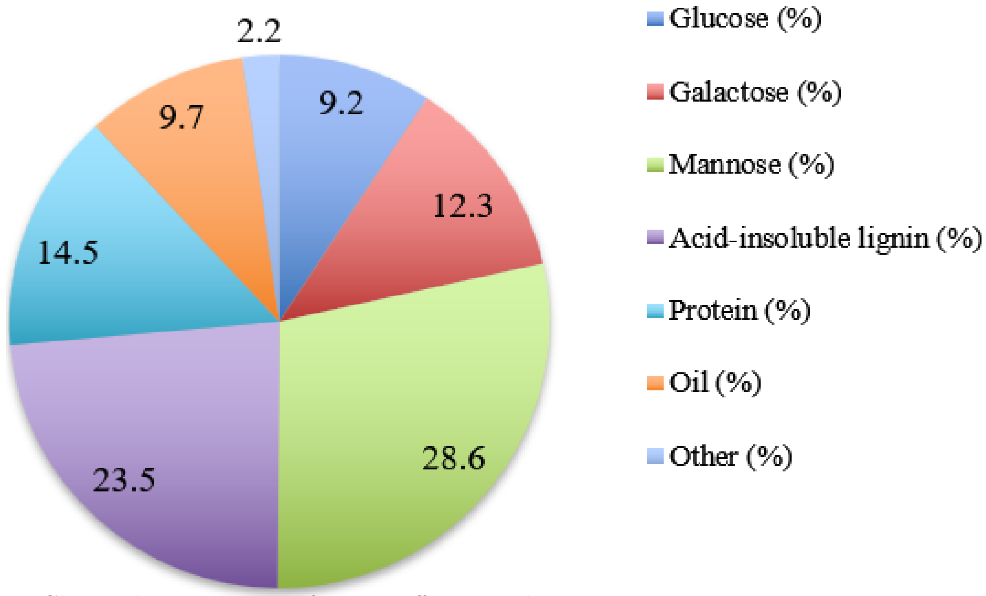

Figure 1. Chemical compositions of spent coffee ground.

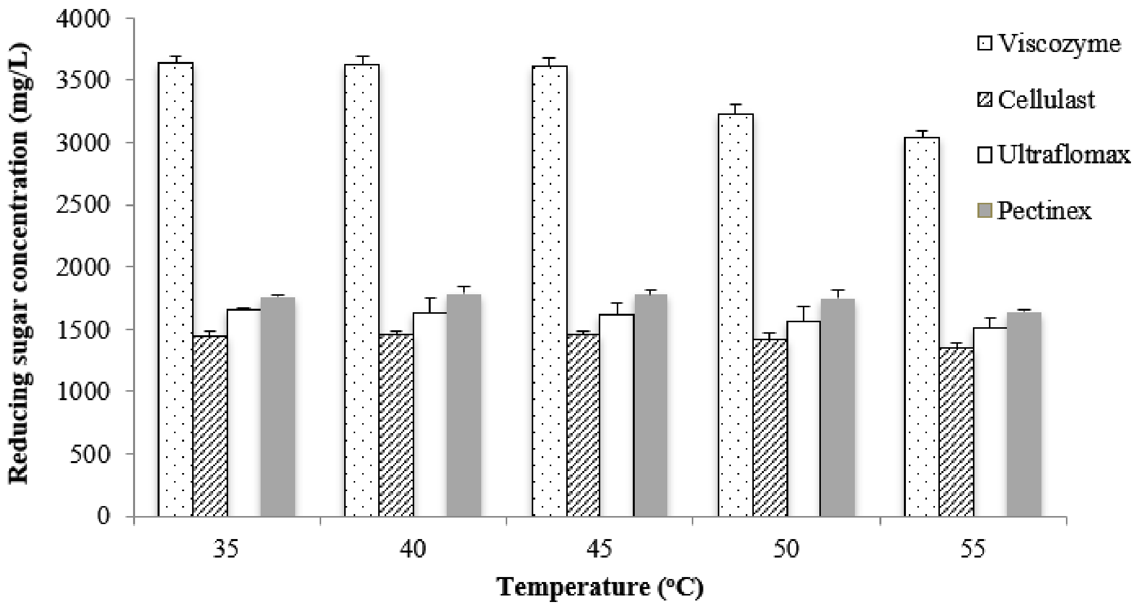

Figure 2. Effect of temperatures on the enzymatic hydrolysis of spent coffee ground.

optimized temperatures were identified within the range recommended by the enzyme manufacturer. Then, further hydrolysis experiments were carried out at 35°C for all enzyme types.

SCGs contain a high fraction of hemicellulose and cellulose, thus the enzymatic hydrolysis process required a mixture of hemicellulase and cellulase. The use of single enzyme was not effective for the hydrolysis of complex lignocellulosic biomass (Cho et al., 2020). In fact, Cellulast is only composed of cellulase leading to the lowest reducing sugar concentration as a result (Figure 3). Pectinex is composed of enzyme activities of pectinase, hemicellulase and beta-glucanase, while Ultraflomax is a cocktail of xylanase and glucanase. The use of Pectinex and Ultraflomax showed higher concentrations of reducing sugars than Cellulast. Viscozyme remarkably improved the yield of reducing sugars, giving 2.2 - 2.7 times higher than other enzymes. The highest concentration of reducing sugar was found to be 6120

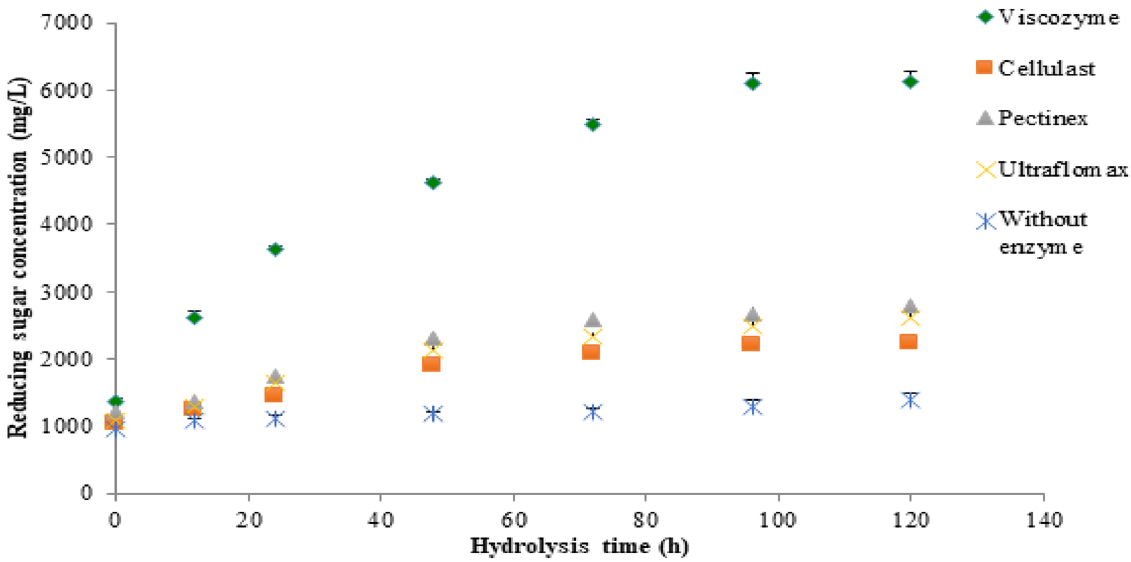

Figure 3. Effect of enzyme types on the hydrolysis of spent coffee ground.

mg/L, while only 1393 mg/L of reducing sugar was released in the experiments without using enzyme. Viscozyme is a mixture of hemicellulase, cellulase, beta-glucanase, arabinase, and xylanase which was widely used for the hydrolysis of various lignocellulose types such as SCG, sugar beet, and apple pomace (Gama et al., 2015; Andlar et al., 2016; Liu et al., 2021). Liu et al. (2021) successfully performed alcoholic fermentation based on SCG hydrolyzed with 6% Viscozyme. Another study prepared a SCG hydrolysate for effective lactic fermentation using a mixture of Viscozyme and Cellulast (Hudecova et al., 2018). Reducing sugar concentration increased rapidly within 96 h but then slowed down. Reducing sugar released at 96 h was not significantly different from that at 120 h when Viscozyme was used.

3.3. Effect of different pretreatments on the hydrolysis performance

The presence of lipid in SCGs limits the access of hydrolytic enzyme to its substrate, therefore the lipid in SCG was removed prior to further pretreatments. Pretreatment is a crucial step in the conversion of lignocellulosic biomass into soluble sugars. Pretreatment aims to decompose the complex biomass matrix, remove lignin, and increase the enzyme accessibility to polysaccharides, subsequently improving the yield of enzymatic saccharification (Trinh et al., 2018). In the study, organosolv and alkali pretreatment showed an improvement in the yield of reducing sugars, achieving 4205 mg/L and 4806 mg/L, respectively, after 120 h hydrolysis. While, enzymatic hydrolysis of untreated sample released 3565 mg/L reducing sugars at the same time (Figure 4). Generally, organosolv pretreatments occur with numerous organic or aqueous solvent mixtures at high temperature to break down the complex structure of lignocellulose and solubilize lignin. Alkali pretreatment is capable of removing lignin and a part of the hemicelluloses by destroying the linkages between lignin and other polymers, thereby facilitating enzyme access to its substrate and improving the production of fermentable sugars (Wongsiridetchai et al., 2018; Jin et al., 2019). The lignin removal and hemicellulose solubilization together facilitate exposing the accessible area of biomass for the subsequent enzymatic hydrolysis process. In this study the pretreatment with acetone was carried out using sonication method which was both effective for the dissolution of lignin and the extraction of polyphenols (Ravindran et al., 2018). The lignin content of organosolv pretreated sample was 23.5%, being lower than untreated sample (26.1%) (Table 1). While, the use of NaOH (1%) also showed a decreased lignin level, giving 21.8% in the pretreated biomass. The alkali treatment of SCG followed by organosolv pretreatment achieved the highest reducing sugar (6120 mg/L) via enzymatic hydrolysis after 120 h, which was 1.7-fold higher than the untreated sample. The two-step pretreatment significantly enhanced the production of sugars compared to the individual

