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Welcome message from Editor-in-Chief

Dear authors, reviewers, and readers

On behalf of the Editorial Board, it is my great pleasure to present Volume 7, Issue 1 of the Journal of Science and Agricultural Technology (JSAT), the official peer-reviewed journal of the Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna (RMUTL), Thailand.

The Journal of Science and Agricultural Technology remains committed to providing an international platform for the dissemination of high-quality, peer-reviewed research and review articles spanning the natural sciences, agricultural sciences, and related applied disciplines. Through rigorous editorial and peer-review processes, JSAT aims to promote scientific innovation, interdisciplinary collaboration, and the exchange of knowledge that addresses both regional and global challenges.

This issue features five original research articles that reflect the breadth and diversity of contemporary scientific inquiry:

- Physicochemical and Pasting Properties of Raw Hom Thong Banana Flour from Northern Thailand
- Effects of Multi-Strain Microbial Inoculants on Fermentation Quality and In Vitro Digestibility of Rice Straw Silage
- Fish Diversity, Ecological Functional Guilds, and IUCN Conservation Status in the Lower Mekong River, Northeastern Thailand
- Case Study: Assessment of Microbial Quantification of Butchered Pork in Fresh Markets and Fresh Pork Shops in Nakhon Pathom, Thailand
- Comparison of Plant Propagation Methods and the Effectiveness of Azolla Combined with Chemical Fertilizer on the Growth of *Episcia cupreata*

These contributions represent valuable advances in agricultural technology, food science, environmental science, microbiology, and biodiversity conservation, demonstrating the continued importance of interdisciplinary research in addressing current scientific and societal needs.

JSAT is published through the Thai Journal Online (ThaiJO) platform and is indexed in Google Scholar, the Thai Citation Index (TCI), and the Digital Object Identifier (DOI) system, with the continued support of the National Research Council of Thailand. The Editorial Board remains dedicated to maintaining high standards of scholarly publishing while enhancing the journal's visibility, accessibility, and international impact.

I would like to express my sincere appreciation to all authors for entrusting JSAT with their valuable research, to our reviewers for their careful and constructive evaluations, and to the Editorial Board and journal staff for their continued dedication and professionalism. Their collective efforts ensure the quality and integrity of every issue we publish.

We warmly invite researchers, scholars, and practitioners from around the world to submit their original manuscripts and join us in advancing scientific knowledge through JSAT. We look forward to your continued support and valuable contributions in future issues.

Best regards,

Assoc. Prof. Dr. Tanongsak Sassa-deepaeng
Editor-in-Chief
Journal of Science and Agricultural Technology

ABOUT THE JOURNAL

Journal of Science and Agricultural Technology (JSAT) publishes original research contributions covering science and agricultural technology such as:

- Natural and applied sciences: biology, chemistry, computer science, physics, material science and related fields. Papers in mathematics and statistics are also welcomed, but should be of an applied nature rather than purely theoretical.
- Agricultural technology: plant science, animal science, aquatic science, food science, biotechnology, applied microbiology, agricultural machinery, agricultural engineering and related fields.

Furthermore, the JSAT journal aims to span the whole range of researches from local to global application.

The JSAT is published two issues a year.

Issue 1: January - June

Issue 2: July - December

Submissions are welcomed from international and Thai institutions. All submissions must be original research not previously published or simultaneously submitted for publication or submitted to other journals. Manuscripts are peer reviewed using the double-blinded review system by at least 3 reviewers before acceptance. There is no publication or processing fee.

The journal financial support is provided by Rajamangala University of Technology Lanna, Thailand.

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INSTRUCTION FOR AUTHORS

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2. Preparation of manuscripts

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The title of manuscript should be supplied with enough information to catch the attention of readers.

2.2) Author names and affiliations

Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Indicate the corresponding author by “*”. If authors have different addresses, numbered superscripts are required to refer to each address provided. The format of authors’ affiliations should be: 1. Department, University, City name, State name, Postal code and Country. Corresponding author must inform email.

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Keywords: – At least 3 to 5 keywords must be given below abstract and use “comma (,)” to separate each.

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2.5) Materials and methods

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2.6) Results and discussion

In this part, the results and discussion can be combined or separated depends on author design. Please use tables, graphs, diagrams, and photographs to provide a clear understanding of the results. Quantitative measures of significance (P-values) should be presented. Authors may use either absolute P-values or a defined significance level as long as usage is consistent. Discussion contains the interpretation of the results into existing literature. It should be clear and concise, address the related mechanisms and their significance.

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Clear and significant conclusions should be provided and related to the results and objectives of your work.

2.8) Acknowledgments

In this section, financial and material support should also be mentioned. Authors should list all funding sources in this acknowledgments section. The names of funding organizations should be written in full. List those individuals who provided support during the research

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2.9.1) In text citations, please use name-year system such as:

a. Before texts: Smith (2018)...., Tsuji and Hayate (2012)...., Smith et al.(2019)...., Smith (2018); Tsuji (2012)...., Smith et al.(2018a); Smith et al.(2008b)....

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Gupta, A.P., and Kumar, V. 2007. New emerging trends in synthetic biodegradable polymers-polylactide: A critique. Eur. Polym. J. 43: 4053-4074.

- **Book**

Carr, R.L. 1976. Powder and granule properties and mechanics. Marcel Dekker Publisher, New York.

- **Chapter in book**

Jackson, M.B. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: Wareing, P.F. (ed) Plant growth substance. Academic Press, London. p.291-301.

- **Proceeding, symposia etc.**

Pratt, A., Gilkes, R.J., Ward, S.C., and Jasper, D.A. 2000. Variations in the properties of regolith materials affect the performance of tree growth in rehabilitated bauxite mine-pits in the Darling Range, SW-Australia. In: Brion, A., and Bell, R.W. (eds) Proceeding of Remade Land 2000, the International Conference on Remediation and Management of Degraded Lands. Fremantle, 30 Nov-2 Dec 2000. Promaco Conventions, Canning Bridge. p.87-88.

- **Dissertation**

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- Keywords
- INTRODUCTION
- MATERIALS AND METHODS
- RESULTS AND DISCUSSION (The results and discussion can be combined or separated depends on author design)
- CONCLUSIONS
- ACKNOWLEDGMENTS (if any)
- REFERENCES

5. After acceptance

Authors would be asked to send the “Copyright Transfer Agreement” to JSAT.

6. Publication or processing fee

There is no publication or processing fee.

Physicochemical and Pasting Properties of Raw Hom Thong Banana (*Musa sapientum* L.) Flour from Northern Thailand

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ABSTRACT

Growing interest in functional foods has increased the demand for alternative flour sources rich in resistant starch and dietary fiber for application in starch-based food products. Raw banana flour has attracted attention as a potential functional ingredient due to its favourable nutritional and physicochemical properties. The aims of this study were to determine proximate composition and pasting properties of raw Hom Thong banana flour (RBF). Hom Thong bananas (*Musa sapientum* L.) from Mae Taeng community, Chiang Mai Province, Thailand were processed into flour by hot-air drying at 60 °C and analyzed for the proximate composition and Rapid Visco Analysis (RVA) properties. The results showed that carbohydrate was the major component of the flour (86.82±0.18 g/100 g) followed by protein (4.20±0.28 g/100 g), ash (2.92±0.10 g/100 g), dietary fiber (2.53±0.14 g/100 g) and fat (0.30±0.05 g/100 g), whereas the moisture content was comparatively low (3.23±0.03 g/100 g) suggesting good storage stability. The RVA results revealed that the raw banana flour has different pasting properties compared to the conventional wheat and cassava flours. Banana flour showed moderate peak viscosity (213.05±16.17 RVU), high trough viscosity (191.35±10.25 RVU), low breakdown viscosity (63.75±1.13 RVU), high final viscosity (283.86±23.27 RVU) and relatively high pasting temperature (84.57±0.93 °C) indicating good thermal stability, limited swelling of the starch granules and strong gel-forming capability. These properties suggested that RBF possesses desirable physicochemical and functional characteristics for noodle processing applications. The physicochemical and pasting properties observed in this study suggested that RBF may be suitable application as a partial substitute for wheat flour in noodle or pasta formulations.