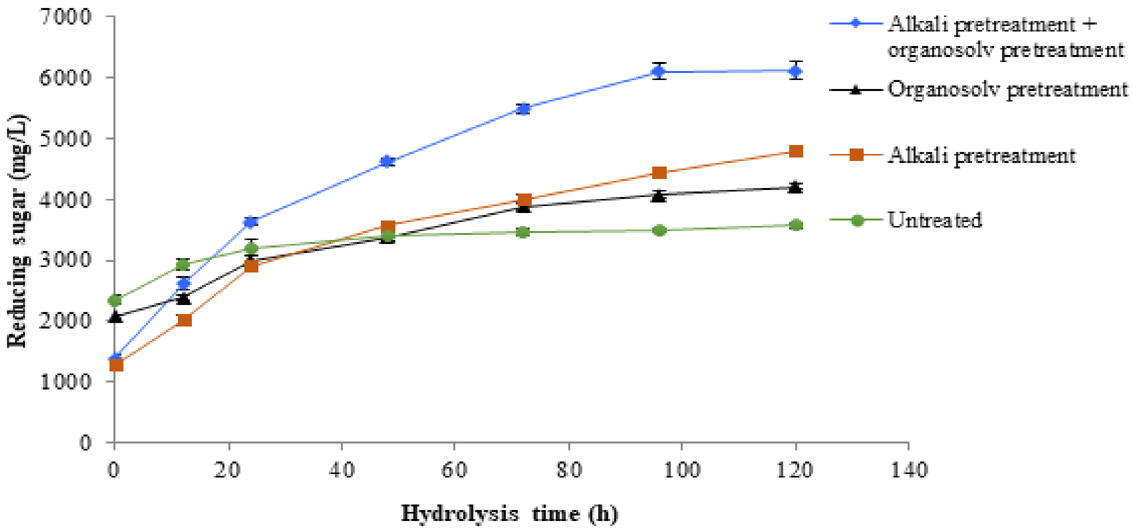

Figure 4. Effect of pretreatment methods on the hydrolysis performance.

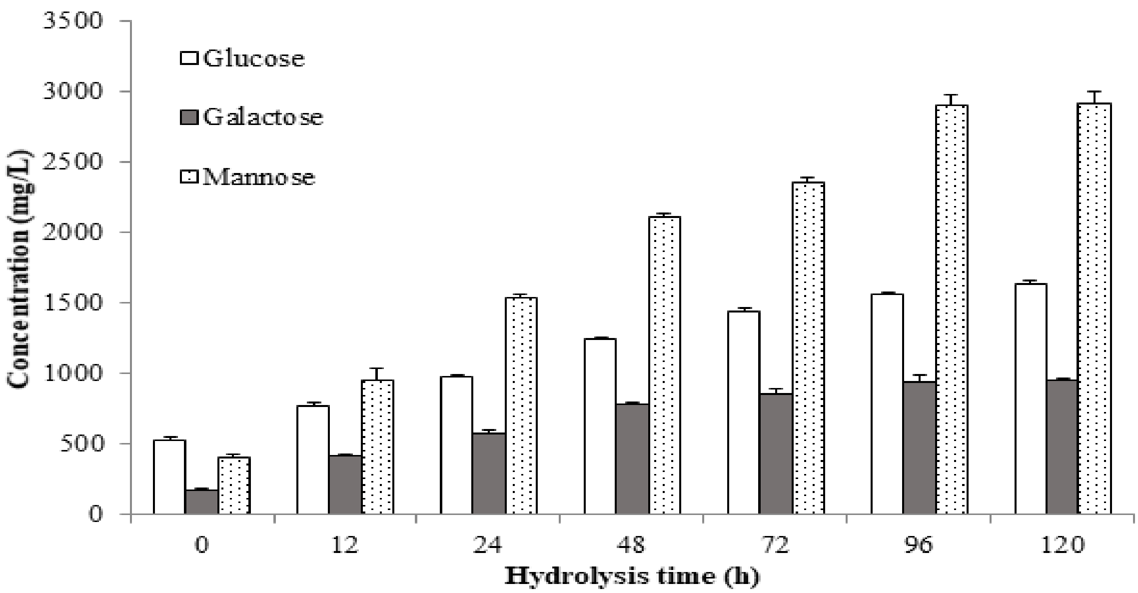

Figure 5. The production of monosaccharides during the enzymatic hydrolysis.

pretreatment process. This is attributed to the effective delignification of both processes compared to the individual step. In fact, the compositional analysis revealed that only 14% of lignin was determined in the SCG pretreated with two-step pretreatment which may explain for the remarkable improvement of biomass digestibility (Table 1). In a previous report, alkali pretreatment with NaOH (0.5 N) has been demonstrated to increase the effectiveness of enzymatic hydrolysis of SCG and produce 526 mg/L of reducing sugar (Wongsiridetchai et al., 2018). Our study successfully developed a two-step process that effectively removed lignin and subsequently enhanced the yield of enzymatic hydrolysis. The use of the combined method has been widely performed previously since single pretreatment couldn't overcome the recalcitrance of biomass (Ravindran et al., 2017; Tang et al., 2020).

3.4. Analysis of the SCG hydrolysate

Spent coffee ground was pretreated by two-step process before applying enzymatic hydrolysis with 5% Viscozyme. Figure 5 shows the release of monosaccharides during the enzymatic hydrolysis within 120 h. Mannose is the most abundant sugar identified in the SCG hydrolysate with the highest concentration of 2917 mg/L after 120 h, which accounted for 47.7% of total reducing sugars. At the same time, the maximum concentration of glucose and galactose were 1633 mg/L and 957 mg/L, respectively. The corresponding yields of mannose, glucose and galactose were 76.8, 43.0 and 25.2 mg/g SCG, respectively. Currently, mannose is used as a starting material to synthesize immune-stimulatory agents, antitumor agents, vitamins, and D-mannitol (Wu et al., 2019). Mannose in the hydrolysate can be separated from other sugars using an established process mentioned in our previous study (Nguyen et al., 2019). SCG hydrolysate is rich in sugars that can be applied as a fermentation medium for the production of bioethanol (Nguyen et al., 2019; Liu et al., 2021); and organic acid (Hudeckdva et al., 2018). Besides, SCG hydrolysate also contained significant amount of polyphenols, giving 174.4 mg GAE/L of total phenolic content after 120 h hydrolysis. Choi et al. (2017) identified the presence of chlorogenic acid, gallic acid, and protocatechuic acid in the SCG, which were responsible for displaying the antioxidant activity. Numerous methods have been reported for the extraction of phenolic compounds. The use of enzymes such as cellulase, hemicellulase, pectinase is capable of breaking down the plant cell walls, thereby facilitating the release of small molecules such as phenolic compounds. Viscozyme contains enzyme activities of hemicellulase, cellulase, beta-glucanase, arabinase and xylanase, which were demonstrated to effectively hydrolyze the cell wall polysaccharides. Therefore, an increase in total phenolic content was observed during the hydrolysis process (Figure 6). Enzyme-assisted extraction is a recently used method that has shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to other methods (Puri et al., 2012). Moreover, the antioxidant activity of the SCG hydrolysate elevated with increasing total phenolic content during the enzymatic hydrolysis with the highest value of 263.2 mg/L ascorbic acid equivalents.

Table 1. Chemical compositions of spent coffee ground pretreated by different methods

Pretreatment method	Cellulose (%)	Galactan (%)	Mannan (%)	Acid-insoluble lignin (%)
Alkali pretreatment + organosolv pretreatment	$13.5^{a} \pm 1.2$	$14.5^{a} \pm 1.3$	$47.7^{a} \pm 1.4$	$14.0^{c} \pm 1.2$
Organosolv pretreatment	$11.4^{ab} \pm 1.1$	$13.5^{a} \pm 0.9$	$34.9^{c} \pm 1.6$	$23.5^{ab} \pm 1.4$
Alkali pretreatment	$9.4^{b} \pm 1.2$	$13.3^{a} \pm 0.6$	$39.6^{b} \pm 1.6$	$21.8^{b} \pm 1.1$
Untreated (defatted)	$10.3^{b} \pm 0.9$	$13.2^{a} \pm 0.9$	$32.8^{c} \pm 1.2$	$26.1^{a} \pm 0.8$

Different letters in a column indicate statistically significant differences ($P < 0.05$).

4. Conclusions

In this study, we demonstrated an efficient pretreatment of SCG initiated by alkali pretreatment using NaOH 1% followed by organosolv

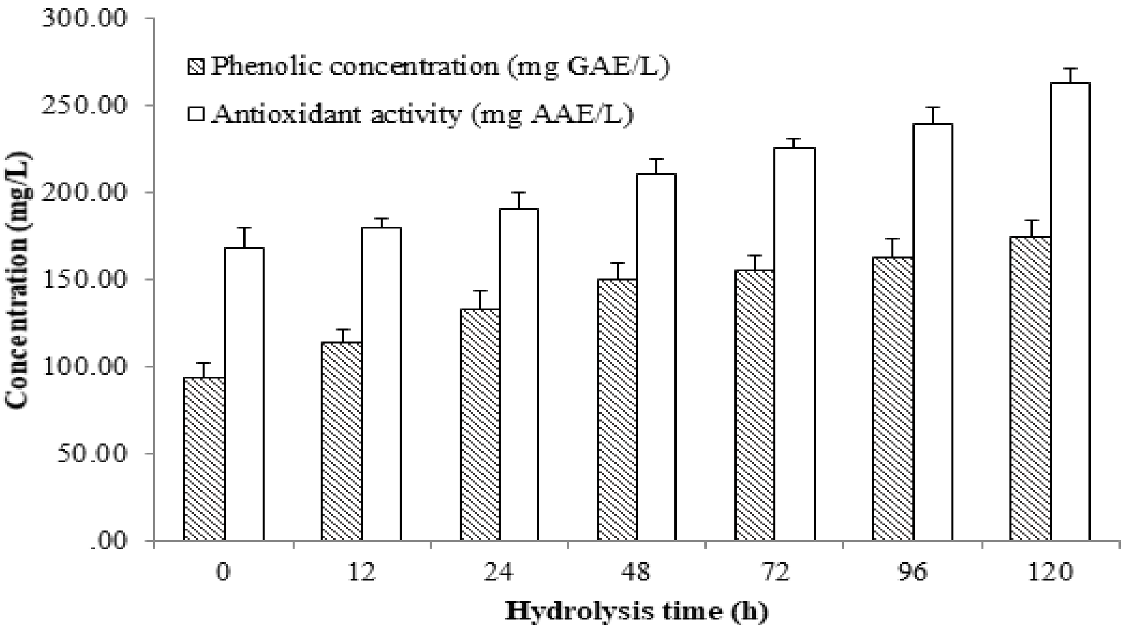

Figure 6. Total phenolic content and antioxidant activity of spent coffee ground hydrolysate.