Keywords: Hom Thong banana flour, banana flour, pasting properties, Rapid Visco Analysis, physicochemical properties

INTRODUCTION

In the last several years, the demand for healthy carbohydrate-based meals has expanded dramatically, due to the increased consumer knowledge of the association between nutrition and chronic non-communicable diseases, including as obesity, diabetes and cardiovascular problems (Baptista *et al.*, 2024). Therefore, the food sector is increasingly interested in developing functional noodle products loaded with dietary fiber, resistant starch and bioactive substances. The strategy of incorporating alternative plant-based flours into traditional wheat-based noodle systems has been reported as an effective way to improve nutritional quality while keeping desired physicochemical and processing qualities (Kim *et al.*, 2021).

Recently, unripe banana flour has attracted much attention as a functional food ingredient due to its high resistant starch, dietary fiber, minerals and bioactive phytochemicals (Shini *et al.*, 2024). Unripe banana is rich in resistant starches, especially the type II resistant starch (RS2), which has compact crystalline structures and is resistant to enzymatic hydrolysis in the small intestine (Zhang *et al.*, 2005). Resistant starch is therefore fermented in the colon to produce short-chain fatty acids which can improve gastrointestinal health, modify the glycemic response and favor the enrichment of gut microbiota (Dong *et al.*, 2025). Furthermore, green banana flour has been revealed to have high amylose content, low digestibility and unique starch granule architectures responsible for its physicochemical and functional features in food processing (Kumar *et al.*, 2019).

Hom Thong banana is one of the most economically important cultivars among banana cultivars planted in Thailand and widely grown in many agricultural areas such as Mae Taeng district, Chiang Mai Province. The use of unripe Hom Thong banana for flour manufacturing might be an alternate option to add value to local agricultural resources and to avoid postharvest losses. Moreover, the conversion of surplus or underutilized bananas into flour products is in line with contemporary trends of sustainable food production and bio-circular economy development.

Kanoklerdrit (2022) investigated the development of noodle products supplemented with banana flour as a partial replacement for wheat flour. The study found that noodles containing Hom Thong banana flour received higher consumer acceptance scores than those prepared with Namwa banana flour. The formulation containing 40% Hom Thong banana flour substitution showed the highest overall acceptability. Banana flour is rich in dietary fiber, which can affect the textural properties of noodle products. Increasing the substitution level beyond 40% resulted in a firmer and coarser texture, leading to lower consumer acceptance.

The incorporation of banana flour and other plant-based flours with similar functional properties, such as whole-grain flours, has gained increasing attention in recent years due to their potential to enhance nutritional value and support the development of functional food products. Such ingredients can increase dietary fiber content and contribute to the production of value-added food products with improved nutritional profiles.

Use of green banana flour in bakery and noodle products has been reported to enhance dietary fiber content, reduce starch digestibility and alter product texture and functional properties (Ovando-Martinez *et al.*, 2009). Pasting characteristics of flour are important in determining the functional properties of flour in starch-based food systems as they give an idea of the extent of starch granule swelling, gelatinization, disintegration and retrogradation events during the heating and cooling processes (Singh *et al.*, 2003). It is known that physicochemical modifications influence notably water absorption, thermal stability, texture, cooking quality and structural integrity of noodle products (Shang *et al.*, 2025). Generally, the suitability of flour for noodles and other starch-based food applications are determined by Rapid Visco Analysis (RVA) to determine the peak viscosity, break down viscosity, final viscosity, setback viscosity and pasting temperature.

The pasting capabilities of banana flour are notably different from those of common wheat and cassava flours due to differences in starch composition, amylose to amylopectin ratio, resistant starch concentration and crystal structure (Xie *et al.*, 2026). The thermal stability, low granule swelling and high retrogradation tendency of banana starch are advantageous for the improvement of the structural stability and cooking tolerance of noodle systems (Khoozani *et al.*, 2019). However, a high level of retrogradation might also affect the textural qualities during storage and processing negatively (Yu *et al.*, 2024).

To our knowledge, there is minimal information on the physicochemical and pasting qualities of RBF in Northern Thailand, but several studies have investigated the nutritional and physicochemical features of RBF. The purpose of this study was to investigate the proximate composition and pasting properties of RBF and to assess its possibility for utilization as an alternative functional ingredient for noodle products.

MATERIALS AND METHODS

Raw material

Raw Hom Thong bananas (*Musa sapientum* L.) were collected from Mae Taeng community, Chiang Mai Province, Thailand. All-purpose wheat flour and cassava flour were purchased from the local market, Chiang Mai, Thailand. All chemicals and reagents used for chemical analyses were of analytical grade.

Preparation of Raw Hom Thong Banana Flour

Raw Hom Thong bananas (*Musa acuminata* AAA group) at ripening stage 3, according to the ripening classification of CSIRO (1972) were thoroughly washed with running water, manually peeled and sliced into pieces of approximately 5 mm thickness. The banana slices were dried in a hot-air oven at 60 °C until the moisture content was lower than 10%. (Ariyasukkosit *et al.*, 2022) The dried banana slices were ground using a laboratory grinder and sieved with an 80-mesh sieve to get fine flour particles (Figure 1). The banana flour was packed in polyethylene bags and stored at room temperature until further analysis.

Proximate Analysis

The proximate composition of RBF, including moisture, ash, crude fat, crude protein, and dietary fiber contents, was determined according to the standard methods of AOAC (2000). Carbohydrate

content was calculated by difference using the equation:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash} + \text{dietary fiber}).$$

All analyses were conducted in triplicate and the results were expressed as g/100 g sample.

Determination of Pasting Properties

The pasting properties of raw banana flour, wheat flour, and cassava flour was determined using a Rapid Visco Analyser (RVA; Newport Scientific Pty. Ltd., Warriewood, Australia) according to the standard RVA method (AACC International, 2009). The parameters evaluated included peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, pasting temperature, and peak time. Flour suspensions were subjected to controlled heating and cooling cycles during analysis to evaluate the rheological and pasting behavior of the flour samples.

Statistical Analysis

All experiments were carried out in triplicate using a completely randomized design (CRD). Data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using analysis of variance (ANOVA), and significant differences among means were determined using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$. Statistical analyses were conducted using SPSS software version (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate Composition of Raw Hom Thong Banana Flour

Table 1 shows the proximate composition of RBF. The results indicated that the major component of the flour was carbohydrate followed by protein, ash, dietary fiber and fat, respectively. The flour also had a relatively low moisture content which indicates good storage stability.

The moisture content of RBF was 3.23 ± 0.03 g/100 g. This showed that drying procedure at 60°C was successful to reduce water content to create stable flour system. The moisture content of RBF obtained in this study was lower than that reported by Jiamjariyatam *et al.* (2019) who found a moisture

content of 11.52%. Differences in moisture content may be attributed to variations in processing and drying conditions during flour production. Low moisture content is important for flour stability as limited water availability might restrict microbiological development and diminish hydrolytic and enzymatic reactions which are associated with quality deterioration during storage. The obtained moisture content was much lower than the moisture limit commonly indicated for commercial flour products (12-14%), hinting that the flour developed in this study should have high shelf stability for dried food applications. Similar findings have been reported by Khoozani *et al.* (2019).

Table 1. Proximate composition of raw Hom Thong banana flour.

Parameters	Results (g/100 g)
Moisture	3.23 ± 0.03
Ash	2.92 ± 0.10
Fat	0.30 ± 0.05
Protein	4.20 ± 0.28
Carbohydrate	86.82 ± 0.18
Dietary fiber	2.53 ± 0.14

The ash level of the flour was 2.92 ± 0.10 g/100 g which was slightly higher than the usual range reported for the refined wheat flour. The finding reveals that unripe banana naturally possesses key mineral components such as potassium, magnesium and phosphorus. Ionic interactions between mineral components and starch molecules can potentially influence the starch functionality and thermal behavior in starch-based systems during processing. The ash content was comparable with raw banana flour of other cultivars (Shini *et al.*, 2024). Hot-air drying conditions have high effect on physicochemical stability and resistant starch retention of raw banana flour (Khoozani *et al.*, 2019).