treatment with acetone. The two-step process effectively removed lignin and improved the production of reducing sugars. The highest sugar concentration reached 6120 mg/L, corresponding to a yield of 161 mg sugar/g substrate. Mannose, the most abundant monosaccharide in the hydrolysate, accounted for 47.7% of the reducing sugars. SCG hydrolysate also contained a total phenolic content of 174.4 mg GAE/L and showed an antioxidant capacity equivalent to 263.2 mg/L of ascorbic acid.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Assessment of surface water quality and some main rivers' capacity of receiving wastewater in Ca Mau province, Vietnam

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ABSTRACT

Surface water from rivers plays a significant role in socio-economic development in Ca Mau province. It supplies freshwater for agriculture and aquaculture. Faced with the pressures of development, surface water quality in Ca Mau province has been being at stake. The aim of this paper was to assess the water quality status and wastewater receiving capacity of the main rivers of Ca Mau province. The obtained results and calculated water quality index (WQI) indicated that almost surface water in Ca Mau province was heavily polluted and did not meet the irrigation purpose. Most rivers and canals in Ca Mau city were not able to receive any more contaminant loads of COD, BOD_5, N-NH_4^+, and P-PO_4^{3-}. To protect the water resource for sustainable development, consequently, it is important to propose water management solutions for the local government to regulate wastewater discharge into surface water bodies in Ca Mau province.

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1. Introduction

Ca Mau, located in the Mekong Delta region of Vietnam, is a coastal province in the southernmost part. This province has a total area of 5,221.2 km^2, a population of 1,229.6 thousand people, and a population density of 236 people/km^2 (GSO, 2018). Ca Mau province is a flat, low-lying area frequently flooded because of its low altitude of –1 to 3 m above sea level and the strong tidal variability of the East Sea and the Gulf of Thailand (Hong & San, 1993). This province is covered by a vast river and canal network, largely contributing to socio-economic development.

In recent years, the process of development and economic restructuring has brought great achievements in the local economy and residents' life quality (GSO, 2018). However, the province's socio-economic development process is continuing at a relatively high pace, which inevitably creates increasing pressures on natural resources and the environment (GSO, 2018). Poor industrial, agricultural and domestic wastewater treatment adds tremendous amounts of nutrients and organic carbon to receiving streams and estuaries, resulting in short-term oxygen loss, fish-killing, and algal bloom proliferation, and long-term creation of dead zones in streams (Reddy & DeLaune, 2008).

Rivers are significant water supply sources for agriculture and aquaculture in Ca Mau province, and particular attention should be paid to water quality and its changing patterns in these rivers. Assessment of water quality by measurement of the WQI is a conventional approach, simple to implement, cost-effective, and widely used in international and domestic studies for stating the general conditions of water quality (Tirkey et al. 2013; Yadav el al. 2015; Nguyen & Nguyen, 2018). Water quality index was designed to provide surface water classification requirements based on standard water characterization parameters (Bordalo et al., 2001; Cude, 2001; Jonnalagadda & Mhere, 2001). It is a clustering algorithm used to turn vast quantities of water characterization data into a single number that enables a casual reader to easily know the water quality status (Akoteyon et al., 2011; and Balan et al., 2012). In addition, the capacity of receiving wastewater of some main rivers and canals in Ca Mau city was determined. On these grounds, the management solutions were proposed to minimize impacts on surface water in Ca Mau province for sustainable development.

2. Materials and Methods

2.1. Monitoring data

To assess the surface water quality in Ca Mau province, water samples from 52 monitoring stations were collected from 2017 to 2018. The samples positions are presented in Table 1 and Figure 1. Each month, samples were taken one day; each day was taken twice - the highest tide peak and the lowest low tide. Water samples were taken midstream 30 cm above the water surface. These samples were grouped into six functional zones with several monitoring parameters presented in Table 2. In addition, the flow rate from the data of hydrological monitoring stations was also used to calculate the wastewater load capacity.

2.2. Method of calculating the WQI

The WQI was calculated according to the formula of VEA (2019) as follows:

$$WQI = \frac{WQI_I}{100} \times \frac{\left(\prod_{i=1}^{n} WQI_{II}\right)^{\frac{1}{n}}}{100} \times \frac{\left(\prod_{i=1}^{m} WQI_{III}\right)^{\frac{1}{m}}}{100} \times \left[\left(\frac{1}{k}\sum_{i=1}^{k} WQI_{IV}\right)^{2} \times \frac{1}{l}\sum_{i=1}^{lWQI_V}\right]^{\frac{1}{3}}$$

Where:

WQI_I: Calculated WQI value for pH parameter

WQI_{II}: Calculated WQI value for Aldrin, BHC, DDT_s, Heptachlor and Heptachlorepoxide

WQI_{III}: Calculated WQI value for As, Cd, Pb, Cr^{6+}, Cu, Zn and Hg

WQI_{IV}: Calculated WQI value for DO, BOD_5, COD, TOC, N-NH_4, N-NO_3, N-NO_2 and P-PO_4

WQI_V: Calculated WQI value total coliforms and *E.coli* parameter

In WQI method, values ranges from 0 – 100 and water qualities are classified as poisoned (< 10), heavily polluted (10 – 25), used for water transport (26 – 50), used for irrigation (51 – 75), used for domestic water supply after adequate treatments (76 – 90), or used for water supply (91 – 100).

2.3. Assessment of capacity of receiving wastewater

The formula for assessing the capacity of receiving wastewater was based on the Circular 76/2017/TT-BTNMT (MONRE, 2017), as follows:

$$L_{tn} = (L_{tđ} - L_{nn} - L_t) \times F_s$$

Where:

L_{tn} (kg/day) is the capacity of receiving the pollution load of water source

$L_{tđ}$ (kg/day) is the maximum pollution load of the water sources for pollutants under review

L_{nn} (kg/day) is the pollution load which is available in the receiving water sources

L_t (kg/day) is the pollution load in the waste source

F_s is the safety coefficient ($0.3 < F_s < 0.7$). The chosen Fs is 0.5 in this study

If L_{tn} is greater than 0, the water sources can still receive contaminants. If L_{tn} is less than or equal 0, it means the water sources are no longer able to receive contaminants.

$$L_{tđ} = C_{qc} \times Q_s \times 86.4$$

Where:

Q_s (m^3/s) is the instantaneous minimum flow measured at water quality monitoring sites

C_{qc} (mg/L) is the concentration limit values of pollutants under consideration specified in water quality standards to ensure the use of water resources (Column B1 of QCVN 08-MT:2015/BTNMT)

Table 1. Locations of samples for surface water quality analysis of Ca Mau province

No.	Coordinates*		Position
	X	Y	
NM-01	09°11'55.0"	105°10'45.2"	Tan Thanh Primary School, Ca Mau city
NM-02	09°10'37.7"	105°08'07.3"	Chua Ba fork, Ca Mau city
NM-03	09°10'27.2"	105°16'44.1"	Tac Van fork, Ca Mau city
NM-04	09°20'50.5"	105°05'17.5"	Xeo Ro fork, Thoi Binh District
NM-05	09°24'53.3"	104°58'07.8"	Khom 3 intersection, U Minh District
NM-06	09°20'35.5"	104°49'33.9"	Khanh Hoi estuary, U Minh District
NM-07	09°04'17.2"	104°58'09.5"	Tran Van Thoi town, Tran Van Thoi District
NM-08	08°56'23.5"	105°00'54.1"	Cai Nuoc fork, Cai Nuoc District
NM-09	08°51'31.7"	104°48'39.9"	Cai Doi Vam town, Phu Tan District
NM-10	08°48'40.5"	104°54'06.0"	Bay Hap estuary, Phu Tan District
NM-11	08°45'22.5"	104°59'28.7"	Tat Nam Can fork, Nam Can District
NM-12	08°42'55.7"	104°49'19.4"	Ong Trang estuary, Nam Can District
NM-13	08°37'16.5"	105°01'13.6"	Rach Goc estuary, Ngoc Hien District
NM-14	08°46'13.1"	105°12'07.8"	Bo De estuary, Ngoc Hien District
NM-15	08°49'32.9"	105°18'15.8"	Ho Gui estuary, Dam Doi District
NM-16	08°58'17.6"	105°19'12.0"	Tan Tien commune fork, Dam Doi District
NM-17	09°01'08.8"	105°24'57.5"	Ganh Hao estuary, Dam Doi District
NM-18	08°59'02.4"	105°12'28.7"	Dam Doi River fork, Dam Doi District
NM-19	08°36'26.5"	104°43'24.4"	Dat Mui restaurant pier, Ngoc Hien District
NM-20	08°57'57.6"	105°06'31.8"	Bay Hap canal, Dam Doi District
NM-21	09°10'13.4"	105°05'14.4"	Confluence of Ong Doc river and Luong The Tran canal, Tran Van Thoi District
NM-22	09°23'52.1"	105°08'29.5"	Ca Mau Sugar Enterprise, Thoi Binh District
NM-23	09°14'35.3"	105°03'51.8"	Gas-Power-Fertilizer Complex, U Minh District
NM-24	09°01'57.4"	104°49'01.7"	Doc estuary, Tran Van Thoi District
NM-25	08°45'41.3"	105°00'08.8"	New Port, Nam Can District
NM-26	09°23'55.3"	105°04'40.8"	Rach Nhum river, Khanh An IZ, U Minh District

*Coordinate system: VN-2000

Table 1. Locations of samples for surface water quality analysis of Ca Mau province (continued)