The fat content of RBF was very low (0.30 ± 0.05 g/100 g) and this can be advantageous for the storage durability of the flour because a low lipid content minimizes the danger of oxidative rancidity and the development of off-flavours during storage. The low-fat content was also seen for green banana flours of other banana cultivars, demonstrating that banana flour is a low fat source of carbohydrates applicable for starch-based food products (Kumar *et al.*, 2019).

The protein content of the flour was 4.20 ± 0.28 g/100 g and lower than that usually seen in wheat flour but greater than reported values for

cassava flour. Banana flour proteins are not involved in the development of gluten networks, but the presence of proteins may affect flour functionality, through interactions with starch and dietary fiber components during thermal processing. In the noodle system these interactions can influence dough consistency, water uptake and textural qualities. The addition of banana flour changed the system structure of dough and the starch-protein interactions in wheat-based systems, according to Hu *et al.* (2025).

The carbohydrate content of RBF was 86.82 ± 0.18 g/100 g. Carbohydrates are the primary component of the flour. Unripe banana flour is claimed to include high quantities of starch and resistant starch which determine its functional and technological features. The high carbohydrate content found in this study indicates that the flour can be used as an alternate source of starch for noodles and other starch-based foods. Green banana flour was found to contain resistant starch associated with lower starch digestion and lower glycemic response, thus enhancing its potential as a functional food ingredient (Pacheco *et al.*, 2025).

The dietary fiber content of RBF was 2.53 ± 0.14 g/100 g. Dietary fiber may affect the functional properties of noodle products through the effect on water absorption, viscosity development and structural stability during processing (Singthong *et al.*, 2026). Starch granules may also compete with fiber components for water uptake that may affect swelling behavior and pasting properties of starch. Functional noodle formulations fortified with plant-derived materials have observed similar observations regarding the functional relevance of dietary fiber in starch-based systems (Singthong *et al.*, 2026).

The proximate composition suggested that RBF has potential as a functional flour ingredient for Khao Soi noodle applications. The flour possessed high carbohydrate, low fat and moderate dietary fiber and mineral content. These may contribute to nutritional improvement and functional qualities in starch-based food.

Pasting Properties of Raw Hom Thong Banana Flour

The pasting qualities of RBF analyzed by a Rapid Visco Analyser (RVA) are shown in Table 2. The RVA parameters of wheat flour and cassava flour reported in earlier studies are presented for comparative reasons.

Table 2. Pasting properties of raw banana flour compared with reported values for wheat flour and cassava flour.

Parameter	Banana flour	Wheat flour*	Cassava flour*
Peak viscosity (RVU)	213.05 ± 16.17	120–180	352.50 ± 4.15
Trough viscosity (RVU)	191.35 ± 10.25	80–140	152.86 ± 3.46
Breakdown (RVU)	63.75 ± 1.13	30–80	199.64 ± 3.16
Final viscosity (RVU)	283.86 ± 23.27	150–250	230.00 ± 2.53
Setback (RVU)	93.58 ± 4.38	50–150	77.14 ± 4.18
Pasting temperature (°C)	84.57 ± 0.93	60–70	67.80 ± 0.05
Peak time (min)	6.71 ± 0.20	5–6	3.65 ± 0.04

Values for banana flour are expressed as mean $\pm$ standard deviation (n = 3).

*Reported values for wheat flour were adapted from Dada *et al.* (2023), whereas cassava flour values were adapted from Ekeledo *et al.* (2024).

Important indicators of properties of starch-based flours are the pasting behavior, swelling characteristics and utility for food processing applications. RVA parameters are commonly used to determine starch gelatinization, paste stability and retrogradation behavior, all of which affect the texture and cooking quality of noodle products (Zhang *et al.*, 2025).

RBF had a peak viscosity of 213.05 ± 16.17 RVU which was higher than the reported range for wheat flour but lower than the value reported for cassava flour. Peak viscosity reflects the maximum swelling capacity of starch granules during heating in excess water. The moderate peak viscosity of banana flour shows its ability of starch granules to absorb water and, at the same time, structural resistance to excessive swelling. The behavior may be due to the presence of resistant starch, relatively high amylose content and compact structure of starch granules commonly found in unripe banana flour (Kumar *et al.*, 2019). The lower peak viscosity compared to cassava flour may suggest stronger internal molecular associations which limit the granule expansion during heating.

The trough viscosity of banana flour was 191.35 ± 10.25 RVU, which was higher compared to the values reported for wheat and cassava flours. Trough viscosity is the resistance of swollen starch granules to prolonged heating and mechanical shear. The higher trough viscosity of banana flour indicates that its starch paste is more stable to heat and shear during processing. This behavior has been observed in starch systems with a higher content of resistant starch and crystalline structures that prevent the granule breakdown upon heating (Pacheco *et al.*, 2025). It is useful in maintaining the structure during

cooking and better paste stability is needed for use in noodles.

Breakdown viscosity is an index of the ease of disintegration of starch granules under the effect of heat and mechanical stress. Compared to cassava flour, raw banana flour had relatively low breakdown viscosity (63.75 ± 1.13 RVU) implying higher resistance of its starch granules to disintegration on heating. The lower breakdown viscosity is related to higher paste stability and less granule swelling, because the interaction between the molecules in the starch structures increases (Xie *et al.*, 2026). This property may lead to reduced cooking losses and increased firmness of noodle products.

The final viscosity of banana flour was 283.86 ± 23.27 RVU which was higher than those reported for wheat and cassava flours. The final viscosity is a measure of the capacity of starch molecules, especially amylose fractions, to reassociate and form a viscous paste on cooling. The increased final viscosity of banana flour reflects high gel forming capacity and stability of the paste on cooling. Similar results were found in green banana starches. Increase in resistant starch and amylose content which increases the molecular reassociation and the paste viscosity (Sharath Kumar *et al.*, 2025). In noodle systems, the increased final viscosity can lead to a firmer texture and improved structural stability on cooking.

Setback viscosity is associated with starch retrogradation and reassociation of starch molecules during cooling. The setback value of banana flour (93.58 ± 4.38 RVU) was higher than that reported for cassava flour and within the reported range for wheat flour. This implies a moderate tendency of starch molecules to re-organize in ordered structures after gelatinization. Higher amylose starches and resistant starches show more distinct retrogradation behavior owing to higher likelihood of re-association of the linear chains of starch during cooling (Rammohan *et al.*, 2025). Moderate retrogradation may be advantageous for the firmness and structural stability of the noodles but excess retrogradation may have adverse effect on the textural softness during storage.

The pasting temperature of banana flour was 84.57 ± 0.93 °C, which was considerably higher than the reported values for wheat and cassava flours. Pasting temperature is the minimum temperature required to initiate starch gelatinization. The higher the pasting temperature, the higher the crystalline stability of the banana starch granules and the stronger the binding within the starch matrix.

Similar increase in gelatinization temperature has been reported for green banana flours with high resistant starch and ordered crystalline structures (Khoozani *et al.*, 2019). Higher pasting temperature may require greater thermal energy during noodle processing but could also improve resistance to overcooking.

Banana flour also exhibited a longer peak time (6.71 ± 0.20 min) than cassava flour and slightly higher values than wheat flour. Longer peak time reflects slower starch granule swelling and delayed gelatinization behavior during heating. A slower gelatinization profile may suggest a better process tolerance with less over-degradation of starch during cooking. Similar findings have been reported for functional noodle systems where fortification with ingredients rich in resistant starch gave slower gelatinization which led to improved cooking stability and texture (Singthong *et al.*, 2026).

The RVA analysis results showed that the RBF had different pasting properties from the typical wheat and cassava flours. The flour showed good thermal stability, moderate swelling, high peak viscosity and high pasting temperature, indicating its potential application in the development of noodle products with higher structural integrity and firmer texture. Moreover, the resistant starch and dietary fiber content may be beneficial in the development of functional noodle products with improved physicochemical properties.

CONCLUSIONS

Raw Hom Thong banana flour (RBF) exhibited distinctive proximate composition and pasting properties. The flour was characterized by high carbohydrate, low fat, moderate dietary fiber and mineral contents and low moisture content, suggesting good storage stability and suitability for dried food applications. The proximate composition data provide baseline information regarding the nutritional characteristics of RBF.