No.	Coordinates*		Position
	X	Y	
NM-27	08°46’38.1”	105°00’58.0”	Nam Can EZ, Nam Can District
NM-28	09°14’25.7”	104°49’47.7”	LFS, Tran Van Thoi District
NM-29	09°09’26.1”	105°09’21.0”	Minh Phu company, Ca Mau city
NM-30	09°22’03.7”	105°13’10.7”	Hung canal, Thoi Binh District
NM-31	09°02’16.3”	105°00’16.6”	Thi Tuong A Hamlet, Hung My Commune, Cai Nuoc District
NM-32	08°39’21.3”	105° 04’24.5”	Tan An Commune, Ngoc Hien District
NM-33	09°09’46.5”	105°13’17.3”	Quoc Viet company, Ca Mau city
NM-34	09°05’13.3”	105°00’20.2”	On Tu canal, Tran Van Thoi District
NM-35	09°02’46.5”	105°01’57.0”	Dai Loi company, Cai Nuoc District
NM-36	08°54’52.5”	104°52’42.6”	Xang Cong Da canal, Phu Tan District
NM-37	08°50’50.7”	105°07’57.4”	Canal 3, Nam Can District
NM-38	08°37’48.1”	105°01’57.8”	Khom 6 fishing port, Ngoc Hien District
NM-39	08°57’42.9”	105°07’22.4”	Lo Cai Nuoc – Tan Duyet, Dam Doi District
NM-40	09°08’10.4”	104°56’36.9”	Cu canal, Tran Van Thoi District
NM-41	09°17’33.2”	105°12’10.5”	Bach Ngu canal, Thoi Binh District
NM-42	09°18’41.7”	105°00’09.0”	Canal 29, U Minh District
NM-43	09°30’36.2”	105°00’50.1”	Hamlet 10, Bien Bach Commune, Thoi Binh District
NM-44	09°00’18.7”	104°53’48.6”	Thi Tuong Lagoon fork, Tran Van Thoi District
NM-45	09°11’51.6”	104°57’37.5”	U Minh Ha NP buffer zone, Tran Van Thoi District
NM-46	09°12’30.1”	104°57’37.9”	Contiguous area between buffer and core zone of U Minh Ha NP, Tran Van Thoi District
NM-47	09°13’24.7”	104°57’32.4”	U Minh Ha NP core zone, Tran Van Thoi District
NM-48	08°36’11.7”	104°49’21.2”	Mui Ca Mau NP buffer zone, Ngoc Hien District
NM-49	08°35’48.7”	104°47’46.5”	Contiguous area between buffer and core zone of Mui Ca Mau NP, Ngoc Hien District
NM-50	08°35’51.3”	104°46’37.3”	Mui Ca Mau NP core zone, Ngoc Hien District
NM-51	09°13’26.3”	104°57’33.5”	U Minh Ha NP, Tran Van Thoi District
NM-52	09°01’52.0”	105°05’17.1”	Landfill site, Tan Hoa Hamlet, Tan Hung Commune, Cai Nuoc District

*Coordinate system: VN-2000

Figure 1. Map of surface water sampling points in Ca Mau province with the water sampling points.

86.4 is dimensional conversion cofficient from $(m^3)/s \times (mg/L)$ to (kg/day).

$$L_{nn} = Q_s \times C_{nn} \times 86.4$$

Where, C_{nn} (mg/L) is the maximum concentration value of the pollutants in the water sources before receiving wastewater based on monitoring data results.

$$L_t = Q_t \text{ x } C_t \text{ x } 86.4$$

Where, Q_t (m^3/s) is the maximum wastewater discharge of wastewater sources based on the discharge permits

C_t (mg/L) is the maximum concentration values of pollutants in wastewater based on the average value of 10 wastewater samples with a sampling frequency of 3 days/sample.

3. Results and Disscussion

3.1. Results and disscussion

3.1.1. Urban areas (residential areas, central markets, tourist areas)

In the urban areas (from NH-01 to NH-20), the pH values ranged from 7.00 to 8.11, and FOG concentrations ranged from 0.32 to 0.47 mg/L,

Table 2. Functional zones with monitoring parameters

No.	Functional zone	Sampling points	Monitoring parameters
1	Urban areas (residential areas, central markets, tourist areas)	NM-01 to NM-20	pH, Dissolved Oxygen (DO), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD5), $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe, Total Coliforms and Fat, Oil & Grease (FOG)
2	Industrial areas (IZs, factories outside IZs and LFS)	NM-21 to NM-28	pH, DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe, As, Hg, Total Coliforms and FOG
3	Aquaculture and processing aquatic products areas	NM-29 to NM-39	pH, Cl^-, DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe, Total Coliforms and FOG
4	Agricultural areas	NM-40 to NM-42	pH, DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe, As, Hg, Total Coliforms, Plant protection products – Organic Phosphorus (Parathion, Malathion)
5	National parks and Thi Tuong Lagoon	NM-43 to NM-51	pH, DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$ and Total Coliforms
6	Landfill site	NM-52	pH, DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe, As, Hg, Cu, Total Coliforms and FOG

both of them met the requirement of irrigation purpose corresponding to Column B1 of QCVN 08-MT:2015/BTNMT (National technical regulation on surface water quality). The DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, Fe concentrations, and total Coliforms in the urban areas were presented in Figure 2. From 2017 to 2018, TTS concentrations at most sampling sites tended to decrease.

However, the TSS concentrations, ranging from 50 to 221 mg/L, still did not reach QCVN 08-MT:2015/BTNMT, column B1. The concentrations of Fe have tended to increase, and most of the sampling points (17/20 points) were not up to quality for irrigation purposes. Significantly at Tat Nam Can fork (NM-11), Ong Trang estuary (NM-12), and Tan Tien commune fork (NM-16), the Fe concentration was tremendous. Half of the sampling points in the urban areas had COD and BOD_5 concentrations that did not meet Column B1 of QCVN 08-MT:2015/BTNMT, but generally, these parameters did not significantly exceed the regulation. Previous study (Simeonov et al., 2003) also indicated that nutrients and metals occurred in surface water from the domestic activities. Total coliforms at most monitoring points (16/20 points) indicated that surface water in these areas could not be used for irrigation. Ammonium and phosphate concentrations at all sampling points in the urban regions met Column B1 of QCVN 08-MT:2015/BTNMT except at Chua Ba fork (NM-02). Concentrations of DO, COD, BOD_5, ammonium, and phosphates at the Chua Ba fork (NM-02) nearly doubled the water quality regulation used for irrigation because the Chua Ba fork received a large amount of wastewater from the market. In general, surface water in urban areas in Ca Mau province was polluted locally by organic matter, especially the section passing through Ca Mau city (NM-01 to NM-03), where residents are highly concentrated, but the domestic wastewater has not been treated and discharged directly into rivers and canals that is consistent with the study of Vo et al. (2015). Besides, the Ganh Hao canal (NM-17), which receives the chitin factory's wastewater, was also contaminated by organic matters.

3.1.2. Industrial areas

In the industrial areas (from NH-21 to NH-28), the pH values ranged from 6.78 to 7.88, and FOG concentrations were below 0.43 mg/L; both met Column B1 of QCVN 08-MT:2015/BTNMT. Concentrations of Hg and As at all sampling points in the area were slight or undetectable. The DO, TSS, COD, BOD_5, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$,

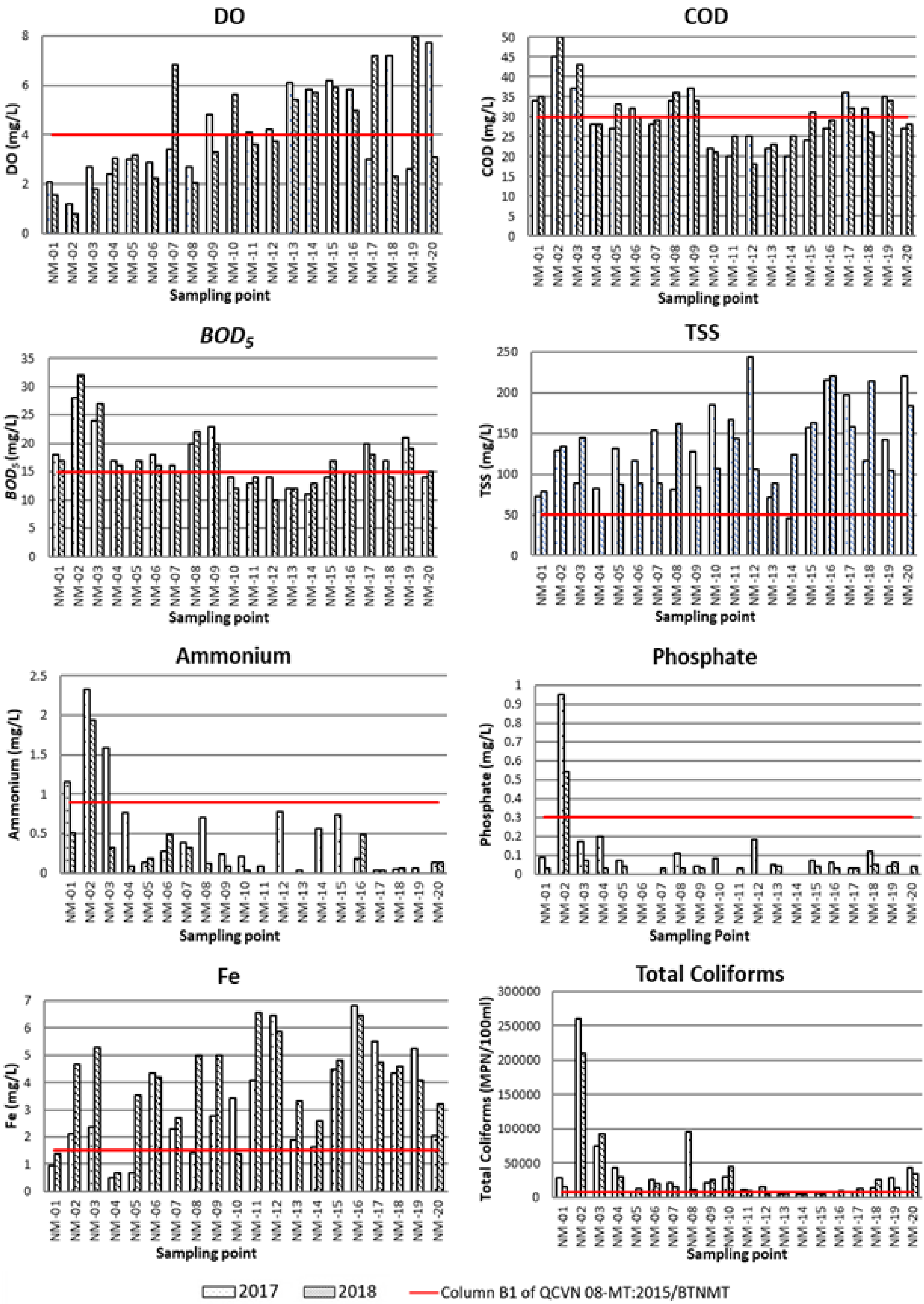

Figure 2. DO, TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-}, Fe concentrations and total coliforms in the urban areas.