The RVA analysis demonstrated that RBF exhibited different pasting properties compared with reported values for wheat flour and cassava flour. RBF showed moderate peak viscosity, high trough viscosity, low breakdown viscosity, high final viscosity, and a relatively high pasting temperature. These characteristics suggest that RBF possesses unique physicochemical properties that may influence its behavior during food processing.

The findings of this study provide fundamental information on the physicochemical

and pasting properties of RBF produced in Chiang Mai Province, Thailand. The results may serve as a basis for future investigations on the application of RBF in noodles and other starch-based food products.

Future studies should be performed to investigate the effects of banana flour incorporation on the cooking quality, textural properties, starch digestibility, sensory acceptance and shelf stability of noodles to support potential commercial applications.

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Effects of Multi-Strain Microbial Inoculants on Fermentation Quality and *In Vitro* Digestibility of Rice Straw Silage

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ABSTRACT

Rice straw is one of the most widely available agricultural by-products in Thailand. However, its direct use as ruminant feed is constrained by low crude protein concentration, high structural fiber content, and the presence of a lignocellulosic matrix that limits microbial degradation in the rumen. This study evaluated the effects of selected microbial inoculant combinations on fermentation quality, chemical composition, fiber fractions, and *in vitro* gas production of rice straw silage. Chopped rice straw was assigned to five microbial treatments in a completely randomized design with three replications: T1, *Trichoderma reesei*; T2, *Saccharomyces cerevisiae*; T3, *T. reesei* + *S. cerevisiae*; T4, *T. reesei* + *S. cerevisiae* + *Bacillus licheniformis*; and T5, *T. reesei* + *S. cerevisiae* + *Lactobacillus plantarum*. The treated materials were ensiled under anaerobic conditions and evaluated after 7, 14, 21, and 28 days of fermentation. Microbial inoculation significantly influenced fiber degradation, particularly neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) ($P < 0.001$). Among the treatments, T5 showed the most favorable response after 28 days of fermentation, with the lowest NDF (69.47%), ADF (47.66%), and ADL (19.02%) values. The lowest pH value was also observed in T5 at 21 days (4.65), suggesting improved acidification during ensiling. *In vitro* gas production differed significantly among treatments at 28 days ($P < 0.01$), with T5 producing the highest gas volume (74.5 mL), indicating greater ruminal fermentability. These results suggest that the combined application of cellulolytic fungi, yeast, and lactic acid bacteria can enhance lignocellulose degradation and improve the feeding value of rice straw silage. Therefore, the inoculant combination of *T. reesei* + *S. cerevisiae* + *L. plantarum* may be considered a promising biological strategy for improving the utilization of rice straw in ruminant production systems.

Keywords: Rice straw silage; Multi-strain Inoculants; Lignocellulose degradation; *In vitro* gas production; Rumen fermentation

INTRODUCTION

Rice straw is a major crop residue generated from rice production in Thailand and other rice-producing regions. Owing to the large annual volume of rice cultivation, rice straw represents an important potential roughage resource for ruminant production. Nevertheless, its practical use as feed remains limited because untreated rice straw is characterized by low nutritive value, poor palatability, and low digestibility. In many production areas, rice straw is left unused or burned in the field, which contributes to air pollution and the loss of potentially valuable biomass. Improving the feeding value of rice straw is therefore relevant not only for reducing feed costs but also for promoting more efficient resource use and sustainable livestock production.

The main nutritional limitation of rice straw is its high concentration of lignocellulosic fiber and low crude protein content. The plant cell wall of rice straw consists primarily of cellulose, hemicellulose,

and lignin. Among these components, lignin is particularly resistant to microbial degradation and forms complex physical and chemical associations with cellulose and hemicellulose. These associations restrict the access of rumen microorganisms and fibrolytic enzymes to fermentable carbohydrates, resulting in reduced voluntary intake and poor digestibility. For this reason, several physical, chemical, and biological treatments have been investigated to improve the feeding value of low-quality roughages. Among these approaches, biological treatment using microorganisms has received increasing attention because it is relatively safe, environmentally friendly, and potentially suitable for farm-level application.

Microbial inoculants may improve silage quality and fiber utilization through different mechanisms. *Trichoderma reesei* is a cellulolytic fungus capable of producing cellulase and hemicellulase enzymes, which can hydrolyze

structural carbohydrates in plant cell walls. *Saccharomyces cerevisiae* may contribute to the fermentation process by consuming residual oxygen, supporting the establishment of anaerobic conditions, and stimulating beneficial microbial activity. *Bacillus licheniformis* is known to produce extracellular enzymes, including protease and xylanase, which may assist in protein and hemicellulose degradation. In contrast, *Lactobacillus plantarum* is a homofermentative lactic acid bacterium that rapidly produces lactic acid, reduces pH, and inhibits undesirable microorganisms during ensiling. These distinct functional characteristics indicate that combinations of microorganisms with complementary activities may be more effective than single-strain inoculation.

Although previous studies have reported beneficial effects of individual microbial inoculants on silage fermentation, information on the combined use of cellulolytic fungi, yeast, *Bacillus* spp., and lactic acid bacteria for improving rice straw silage remains limited. In particular, few studies have examined how different microbial combinations influence fiber fractions and *in vitro* ruminal fermentability across different fermentation periods. Therefore, further evaluation is needed to clarify whether multi-strain inoculation can improve lignocellulose degradation and enhance the digestibility of rice straw silage.

This study was based on the hypothesis that multi-strain microbial inoculation would improve fermentation quality, reduce fiber fractions, and increase *in vitro* gas production of rice straw silage compared with single-strain inoculation. The objective of this study was to evaluate the effects of different microbial inoculant combinations on chemical composition, pH, NDF, ADF, ADL, and *in vitro* gas production of rice straw silage after 7, 14, 21, and 28 days of fermentation.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was arranged in a completely randomized design (CRD) with five microbial treatments and three replications per treatment. Rice straw was chopped into approximately 3–5 cm lengths to improve mixing uniformity and increase the contact surface between the substrate and microbial inoculants. The chopped rice straw was thoroughly mixed and randomly allocated to the following treatment groups.

T1: *Trichoderma reesei*

T2: *Saccharomyces cerevisiae*

T3: *Trichoderma reesei* + *Saccharomyces cerevisiae*

T4: *Trichoderma reesei* + *Saccharomyces cerevisiae* + *Bacillus licheniformis*

T5: *Trichoderma reesei* + *Saccharomyces cerevisiae* + *Lactobacillus plantarum*

Preparation and ensiling of rice straw

Each microbial inoculant was prepared as a suspension and applied uniformly to the rice straw at approximately 1×10^6 CFU/g fresh matter. After inoculation, the treated rice straw was mixed thoroughly to ensure an even distribution of microorganisms throughout the material. The samples were then packed tightly into airtight plastic containers or fermentation bags to minimize oxygen exposure and promote anaerobic conditions. All silage samples were stored at room temperature and opened for analysis after 7, 14, 21, and 28 days of fermentation.

Chemical composition and fiber analysis

At each fermentation period, representative silage samples were collected from each replicate. The samples were analyzed for dry matter, ash, crude protein, and ether extract according to standard AOAC procedures. Fiber fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were determined using the detergent fiber method. Silage pH was measured by mixing a representative sample with distilled water, filtering the extract, and measuring the filtrate using a calibrated pH meter.

In vitro gas production

In vitro gas production was used as an indicator of ruminal fermentability and potential digestibility. The procedure was conducted according to the principle of the *in vitro* gas production technique. Rumen fluid was collected before morning feeding and maintained under anaerobic conditions. The rumen fluid was filtered through warm cheesecloth and mixed with buffer solution at a ratio of 1:2 under continuous CO₂ flushing. Approximately 200 mg of dried and ground silage sample was incubated with buffered rumen fluid in calibrated incubation bottles or syringes at 39 °C. Gas production was recorded after incubation using

a gas-measuring device or pressure transducer. Gas volume was expressed as milliliters per sample and used to compare the fermentability of rice straw silage among treatments.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) according to a completely randomized design. When significant treatment effects were detected, treatment means were compared using an appropriate multiple comparison test. Statistical significance was declared at $P < 0.05$, while highly significant differences were declared at $P < 0.01$ or $P < 0.001$. Results are presented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Chemical composition and pH

The effects of microbial inoculation on chemical composition and pH of rice straw silage are presented in Table 1. Dry matter content ranged from approximately 94.35% to 96.92% across treatments and fermentation periods. Significant differences among treatments were observed at all sampling times ($P < 0.01$ or $P < 0.001$). Ash content also differed significantly among treatments throughout fermentation ($P < 0.001$). Crude protein content showed a significant treatment effect only at 14 days

($P < 0.01$), whereas no significant differences were detected at 7, 21, or 28 days. Ether extract differed significantly at 7, 14, and 28 days ($P \leq 0.01$), but not at 21 days. The pH values varied among treatments, with significant differences observed at 14 days ($P < 0.01$). Although treatment differences were not significant at 21 days, the lowest pH value was recorded in T5 (4.65), indicating a stronger acidification response in this treatment

Table 1. Effects of multi-strain microbial inoculation on chemical composition and pH of rice straw silage during different fermentation periods

Item	Time	T1	T2	T3	T4	T5	P-value
Dry matter	7 d	96.92 $\pm$ 0.21 ^a	95.72 $\pm$ 0.70 ^b	96.20 $\pm$ 0.03 ^c	95.24 $\pm$ 0.09 ^d	95.11 $\pm$ 0.20 ^d	<0.001
	14 d	95.07 $\pm$ 0.27 ^a	95.67 $\pm$ 0.02 ^b	95.45 $\pm$ 0.17 ^{ab}	96.44 $\pm$ 0.21 ^c	95.51 $\pm$ 0.23 ^{ab}	<0.001
	21 d	96.24 $\pm$ 0.31 ^a	94.35 $\pm$ 0.92 ^b	95.41 $\pm$ 0.22 ^{ab}	96.15 $\pm$ 0.02 ^a	96.14 $\pm$ 0.01 ^a	<0.01
	28 d	95.66 $\pm$ 0.54 ^a	95.31 $\pm$ 0.48 ^{ab}	95.27 $\pm$ 0.08 ^{ab}	94.41 $\pm$ 0.33 ^b	95.73 $\pm$ 0.24 ^a	<0.01
Ash	7 d	3.38 $\pm$ 0.56 ^a	4.28 $\pm$ 0.07 ^{bc}	3.80 $\pm$ 0.03 ^{ab}	4.76 $\pm$ 0.09 ^{cd}	5.02 $\pm$ 0.95 ^d	<0.001
	14 d	4.93 $\pm$ 0.27 ^a	4.33 $\pm$ 0.02 ^b	4.56 $\pm$ 0.18 ^{ab}	3.56 $\pm$ 0.21 ^c	4.49 $\pm$ 0.23 ^{ab}	<0.001
	21 d	3.76 $\pm$ 0.31 ^a	5.99 $\pm$ 0.71 ^b	4.59 $\pm$ 0.22 ^a	3.85 $\pm$ 0.02 ^a	3.86 $\pm$ 0.01 ^a	<0.001
	28 d	3.56 $\pm$ 0.25 ^a	4.69 $\pm$ 0.48 ^b	4.59 $\pm$ 0.26 ^b	5.59 $\pm$ 0.33 ^c	4.27 $\pm$ 0.24 ^{ab}	<0.001
Crude protein	7 d	2.87 $\pm$ 0.00	2.58 $\pm$ 0.34	2.75 $\pm$ 0.33	3.16 $\pm$ 0.42	2.63 $\pm$ 0.39	0.28
	14 d	3.10 $\pm$ 0.10 ^a	2.31 $\pm$ 0.13 ^b	2.98 $\pm$ 0.41 ^{ab}	2.36 $\pm$ 0.06 ^b	2.38 $\pm$ 0.34 ^b	<0.01
	21 d	2.67 $\pm$ 0.14	2.43 $\pm$ 0.24	2.52 $\pm$ 0.22	2.45 $\pm$ 0.41	2.15 $\pm$ 0.16	0.22
	28 d	2.54 $\pm$ 0.44	2.63 $\pm$ 0.29	2.33 $\pm$ 0.30	2.69 $\pm$ 0.03	2.49 $\pm$ 0.19	0.61
Ether extract	7 d	0.76 $\pm$ 0.00 ^a	0.78 $\pm$ 0.09 ^a	0.82 $\pm$ 0.07 ^a	0.77 $\pm$ 0.15 ^a	0.53 $\pm$ 0.03 ^b	0.01
	14 d	1.89 $\pm$ 0.44 ^a	1.68 $\pm$ 0.24 ^{ab}	0.83 $\pm$ 0.20 ^c	1.81 $\pm$ 0.23 ^{ac}	1.03 $\pm$ 0.08 ^{bc}	<0.001
	21 d	0.88 $\pm$ 0.08	0.79 $\pm$ 0.21	0.98 $\pm$ 0.17	1.19 $\pm$ 0.39	1.37 $\pm$ 0.50	0.21
	28 d	0.83 $\pm$ 0.09 ^a	0.67 $\pm$ 0.14 ^{ab}	0.48 $\pm$ 0.01 ^b	0.77 $\pm$ 0.01 ^a	0.81 $\pm$ 0.01 ^a	<0.001
pH	7 d	6.90 $\pm$ 0.17	5.42 $\pm$ 1.44	6.35 $\pm$ 0.80	6.74 $\pm$ 0.11	6.65 $\pm$ 0.83	0.27
	14 d	6.04 $\pm$ 0.61 ^{ab}	5.82 $\pm$ 0.77 ^{ab}	5.59 $\pm$ 0.70 ^a	7.05 $\pm$ 0.11 ^b	7.11 $\pm$ 0.10 ^b	<0.01
	21 d	4.97 $\pm$ 0.15	5.15 $\pm$ 0.60	5.46 $\pm$ 1.23	5.03 $\pm$ 0.89	4.65 $\pm$ 0.08	0.75
	28 d	5.80 $\pm$ 0.18	5.12 $\pm$ 0.31	5.75 $\pm$ 1.61	5.53 $\pm$ 1.51	5.51 $\pm$ 0.66	0.93

Values are presented as mean $\pm$ standard deviation. Different superscript letters within the same row indicate significant differences among treatments ($P < 0.05$). T1 = *Trichoderma reesei*; T2 = *Saccharomyces cerevisiae*; T3 = *T. reesei* + *S. cerevisiae*; T4 = *T. reesei* + *S. cerevisiae* + *Bacillus licheniformis*; T5 = *T. reesei* + *S. cerevisiae* + *Lactobacillus plantarum*.

Fiber fractions

The effects of microbial inoculation on NDF, ADF, and ADL are shown in Table 2. Significant differences among treatments were observed for all fiber fractions at all fermentation periods ($P < 0.05$ to $P < 0.001$). At 28 days, T5 had the lowest NDF value (69.47%), followed by T4

(72.82%) and T2 (72.93%). For ADF, T5 and T4 had lower values than T1 and T2 at 28 days. A particularly clear reduction was observed in ADL, where T5 showed the lowest value at 28 days (19.02%). These results indicate that the T5 combination was the most effective treatment for reducing structural fiber components in rice straw silage.

Table 2. Effects of multi-strain microbial inoculation on fiber fractions of rice straw silage during different fermentation periods.