Fe concentrations, and total Coliforms in the industrial areas were presented in Figure 3. From 2017 to 2018, TTS and Fe concentrations at almost sampling sites tended to increase and did not reach QCVN 08-MT:2015/BTNMT, column B1. DO, COD, and BOD5 concentrations indicated that surface water at Gas – Power – Fertilizer Complex (NM-23) and LFS (NM-28) was heavily polluted by organic matters because of fertilizers productions and Petrovietnam's activities. Ammonium concentrations at all sampling points in the industrial areas were up to quality for irrigation purposes except at Gas – Power – Fertilizer Complex (NM-23). All sampling points had phosphate concentrations that met Column B1 of QCVN 08-MT:2015/BTNMT, while total Coliforms did not satisfy the regulation. Effects of some water quality parameters especially total coliform and fecal coliform in surface water were analyzed and confirmed in waste water from industrial zones (Diviya and Solomon, 2016).

3.1.3. Aquaculture and aquatic products processing areas

In the aquaculture and aquatic products processing areas (from NH-29 to NH-39), the pH values ranged from 7.46 to 8.56, and FOG concentrations were below 0.47 mg/L, both of them met Column B1 of QCVN 08-MT:2015/BTNMT. Total Coliforms at almost sampling points (10/11) were huge, so water quality in these areas was only suitable for transport purposes. The DO, TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-}, Fe, and Chloride concentrations in the areas were presented in Figure 4. At almost sampling points (10/11), TSS concentrations did not reach the irrigation purposes. The discharge of wastewater from fisheries activities to the surface water increased the TSS concentration, and besides, TSS concentrations in the areas were affected by other factors such as silt content, tidal regime, transportation, . . . Chloride concentrations at all sampling points were very high and did not meet the irrigation purposes. DO, COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations indicated that surface water at Minh Phu company (NM-29) and Quoc Viet company (NM-33) was heavily polluted by organic matters. Because the large fisheries facilities are located in these areas, and the direct discharge without treatment into the canals and rivers is from small and unplanned fisheries companies.

3.1.4. Agricultural areas

The main types of agricultural production of Ca Mau province, such as rice, vegetables, short-day crops (corn, sugarcane, bananas, ...) were located in the farming areas (from NM-40 to NM-42). The pH values ranged from 7.21 to 8.56 that met Column B1 of QCVN 08-MT:2015/BTNMT. Concentrations of Hg, As, and organic phosphorus (plant protection products) at all sampling points in the agricultural area were undetectable. The TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations and total Coliforms in the areas were presented in Figure 5. From 2017 to 2018, ammonium concentrations tended to increase while phosphate concentrations tended to decrease. All agricultural sampling points were lightly polluted by organic compounds. Nutrient emissions from agricultural activities have become the dominant source of nutrient loads to freshwater in the Netherlands. The research focused on nutrient emissions from agriculture, emphasizing nutrient loads to surface waters, and strategies and perspectives to reduce these emissions (Diederik et al., 1998).

3.1.5. National parks and Thi Tuong Lagoon

In the national parks and Thi Tuong Lagoon areas (from NM-43 to NM-51), the pH values ranged from 6.03 to 8.58 that met Column B1 of QCVN 08-MT:2015/BTNMT. The DO concentrations ranged from 0.59 to 4.98 mg/L; in canals in U Minh Ha NP, the DO concentrations were quite low due to the influence of vegetation decomposition in water. The TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations and total Coliforms in the areas were presented in Figure 5. At all sampling points, the TSS concentrations were tremendous and did not reach the irrigation purposes. The total Coliforms at almost sampling points did not meet QCVN 08-MT:2015/BTNMT, Column B1 and tended to increase. The COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations tended to decrease and almost met the irrigation purposes except at U Minh Ha NP (NM-45, NM-46, and NM-47).

3.1.6. Landfill site

The pH value at the landfill site (NM-52) raged from 7.61 to 8.19, meeting Column B1 of QCVN 08-MT:2015/BTNMT. The As, Cu, and

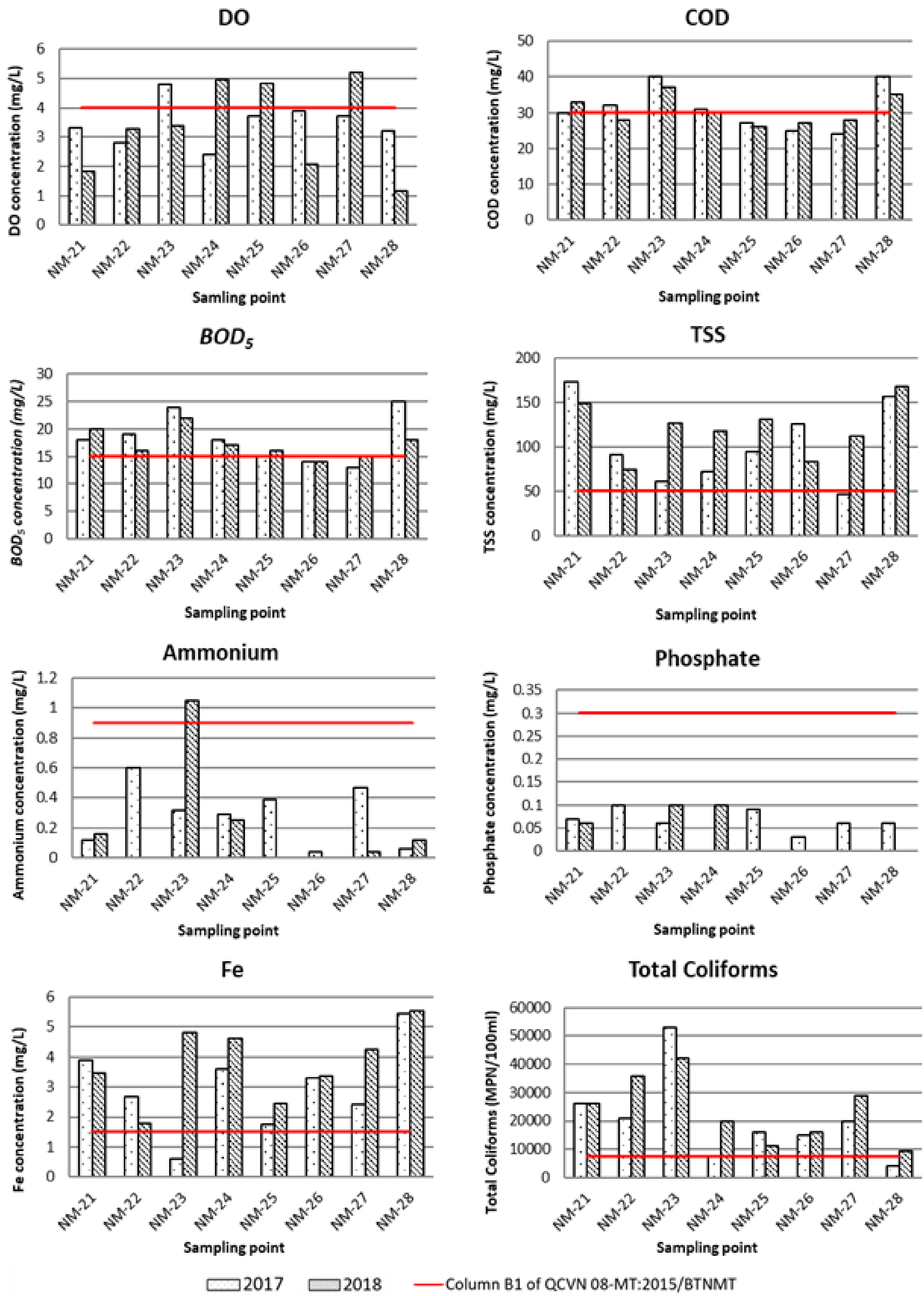

Figure 3. DO, TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-}, Fe concentrations and total coliforms in the industrial areas.

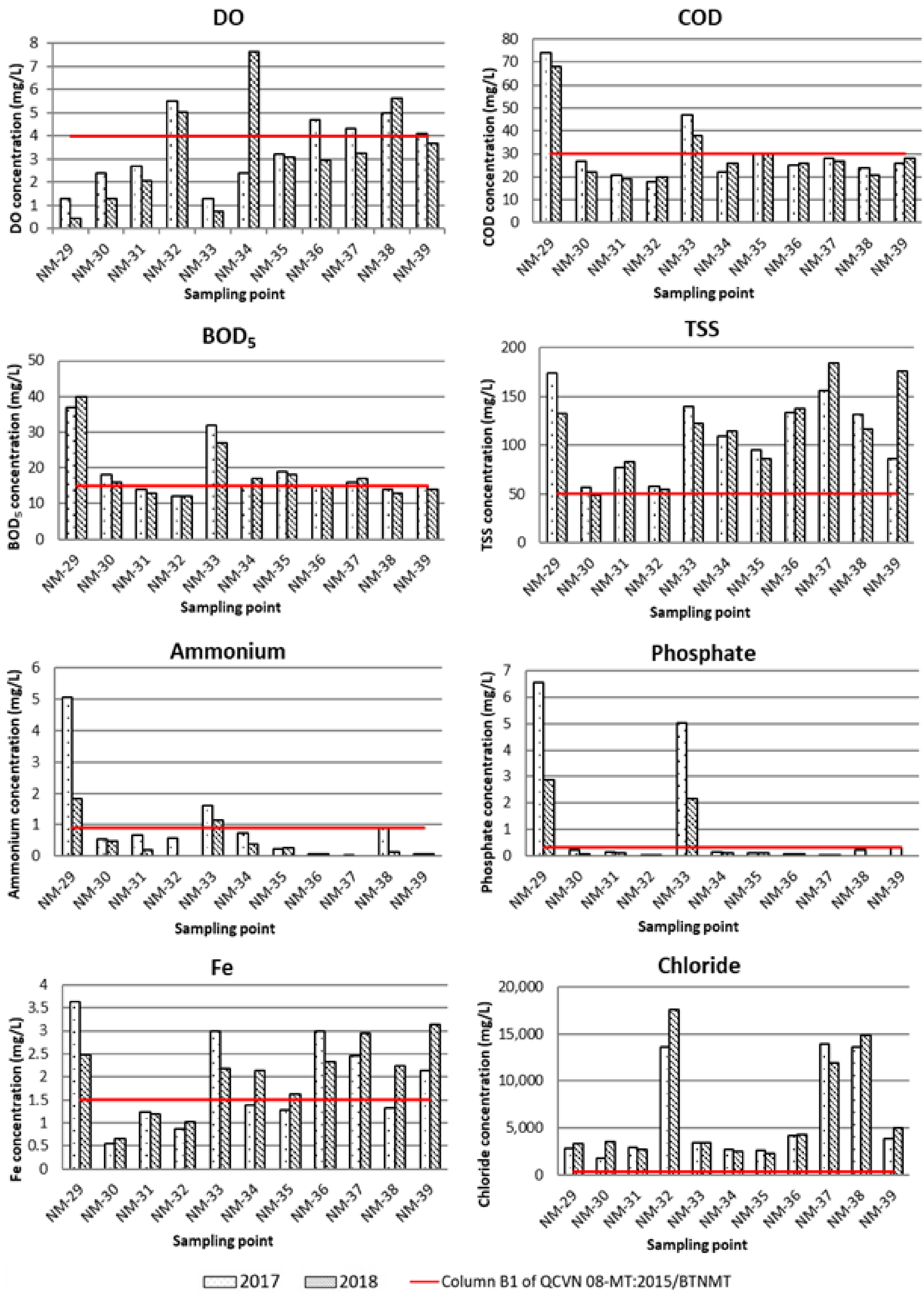

Figure 4. DO, TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-}, Fe and chloride concentrations in the fisheries areas.

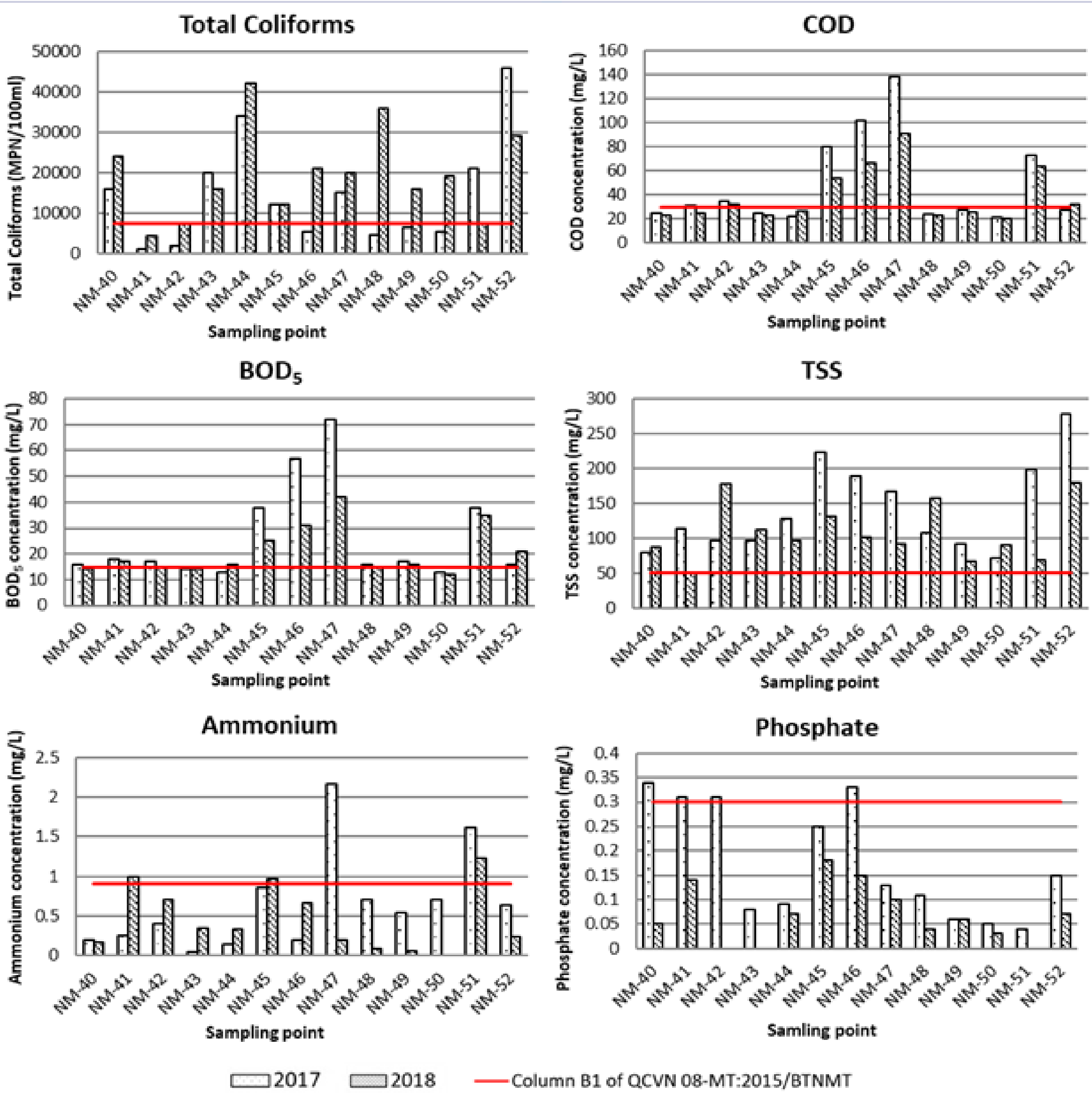

Figure 5. TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations and total coliforms in the agricultural areas, the National Parks, Thi Tuong Lagoon and landfill site.

FOG concentrations were undetectable. The TSS, COD, BOD_5, N-NH_4^+, P-PO_4^{3-} concentrations and total Coliforms at the landfill site were presented in Figure 5. The COD and BOD5 concentrations tended to increase and did not reach the irrigation purpose, while the N-NH_4^+ and P-PO_4^{3-} concentrations tended to decrease and met the intent.

3.1.7. Water quality index values

The calculated WQI values at 52 sampling points in 2018 were presented in Figure 6. The WQI indicates that: Surface water at 05/52 monitoring stations (NM-02, NM-29, NM-33, NM-45, and NM-46) has been heavily polluted that requires timely remediation and recovery solutions; Surface water at 35/52 sampling points has been polluted, which need adequate treatment in the future; Water quality at 09/52 sampling points could be used for transportation; Only 03/52 sampling points (NM-13, NM-15, and NM-41) had water quality reaching the irrigation purpose. One of the main reasons for the low WQI in Ca Mau was the high total Coliforms at most monitoring points. In general, based on WQI, the sur-

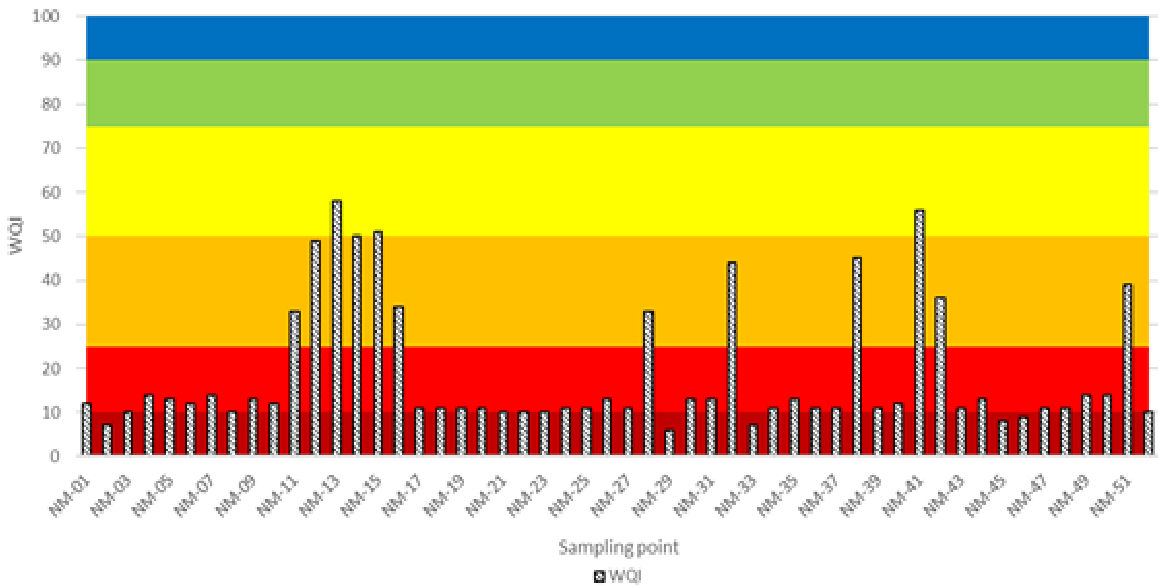

Figure 6. Water quality index of some major rivers in Ca Mau province in September 2018.

face water in Ca Mau province needs to be improved to achieve the irrigation purpose that is consistent with the study of Vo et al. (2015).

3.2. Assessing the capacity of receiving wastewater of some main rivers and canals in Ca Mau city

The main wastewater sources in Ca Mau city are domestic, industrial, and hospital wastewater. Wastewater discharge volume and receiving areas are presented in Table 3, Table 4, and Table 5. The wastewater parameters discharging to the main rivers and canals in Ca Mau city are shown in Table 6.

The capacity of receiving wastewater of 04 main rivers and canals in Ca Mau city was assessed through 04 pollution indicators: COD, BOD_5, N-NH_4^+ and P-PO_4^{3-} concentration (Table 7). All rivers and canals in Ca Mau city were not able to receive any more contaminant load of COD and BOD5. Almost rivers and canals could not acquire any more pollutant load of N-NH_4^+ and P-PO_4^{3-}, only Ca Mau river was able to receive wastewater with pollutant load of N-NH_4^+ and P-PO_4^{3-} is 273.75 and 130.60 kg/day. The calculated capacity of receiving wastewater reflects the actual situation of surface water quality in Ca Mau city, showing signs of severe decline. In the long term, to ensure the quality of water resources for irrigation, it is necessary to take adequate management solutions to protect water sources against the pressure of socio-economic development demands.