Item	Time	T1	T2	T3	T4	T5	P-value
NDF	7 d	77.68±0.04 ^a	75.11±0.04 ^b	76.18±0.04 ^c	76.82±0.04 ^d	76.81±0.04 ^d	<0.001
	14 d	75.01±0.04 ^a	75.71±0.04 ^b	75.68±0.04 ^b	75.28±0.04 ^c	78.34±0.04 ^c	<0.001
	21 d	77.11±0.04 ^a	73.92±0.04 ^b	72.38±0.04 ^c	72.65±0.04 ^d	70.14±0.05 ^e	<0.001
	28 d	75.73±0.04 ^a	72.93±0.04 ^b	74.12±0.04 ^c	72.82±0.04 ^b	69.47±0.04 ^d	<0.001
ADF	7 d	53.64±0.04 ^a	52.31±0.04 ^{bc}	51.48±0.36 ^b	49.36±0.04 ^d	52.68±0.31 ^c	0.016
	14 d	52.18±0.04 ^a	52.18±0.06 ^{ab}	50.66±0.04 ^c	51.07±0.04 ^d	51.92±0.04 ^b	<0.001
	21 d	51.67±0.14 ^a	50.67±0.00 ^b	49.18±0.06 ^c	45.01±0.01 ^d	44.06±0.03 ^c	<0.001
	28 d	50.81±0.24 ^a	50.19±0.16 ^a	48.68±0.40 ^b	47.69±0.41 ^b	47.66±0.13 ^b	<0.001
ADL	7 d	33.54±0.01 ^a	38.27±0.30 ^b	34.52±0.52 ^a	35.93±0.15 ^c	30.28±0.34 ^d	<0.001
	14 d	26.27±0.02 ^a	28.12±0.50 ^b	27.54±0.10 ^{bc}	26.52±0.35 ^{ac}	23.44±0.24 ^d	<0.001
	21 d	25.23±0.54 ^a	21.68±0.03 ^b	21.32±0.11 ^b	24.51±0.14 ^a	21.18±0.00 ^b	<0.001
	28 d	22.18±0.36 ^a	20.17±0.07 ^{bc}	20.41±0.24 ^b	22.55±0.44 ^a	19.02±0.34 ^c	<0.001

Values are presented as mean ± standard deviation. Different superscript letters within the same row indicate significant differences among treatments ($P < 0.05$). T1 = *Trichoderma reesei*; T2 = *Saccharomyces cerevisiae*; T3 = *T. reesei* + *S. cerevisiae*; T4 = *T. reesei* + *S. cerevisiae* + *Bacillus licheniformis*; T5 = *T. reesei* + *S. cerevisiae* + *Lactobacillus plantarum*.

In vitro gas production

The effects of microbial inoculation on in vitro gas production are presented in Table 3. No significant differences among treatments were observed at 7, 14, or 21 days of fermentation ($P > 0.05$). However, treatment effects became significant

at 28 days ($P < 0.01$). At this time, T5 produced the highest gas volume (74.5 mL), whereas T2 and T3 produced comparatively lower values. These results suggest that the influence of microbial inoculation on ruminal fermentability became more evident after a longer fermentation period.

Table 3. Effects of multi-strain microbial inoculation on in vitro gas production of rice straw silage during different fermentation periods

Item	Time	T1	T2	T3	T4	T5	P-value
Gas production	7 d	71.0±1.41	70.5±0.71	70.5±0.71	70.0±0.00	71.0±0.00	0.69
	14 d	74.5±2.12	74.0±2.83	77.5±0.71	74.0±2.83	72.0±0.00	0.25
	21 d	73.0±1.41	73.0±4.24	75.0±1.41	72.0±0.00	71.0±1.41	0.52
	28 d	71.0±1.41 ^{ab}	70.0±0.00 ^b	69.0±1.41 ^b	72.0±0.00 ^{ab}	74.5±0.71 ^a	<0.01

Values are presented as mean ± standard deviation. Different superscript letters within the same row indicate significant differences among treatments ($P < 0.05$). T1 = *Trichoderma reesei*; T2 = *Saccharomyces cerevisiae*; T3 = *T. reesei* + *S. cerevisiae*; T4 = *T. reesei* + *S. cerevisiae* + *Bacillus licheniformis*; T5 = *T. reesei* + *S. cerevisiae* + *Lactobacillus plantarum*.

Effects of microbial inoculation on fermentation quality and chemical composition

The results of the present study indicate that microbial inoculation influenced several chemical characteristics of rice straw silage. Dry matter content remained within a relatively narrow range during fermentation, suggesting that the ensiling process did not result in excessive material loss. This finding is important because substantial dry matter loss can reduce the amount of recoverable feed and may reflect undesirable fermentation. The significant differences in ash content among treatments may be associated with proportional changes in organic matter utilization during fermentation rather than direct changes in mineral concentration. Therefore, ash responses should be interpreted cautiously as part of the overall compositional changes occurring during ensiling.

Crude protein content differed significantly only at 14 days. The higher crude protein value observed in some treatments during this period may be related to microbial biomass accumulation in the early stage of fermentation. However, the subsequent decline or stabilization of crude protein content may be explained by microbial protein turnover, proteolysis, and the use of soluble nitrogen by fermentative microorganisms. The crude protein response was therefore less consistent than the fiber response, indicating that the primary contribution of microbial inoculation in this study was associated more with structural fiber modification than with protein enrichment.

Silage pH is an important indicator of fermentation quality because rapid acidification helps suppress undesirable microorganisms and preserve nutrients. The lowest pH value observed in T5 at 21 days suggests that the inclusion of *Lactobacillus plantarum* promoted lactic acid fermentation. Lactic acid bacteria convert available water-soluble carbohydrates into lactic acid, resulting in pH reduction and improved fermentation stability. Although rice straw contains relatively low levels of readily fermentable carbohydrates, the combined activity of *T. reesei* and *S. cerevisiae* may have increased substrate availability and created a more favorable environment for lactic acid production in T5.

Superior fiber degradation in T5

The most notable finding of this study was the greater reduction of NDF, ADF, and ADL in T5, particularly at 21 and 28 days of fermentation. This response can be explained by the complementary activities of *T. reesei*, *S. cerevisiae*, and *L. plantarum*.

T. reesei is known for its ability to produce cellulolytic and hemicellulolytic enzymes, which can break down cellulose and hemicellulose in plant cell walls. Partial degradation of the fiber matrix may increase the availability of fermentable substrates during ensiling and improve the accessibility of rumen microorganisms to structural carbohydrates.

S. cerevisiae may further support the fermentation process by consuming residual oxygen and helping establish anaerobic conditions. Oxygen removal during the early stage of ensiling is essential because prolonged aerobic conditions can delay lactic acid fermentation and promote the growth of undesirable microorganisms. In addition, yeast metabolites such as vitamins, peptides, and growth factors may stimulate beneficial microbial populations. Thus, the inclusion of *S. cerevisiae* may have contributed to a more favorable microbial environment for both enzymatic fiber modification and acid fermentation.

The addition of *L. plantarum* in T5 likely provided a further advantage over treatments that did not include lactic acid bacteria. *L. plantarum* is a strong lactic acid producer and can rapidly reduce pH, stabilize silage fermentation, and limit nutrient losses. A lower pH may also help preserve the products of fungal and yeast activity during fermentation. Therefore, the superior performance of T5 was likely the result of a synergistic interaction among the three microorganisms: *T. reesei* promoted fiber hydrolysis, *S. cerevisiae* improved the anaerobic environment and supported microbial activity, and *L. plantarum* stabilized fermentation through lactic acid production.

The reduction in ADL is particularly meaningful because lignin is a major barrier to ruminal degradation of rice straw. Lower ADL values suggest modification of the lignified structure, which may allow rumen microorganisms greater access to cellulose and hemicellulose. This interpretation is consistent with the observation that T5 had both the lowest ADL value and the highest *in vitro* gas production at 28 days.

Relationship between fiber fractions and *in vitro* gas production

In vitro gas production reflects the fermentation of soluble and structural carbohydrates by rumen microorganisms. The absence of significant differences at 7, 14, and 21 days suggests that the early fermentation period was not sufficient to create clear differences in ruminal fermentability among treatments. However, after 28 days, T5 produced a significantly higher gas volume than several other

treatments. This result indicates that a longer fermentation period allowed the multi-strain inoculant to modify the fiber structure more effectively, thereby improving the fermentability of rice straw silage.

The relationship between reduced fiber fractions and increased gas production supports the interpretation that microbial pretreatment improved substrate availability. As NDF, ADF, and ADL decreased, rumen microorganisms likely gained greater access to fermentable carbohydrates. Therefore, the higher gas production observed in T5 at 28 days is consistent with its lower NDF, ADF, and ADL values. These findings also support the usefulness of *in vitro* gas production as a practical indicator of improved digestibility in biologically treated rice straw.