3.3. Management solutions proposals

From the analytical process above and for adequate protection and management of surface water sources in Ca Mau province, the synchronous implementation of solutions is urgently required, such as: (i) Investing in building centralized domestic wastewater collection and treatment systems, especially in Ca Mau city (corresponding to NM-01 to NM-03), to reduce the organic pollutants and total coliforms of domestic wastewater discharging to surface areas; (ii) Relocating the factories outside industrial zones (especially the aquatic products processing factories around NM-29 and NM-33, and in Ganh Hao river, corresponding to NM-17) into industrial parks and industrial clusters to collect and treat all industrial wastewater before discharging into the rivers; (iii) Tightening factories' wastewater treatment implementation, especially at Gas – Power – Fertilizer Complex (NM-23) and LFS (NM-28), to efficiently manage industrial wastewater discharge; (iv) Improving the capacity of receiving wastewater by periodically dredging to increase the flows of rivers and canals (Q_s); and (v) Determining the capacity of receiving wastewater of all rivers and canals throughout Ca Mau province to issue appropriate discharge permits to ensure the water quality.

Table 3. The domestic wastewater discharge volume and receiving areas in Ca Mau city

No.	Wastewater receiving areas	Number of households	Discharge volume (m^3/day)
1	Ca Mau river	5,655	2,714.4
2	Quan Lo – Phung Hiep canal	7,421	3,562.4
3	Ca Mau – Bac Lieu canal	8,115	3,895.2
4	Ganh Hao river	4,251	2,040.0
	Total:		12,212

Table 4. The industrial wastewater discharge volume and receiving areas in Ca Mau city

No.	Wastewater receiving areas	Number of factories	Discharge volume (m^3/day)
1	Ca Mau – Bac Lieu canal	09	3,891
2	Ganh Hao river	09	5,960
	Total:		9,851

Table 5. The hospital wastewater discharge volume and receiving areas in Ca Mau city

No.	Wastewater receiving areas	Number of hospitals	Discharge volume (m^3/day)
1	Ca Mau – Bac Lieu canal	04	525
2	Ca Mau river	02	60
3	Quan Lo – Phung Hiep canal	02	35
	Total:		620

Table 6. The maximum concentration values of wastewater discharging to the main rivers and canals in Ca Mau city

No.	Parameter	Maximum concentration values of wastewater C_t (mg/L)			
		Quan Lo – Phung Hiep canal	Ca Mau – Bac Lieu canal	Ca Mau river	Ganh Hao river
1	BOD_5	59.0	124.9	86.0	303.0
2	COD	-	300.1	197.0	764.0
3	$N\text{-}NH_4^+$	31.6	11.7	16.0	27.9
4	$P\text{-}PO_4^{3-}$	1.0	1.6	1.1	0.7

Table 7. The capacity of receiving wastewater of some main rivers in Ca Mau city

No.	Parameter	Capacity of receiving wastewater L_{tn} (kg/day)			
		Quan Lo – Phung Hiep canal	Ca Mau – Bac Lieu canal	Ca Mau river	Ganh Hao river
1	BOD_5	-122.28	-7,810.60	-6,951.72	-28,059.50
2	COD	-43.20	-5,587.40	-7,477.28	-42,429.83
3	$N\text{-}NH_4^+$	-53.18	-227.55	273.75	-1,148.23
4	$P\text{-}PO_4^{3-}$	0.48	-1,129.03	130.60	-2,743.73

4. Conclusions

The river and canal system in Ca Mau province play a particularly important role in the socio-economic development of the province. Facing development pressures, surface water quality in Ca Mau province is being seriously threatened, especially in Ca Mau city. Surface water quality was polluted by organic matters, nutrients, and microorganisms, only meeting transportation purposes. According to the calculated WQI, most of the rivers and canals in Ca Mau were heavily polluted and need to be treated in the future. Most rivers and canals in Ca Mau city could no longer receive more BOD5 and COD in wastewater, the capacity to receive N-NH_4^+ and P-PO_4^{3-} was no longer or very low. Proposed solutions need to be applied to improve the surface water quality in Ca Mau province.

Conflict of interest

The authors declare no conflict of interest.

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Evaluation of the production process of biodegradable drinking straws from corn kernel

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ABSTRACT

New product development is one of the most effective methods to expand the economic value of corn, which is currently a low-cost agricultural material. The present study was conducted to determine the production process of straws from corn kernel under the laboratory scale. The influences of mixing formula, steaming time, screw speed, and drying temperature on hardness and expansion time of the drinking straws from corn kernels were investigated. In addition, the biodegradability of the resultant straws was also tested in the natural environment. The study determined the most suitable recipe for producing the drinking straw product, including corn flour (75%), tapioca starch (20%), rice flour (5%), with the addition of 0.5% xanthan gum and 0.2% potassium sorbate (w/w). The operating conditions of steaming time (10 min), screw speed (40 rpm), and air drying temperature (40°C) were found to be the most appropriate. For the decomposition study, the results indicated that the drinking straw product was capable to be decomposed after 40 days at a temperature of 30 ± 2°C. As such, it can be concluded that it is highly potential to build up the practical production process for making environmentally friendly drinking straws from corn kernels.

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1. Introduction

One of the most important factors causing environmental pollution is the increase in plastic waste. Plastic straws have gained global attention due to growing concerns about their impact on human health and the environment. The amount of plastic waste in the oceans is estimated to double between 2010 and 2025, increasing from about 8 million tons in 2010 to 9 million tons in 2015, and to 16 million in 2025 (Jambeck et al., 2015; Mortillaro, 2017). More than 50% of manufactured plastics, including plastic straws, are disposed of after single-use, leading to 150 million tons of annual plastic waste worldwide (James et al., 2019). The average decomposition time of plastic straws was 100 - 200 years, which causes many negative impacts on the environment (Garcia & Robertson, 2017; Ramirez & George, 2019). So far, many researchers have focused on the development of biodegradable materials that could substitute synthetic polymers. Natural materials, such as paper, leaves, bamboo, grass, among others, showed the potential to apply plant-based materials, possibly replacing plastic due to the safety in usage and the friendliness to the environment. Global agriculture aims to minimize its impacts on the environment and human health. Thus, a broader beneficial effect on the environment will only be attained through consumer acceptance and behavioral changes.

Corn (*Zea mays* L.) has been a popular food crop in the world. It is grown on soils of widely different properties from the raw sands to the clays of delta regions and from shallow soils on residual material to those very deep on loess, till, or alluvium. Moreover, corn was grown in areas with rainfall from 250 mm to more than 5000 mm per year and a growth cycle of 3 to 13 months (Murdia et al., 2016). In Vietnam, corn is grown a lot in the northern mountainous areas, the Central Highlands, and the Southeast region. The total corn production in Vietnam in the 2015 - 2016 crop year reached up to 5.28 million tons (Dang & Nguyen, 2020). It is well-known that corn has a high content of starch. However, the focus of research on products that can replace single-use plastic from starch, to reduce environmental pollution, is still limited.

The extrusion technique is one of the most appropriate technologies for the production of extruded products such as straw, pasta, and noodle, among others (Marti & Pagani, 2013). According to Zieliński et al. (2006) and Oniszczuk et al. (2019), extrusion technology does not degrade the valuable compounds present in raw materials and improves the physicochemical properties of the extruded products. As such, the physical properties of the straw such as hardness and expansion time could be positively obtained by optimizing the processing parameters, including steaming time, screw speed, and drying temperature. There is a need to determine the most suitable operating variables.

Therefore, this study aimed to determine the production process for making straws from corn kernels on a laboratory scale. Specific objectives were to determine the mixing recipe, extrusion conditions, and biodegradable time of drinking straw from the corn kernel.

2. Materials and Methods

2.1. Materials

The dried corn kernels (Zea may L.) used in the present study, that was the feed grain type, were purchased in District 12, Ho Chi Minh City, Vietnam. The dried kernels without pest and deterioration were selected. Then, the dried kernels were ground and sieved through a 0.5 mm open diameter to collect the powdery homogeneous sample. The powdery sample referred to as corn flour (moisture content $\leq$ 10%) was stored in an aluminum foil bag at ambient temperature (30 $\pm$ 2°C).

Tapioca starch and rice flour were purchased in Co.opXtra supermarket Thu Duc city, Ho Chi Minh City, Vietnam. Xanthan gum and potassium sorbate were purchased from PATH Co., Ltd (Vietnam).

Equipment used in this study included a single screw laboratory extruder (made in Vietnam), a texture analyzer (Zwick Roell, Germany), and a moisture balance MX-50 (Sartorius, Japan).

2.2. Experimental design

2.2.1. The effect of mixing recipe on hardness and expansion time of drinking straw

The corn kernel flour as described in Section 2.1 was mixed with four different ingredients, including tapioca starch, rice flour, xanthan gum, and potassium sorbate. The ratio of the corn flour: tapioca starch: rice flour was 70%: 25%: 5% (Formula 1); 75%: 20%: 5% (Formula 2); 70%: 20%: 10% (Formula 3) (w/w). Then, an amount of 0.5% xanthan gum and 0.2% potassium sorbate (w/w) was added to each formula. Next, a fixed amount of water was added into the mixture at the ratio of 3:2 (w/w). The slurry (or dough-like) obtained was fed into the extruder. The obtained straws were evaluated the hardness and the expansion time.

The one factor (mixing recipe) was randomly designed to investigate the effect of different materials on the hardness and expansion time. The mixing recipe runs and subsequent measurements were carried out in triplicate. A total of 9 runs were conducted.

2.2.2. Effect of steaming time, screw speed, and drying temperature on hardness and expansion time of drinking straw

In the next step of the production process of straws, the one-factor experiments were designed at a time. The best formula (the results obtained from Section 2.2.1) was applied. After mixing all materials, steaming time was investigated at three levels (5, 10, and 15 min). After that, the screw speed was surveyed at three levels (40, 80, and 120 rpm). Finally, straws were air-dried at three temperatures (40, 50, and 60°C) in a hot air oven. The air drying was terminated when the final moisture content of each straw sample was constant (approximately 10%). The obtained straws were sealed into a high barrier bag, using a hot sealing machine, and analyzed hardness and expansion time. All experiments and subsequent analyses were performed in triplicate.