Optimal fermentation period

The results suggest that a fermentation period of 21–28 days is appropriate for rice straw treated with multi-strain microbial inoculants. At 21 days, pH reduction was most evident in T5, indicating active lactic acid fermentation. At 28 days, fiber degradation and *in vitro* gas production were most improved. Therefore, fermentation for at least 21 days may be required to achieve effective acidification, whereas 28 days appears more suitable when the primary objective is to maximize fiber modification and ruminal fermentability.

Conclusions

Multi-strain microbial inoculation improved fermentation characteristics, fiber degradation, and *in vitro* fermentability of rice straw silage. Among the tested treatments, T5 (*T. reesei*, *S. cerevisiae*, and *L. plantarum*) showed the most favorable response, as indicated by the lowest NDF, ADF, and ADL values and the highest *in vitro* gas production at 28 days of fermentation. The superior performance of T5 was likely associated with synergistic interactions among cellulolytic fungi, yeast, and lactic acid bacteria. These findings suggest that multi-strain microbial inoculation is a promising biological approach for improving the nutritive value of rice straw and increasing its potential use as ruminant feed. For practical application, fermentation for 21–28 days is recommended, with 28 days providing the clearest improvement in fiber degradation and ruminal fermentability.

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Fish Diversity, Ecological Functional Guilds and IUCN Conservation Status in the Lower Mekong River, Northeastern Thailand

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ABSTRACT

This study aimed to quantify spatial and seasonal variation in fish diversity, classify fish into ecological functional guilds, and assess conservation status using the IUCN Red List in the Mekong River across three provinces in northeastern Thailand: Loei, Nakhon Phanom, and Ubon Ratchathani. Fish sampling was conducted during three seasonal periods. A total of 84 fish species belonging to 61 genera and 21 families were recorded. The highest diversity was the family Cyprinidae, with 45 species, followed by the families Bagridae and Siluridae, each represented by six species. The assessment of species diversity and evenness indicated that the fish community exhibited a moderate level of diversity with a relatively even distribution of species. Classification of fish based on ecological functional guilds showed that most species belonged to group G3, which inhabits and spawns in the main river channel, accounting for 29 species. The conservation status assessment based on the International Union for Conservation of Nature (IUCN) Red List revealed that most species were categorized as Least Concern (LC), with 65 species, followed by Vulnerable (VU), with six species. One species was classified as Endangered (EN), namely *Probarbus jullieni*. The findings of this study provide important baseline information for fisheries management and biodiversity conservation in the Mekong River ecosystem of Thailand.

Keywords: Mekong River, Fish diversity, Ecological Functional Guilds, Conservation status

INTRODUCTION

The Mekong River is a major global river system and an important freshwater ecosystem that supports both biodiversity and the livelihoods of millions of people in Southeast Asia. Originating on the Tibetan Plateau, the river flows through six countries, including China, Myanmar, Lao PDR, Thailand, Cambodia, and Vietnam, before eventually discharging into the South China Sea. In total, the Mekong River extends for approximately 4,880 km and drains a basin of about 795,000 km² (Mekong River Commission, 2009).

The lower Mekong basin has diverse topography and habitats, such as the Mekong mainstream, tributaries, floodplains, wetlands, and deep pools. These habitats provide important ecological conditions that sustain many freshwater organisms. (Valbo-Jørgensen *et al.*, 2009, Hurtle and Bamrungrach, 2015). The high diversity of ecosystems has led to the Mekong River basin being classified as a globally important biodiversity area within the Indo-Burma biodiversity hotspot (Critical Ecosystem Partnership Fund, 2020). Changes in ecosystems, water levels, and flooded areas in each

season affect the structure of habitats and the life cycles of aquatic animals, especially fish. Many fish species undertake seasonal migrations to access feeding grounds, spawning habitats, and nursery areas at different times of the year. (Mekong River Commission, 2009, Valbo-Jørgensen *et al.*, 2009)

The Mekong River supports a highly diverse fish species; approximately 1,100 species have been recorded throughout the basin, with at least 850 species occurring in the Lower Mekong Basin (Rainboth *et al.*, 2012, Hurtle, 2009). In 2016, about 540 fish species were reported from the Mekong River (Nagao Natural Environment Foundation, 2021). The Cyprinidae family represents the most diverse assemblage in the Mekong Basin, accounting for over 40 percent of all recorded fish species in the basin (Mekong River Commission, 2021). Moreover, more than 693 species have been assessed and documented in the IUCN Red List of Threatened Species; the *Platyptropius siamensis* has been declared extinct (Mekong River Commission, 2019).

The Mekong River flows along the border between Thailand and Lao PDR for about 917 km. It passes through eight provinces: Chiang Rai, Loei,

Nong Khai, Bueng Kan, Nakhon Phanom, Mukdahan, Amnat Charoen, and Ubon Ratchathani (National Water Resources Office, 2023). Northeastern is part of the Lower Mekong Basin and is an important place for fish resources and diversity. For instance, a survey of fish diversity in the provinces of Ubon Ratchathani, Amnat Charoen, Mukdahan, and Nakhon Phanom found 164 species from 97 genera and 32 families (Rotmongkoldee *et al.*, 2012). A study in the Mekong River in Nakhon Phanom Province found 82 fish species from 56 genera, 19 families, and 11 orders, with Cypriniformes being the most common group (Nuntawan *et al.*, 2019). At present, infrastructure development such as hydropower dam construction, overfishing, and climate change may have long-term impacts on the ecosystem and fish diversity of the Mekong River. Therefore, assessing fish diversity and conservation status in the Mekong River is important for monitoring changes in fisheries resources and supporting the sustainable management of aquatic resources.

However, previous studies have primarily focused on species inventories without integrating ecological functional guilds and updated conservation status. Therefore, this study aims (1) to quantify spatial and seasonal variation in fish diversity, (2) to classify fish into ecological functional guilds, and (3) to assess conservation status using the IUCN Red List.

MATERIALS AND METHODS

Study Area

Fish sampling was carried out in the Lower Mekong River at three sites in Northeastern Thailand: Ban Huai Hiem, Hat Khamphi Subdistrict, Pak Chom District, Loei Province (18.211687/102.073306); Ban That Phanom Nuea, That Phanom Subdistrict, That Phanom District, Nakhon Phanom Province (16.044130/104.729622); and Hat Chom Dao, Ban Natal, Natal Subdistrict, Natal District, Ubon Ratchathani Province (15.907450/105.341432). A stratified random sampling design was used to cover three hydrological periods to capture seasonal variation: peak flood season (September–October; S1), receding flood season (November–December; S2), and low-water season (January–February; S3). At each site and in each season, three replicates were conducted to ensure good representation of fish assemblages.

Fish sampling

Fish specimens were collected at three sampling stations using trawl nets. A trawl net with a mesh size of 2×2 cm, 40 m in length, and approximately 8 m in width, as well as a fine-mesh trawl net with a mesh size of 0.2 cm, 12 m in length, and 3 m in width, were towed along the riverbank. At each sampling station, trawling was conducted three times. Specimens were weighed using a digital balance with a precision of 0.1 g. Standard length (SL) and total length (TL) were measured to the nearest 0.1 cm. Fresh specimens were photographed using a digital camera and subsequently preserved in a 10% formalin solution for further analysis and taxonomic identification at the laboratory, Mahasarakham University.

The combined use of trawl nets with different mesh sizes was intended to reduce gear-specific bias and enhance overall sampling efficiency. Larger trawl nets primarily target large-bodied fishes, nektonic, and pelagic species, whereas smaller trawl nets were more effective for capturing small-bodied and juvenile fishes in littoral zone or floodplain. This complementary approach is widely recommended to improve the representativeness of fish assemblage data, as different gear types selectively sample distinct components of the community (Guest *et al.*, 2003).

Nevertheless, several limitations remain. Both gear types may underrepresent benthic and structure-associated species, and gear selectivity can influence the observed species composition and relative abundance. In addition, catch efficiency may vary with environmental conditions, such as flow velocity, turbidity, and habitat complexity, as well as fish behavior, potentially introducing additional sampling bias.

Fish sampling conducted for this study was approved under the ethical guidelines for the use of animals in scientific research and biosafety by the Institutional Animal Care and Use Committee of Mahasarakham University (Approval No. IACUC-MSU-09/2024).