2.2.3. Investigation of the biodegradability of drinking straws in the natural environmental conditions

After the determination of the best parameters for the production process of straws from corn kernels, the biodegradability of the straws in the natural environment was evaluated. The straw samples were cut into short pieces in 5 - 7 cm and buried in the soil at a depth of 8 - 10 cm. The decomposition time of the straws in the environment from the beginning to the complete decomposition was periodically recorded. The plastic straws were also used as a negative control. In addition, the experimented straws were evaluated at ambient temperature (30 ± 2°C). The decomposition time of straws was recorded on the first day and after every 5 days for up to 45 days. The experiment was carried out in duplicate.

2.2.4. Moisture content

The moisture was determined by infrared moisture drying balance MX-50 (0.01%/max 51 g), (Sartorius, Japan). One gram of sample was weighed and dried until a constant weight was obtained.

2.2.5. Hardness

The hardness of the tested straws was measured by using a texture analyzer (Zwick Roell, Germany). The probe moved to the point of contact with the sample, applied force to the sample, and penetrated. During penetration, the force value increased gradually and started to decrease at the point where the suction straw's texture was broken. The recorded force values continued when the penetration reached the specified distance. The maximum force value reflected the hardness of the suction straws.

2.2.6. Expansion time

The tested straws with an equal length of approximately 20 cm were placed in 300 - 350 mL of water, and the expansion time at 30 ± 2°C was measured. The time of use was counted from the initial time when the straw was submerged in the water until the signs of cracking, breaking, or any non-integrity were observed.

2.3. Statistical analysis

The experiments were carried out in triplicate and the results were presented as mean values with standard deviations. Different mean values were analyzed by analysis of variance method (ANOVA) and least significant difference (LSD) using Statgraphics software.

3. Results and Discussion

3.1. The effect of mixing recipe on hardness and expansion time of drinking straw

This study aimed to investigate the effect of different mixing recipes on the hardness and expansion time of the straws from corn kernels under the laboratory scale. The most suitable recipes would be chosen according to the highest value of hardness and longer expansion time. The results of this experiment are shown in Table 1.

Table 1 shows that each hardness and expansion time was displayed differently under the different mixing recipes. The hardness of the test straws was in decreasing order of formula 2 (18.83 N), formula 1 (16.27 N), and formula 3 (14.77 N). For the expansion time, the effect of formulas used to mix was found to be decreased as follows: Formula 2 (18.94 h), Formula 1 (4.26 h), and Formula 3 (2.64 h). These values were statistically significant at the confidence level $\alpha < 0.05$.

The investigated drinking straws became harder and the expansion time was longer due to an increase in the percentage of corn flour and a decrease in the ratio of tapioca and rice flour. The molecular structures of amylose and amylopectin may have an impact on the functional properties of the starch-based product. When the flours were heated, starch gelatinization occurred, the amylose inside the grain was likely to be released and created a three-dimensional network structure. As such, changing the flour ratio affected the ratio of amylose: to amylopectin, which affected starch gelatinization, causing the network structure to be altered (Cai et al., 2014; Li et al., 2018). Therefore, it can be concluded that formula 2, which was corn flour: tapioca starch: rice flour of 75: 20: 5 (%, w/w) should be the most

Table 1. The effect of mixing recipes on structured and expansion time of drinking straw

Formula	Hardness (N)	Expansion time (h)
Corn flour: tapioca starch: rice flour: 70: 25: 5 (%, w/w); (Formula 1)	$16.27^{ab} \pm 1.95$	$4.26^{b} \pm 1.58$
Corn flour: tapioca starch: rice flour: 75: 20: 5 (%, w/w); (Formula 2)	$18.83^{a} \pm 0.97$	$18.94^{a} \pm 6.06$
Corn flour: tapioca starch: rice flour: 70: 20: 10 (%, w/w); (Formula 3)	$14.77^{b} \pm 0.80$	$2.64^{b} \pm 2.78$

Values are mean ± SD (triplicates) after statistical analyses.
The values in the same column followed by different superscripts (a, b) were significantly different ($P < 0.05$).

suitable choice for the following experiments.

3.2. The effect of steaming time, screw speed, and drying temperature on hardness and expansion time of drinking straw

To build up the production process for making straws from corn kernels, it is important to investigate key operating conditions of steaming time, screw speed, and drying temperature. The impacts of steaming time, screw speed, and drying temperature on the hardness and expansion of the resultant straws are presented in Tables 2, 3, and 4, respectively.

Table 2. The effect of steaming time on hardness and expansion time of drinking straw

Steaming time (min)	Hardness (N)	Expansion time (h)
5	$16.8^{a} \pm 1.57$	$3.11^{b} \pm 1.0$
10	$17.33^{a} \pm 1.42$	$11.14^{a} \pm 4.46$
15	$17.43^{a} \pm 0.60$	$11.25^{a} \pm 5.23$

Values are mean ± SD (triplicates) after statistical analyses. The values in the same column followed by different superscripts (a, b) were significantly different ($P < 0.05$).

Table 3. The effect of screw speed on hardness and expansion time of drinking straw

Screw speed (rpm)	Hardness (N)	Expansion time (h)
40	$19.93^{a} \pm 0.35$	$17.31^{a} \pm 5.94$
80	$16.17^{b} \pm 0.32$	$7.42^{ab} \pm 5.96$
120	$15.53^{b} \pm 1.59$	$2.86^{b} \pm 2.37$

Values are mean ± SD (triplicates) after statistical analyses. The values in the same column followed by different superscripts (a, b) were significantly different ($P < 0.05$).

For a steaming time, the hardness of straws was found to be statistically non-significant ($P > 0.05$). The corn flour had high amylose content, about 24% (Pereira et al., 2021), so the gelatinization time took longer, the investigated time duration did not differ much. As such, it did not have a great influence on the amount of gelatinized starch. In contrast, the steaming time significantly influenced the expansion time of straws ($P < 0.05$). The time used when steaming at 10 min and 15 min resulted in expansion time ranging from 11.14 h to 11.25 h, which was found to be a non-significant difference ($P > 0.05$). However, an expansion time of 3.11 h was obtained when the steaming time was 5 min and was statistically different compared to the other two. Therefore, it can be seen that the steaming time of 10 min gave the best results in the production process of straws because of its economic relevance.

Table 4. The effect of drying temperature on hardness and expansion time of drinking straw

Drying Temperature (°C)	Hardness (N)	Expansion time (h)
40	$21.2^{a} \pm 1.76$	$14.87^{a} \pm 8.93$
50	$21.9^{a} \pm 3.08$	$1.52^{b} \pm 0.87$
60	$17.73^{a} \pm 5.23$	$0.3^{b} \pm 0.06$

Values are mean ± SD (triplicates) after statistical analyses. The values in the same column followed by different superscripts (a, b) were significantly different ($P < 0.05$).

As can be seen in Table 3, the hardness and expansion time of the resultant straws were significantly affected by the machine's screw speed ($P < 0.05$). The value of hardness of the straws was found to be decreased as increasing the screw speed from 40, 80, and 120 rpm. Similar trends in expansion time were also found to be significant ($P < 0.05$) as the speed was increased.

It can be explained that changes during the extrusion process led to some changes such as loss of crystal structure, disruption of grain structure, glycosidic bond breakage, and new molecular interactions (Zhong & Sun, 2005). Furthermore, according to Wang et al. (2016) the physical and chemical properties of the extruded product may be also positively and negatively affected by extrusion conditions including temperature, pivot speed, and screw structure affected. During the extrusion process, starch partially gelatinizes due to the effect of temperature (Mishra et al., 2012). When the screw speed is too fast, the dough is overworked, which affects the quality of the finished product (Cubadda & Carcea, 2003), resulting in reduced hardness, short expansion time, and easy cracking and breakage. This finding is also in agreement with other studies (Bouasla & Wójtowicz, 2019). Therefore, screw speed at 40 rpm was the best result in the production process of straws from corn kernels.

Table 4 shows the effect of air drying temperature on the hardness of straws was found to be statistically non-significant at $P > 0.05$. However, the value of expansion time of the resultant straws was significantly impacted by the air drying temperature ($P < 0.05$). The expansion time was reduced by increasing the drying temperature from 40, 50, and 60°C.

It is well-known that during the drying process, the air temperature and relative humidity must be controlled appropriately, if the drying process is too slow, which is less than 40°C, the product may become moldy or damaged. However, if the drying process is too fast, the moisture content product will evaporate rapidly, which can cause cracking or breakage of the product (Cubadda & Carcea, 2003). Therefore, the drying temperature at 40°C was found to be the most suitable for drying the corn straw product in terms of hardness and expansion time.

3.3. Investigation of the biodegradability of drinking straws in the natural environmental conditions

The results of the degradability of the resultant corn straws (Figure 1) were periodically recorded at 30 ± 2°C for up to 40 days as presented in Table 5.

At 30 ± 2°C, after 5 days of the track, both samples had not started decomposition. Keeping recording results until the 10^{th} day, the decomposition of the corn straw sample appeared and continued in the following days, for up to 45 days. After 40 days, the corn straws were completely decomposed in the natural environment,

Figure 1. Biodegradable drinking straws made from corn kernel.

Table 5. Decomposition time of the corn straw buried in the soil

Treatments	Time (day)									
	0	5	10	15	20	25	30	35	40	45
Corn straws	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-

(-) absence (+) presence.

however, the control samples were neither. The study proved that corn straws were biodegradable in the natural environment. As such, it can be concluded that agricultural materials such as corn kernels could be used for making biodegradable straws. The degradation characteristics of the straw obtained from this study with the biodegradable straws reported in other studies should be done.

4. Conclusions

The study determined the production process of the drinking straws from corn kernels with the important parameters. The results showed that the most appropriate recipe for making the drinking corn straws was 75% corn flour, 20% tapioca starch, 5% rice flour, 0.5% xanthan gum, and 0.2% potassium sorbate (w/w). The most suitable operating conditions of steaming time, screw speed, and air drying temperature were found to be 10 min, 40 rpm, and 40°C, respectively. The resultant drinking straws product were capable of decomposing after 40 days at a temperature of 30 ± 2°C. Therefore, it can be concluded that it is highly potential to build up the practical production process for making environmentally friendly drinking straws from corn kernels.

Conflict of interest

The authors declare no conflict of interest.

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