Water quality measurement

Water quality was conducted at Mekong River fish sampling stations throughout three seasons, pH, electrical conductivity (EC), water temperature (T), and dissolved oxygen (DO), using the Eutech PCD 650 (OAKTON) automatic water quality meter.

Data analysis

Fish species were identified using standard taxonomic references, including Saenjandaeng (2014), Metheethananwat (2012), and the Nagao Natural Environment Foundation (2021), as well as the online database FishBase (www.fishbase.org). The conservation status of each recorded species was verified based on the IUCN Red List of Threatened Species. Fish guilds were classified according to their migratory behavior and habitat use patterns following the Mekong River Commission (2021).

Ecological analyses were conducted to evaluate fish community structure and diversity. The diversity index (H') (Shannon and Wiener, 1949), dominance index (D) (Simpson, 1949), species richness index (R) (Margalef, 1968), and evenness index (J') (Pielou, 1966) were calculated for each sampling site and season.

Statistical Analysis

Data were assessed for normal distribution using the Shapiro–Wilk test. Differences in fish yields by province and season were evaluated using one-way analysis of variance (ANOVA) when data were normal; otherwise, the non-parametric Kruskal–Wallis test was used when data violated normality. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics version 29.

RESULTS

A survey of fish diversity in the Mekong River was done over three seasons in three provinces in northeastern Thailand: Loei, Nakhon Phanom, and Ubon Ratchathani. A total of 1,690 fish specimens were collected, representing 84 species, 61 genera, and 21 families. The family Cyprinidae showed the highest species richness with 45 species, accounting for 54% of the total species recorded. This was followed by the families Bagridae and Siluridae, each represented by six species (7% of the total species) (Figure 1).

Species composition analysis indicated that *Barbonymus altus* was the most abundant species, comprising 20.5% of all individuals collected. Other dominant species included *Puntius proctozystron*, *Oreochromis niloticus*, *Sikukia gudgeri*, and *Parambassis siamensis*, accounting for 13%, 6.5%, 6.2%, 6.2%, and 5.2% of the total individuals, respectively. In contrast, several species were rarely encountered, including *Puntius waandersi*,

Rasbora myersi, *Pristolepis fasciatus*, and *Wallago attu*, each representing only 0.1% of the total individuals recorded.

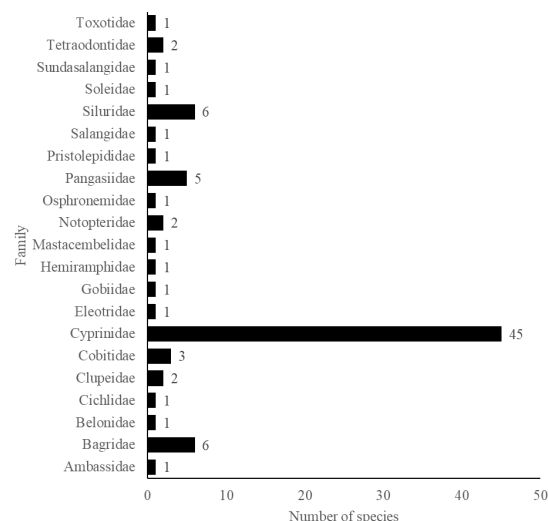


Figure 1. Distribution of fish species richness by family in the Lower Mekong River, northeastern Thailand.

When fish diversity was examined by sampling area, Ubon Ratchathani showed the highest species richness with 52 species, followed by Nakhon Phanom with 50 species and Loei with 37 species (Figure 2). In Loei Province, a total of 395 individuals were collected. The most abundant species was *Oreochromis niloticus*, representing 23.48% of the total individuals, followed by *Cyclocheilichthys enoplos* (15.58%) and *Parambassis siamensis* (14.65%). In Nakhon Phanom Province, 865 individuals were recorded. *Barbonymus altus* was the most abundant species, accounting for 25.78% of the total individuals, followed by *Puntius proctozystron* (21.38%) and *Mystacoleucus obtusirostris* (8.20%). In Ubon Ratchathani Province, 430 individuals were collected. The dominant species was *Barbonymus altus* (29.11%), followed by *Puntius proctozystron* (7.34%) and *Raiamas guttatus* (6.08%). Examination of species distribution among sampling areas indicated that 13 species occurred in all three provinces. These included *Barbonymus altus*, *Barbonymus gonionotus*, *Probarbus jullieni*, *Puntius proctozystron*, *Raiamas guttatus*, *Sikukia gudgeri*, *Henicorhynchus siamensis*, *Acantopsis runghthipae*, *Hemibagrus spilopterus*, *Xenentodon canciloides*, *Mastacembelus armatus*, *Parambassis siamensis*, and *Brachirus harmandi*. Fish abundance was compared among the three sampling areas using the Kruskal Wallis test. The

results indicated no significant difference in fish abundance among areas at the 0.05 significance level ($H = 3.289$, $p = 0.193$).

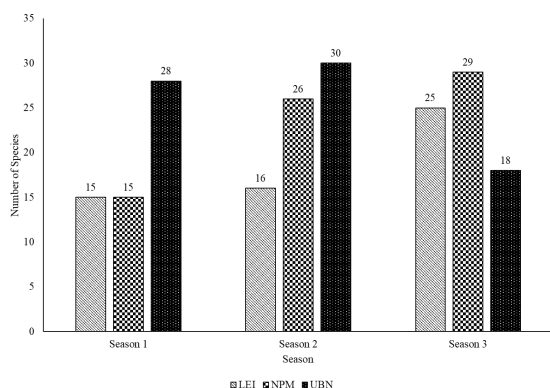


Figure 2. Variation in the number of fish species recorded in the Mekong River across three seasonal periods.

When seasonal variation was examined, the receding flood season (S2) showed the highest species richness with 50 species, followed by the low-water season (S3) with 49 species and the peak flood season (S1) with 43 species. During the peak flood season (S1), the most abundant species was *Barbonymus altus*, representing 34.70% of the total individuals, followed by *Cyclocheilichthys enoplos* (13.13%) and *Mystacoleucus obtusirostris* (12.94%). During the receding flood season (S2), *Barbonymus altus* remained the most abundant species, accounting for 21.78% of the total individuals. Other common species included *Oreochromis niloticus*

(17.61%) and *Sikukia gudgeri* (13.63%). In the low water period (S3), *Puntius proctozystron* was the most abundant species (29.60%), followed by *Barbonymus altus* (8.28%) and *Cosmochilus harmandi* (6.28%). Fish abundance among seasons was compared using the Kruskal Wallis test. The results indicated no significant difference in fish abundance among seasons at the 0.05 significance level ($H = 0.622$, $p = 0.733$).

Ecological indices including the diversity index (H'), dominance index (D), species richness (R), and evenness index (J') were also calculated for each province. Results were presented as mean $\pm$ standard error (SE). In Loei Province, H' , D , R and J' were 2.11 ± 0.62 , 0.21 ± 0.12 , 3.62 ± 1.33 , and 0.72 ± 0.13 , respectively. In Nakhon Phanom Province, H' , D , R , and J' were 1.96 ± 0.42 , 0.24 ± 0.07 , 3.97 ± 1.09 , and 0.62 ± 0.06 , respectively. In Ubon Ratchathani Province, H' , D , R , and J' were 2.52 ± 0.15 , 0.13 ± 0.04 , 5.04 ± 1.01 , and 0.78 ± 0.06 , respectively (Figure 3).

When ecological indices were examined across seasons (mean $\pm$ SE), the peak flood season (S1) showed an H' , D , R and J' were 1.90 ± 0.47 , 0.26 ± 0.08 , 3.55 ± 1.32 , and 0.65 ± 0.09 , respectively. During the receding flood season (S2), H' increased to 2.17 ± 0.49 , whereas D , R and J' were 0.21 ± 0.08 , 4.52 ± 1.60 , and 0.69 ± 0.09 , respectively. And, during the low-water season (S3), H' further increased to 2.54 ± 0.26 , while D decreased to 0.12 ± 0.06 , R was 4.57 ± 0.58 , and J' reached 0.81 ± 0.10 , respectively (Figure 4).

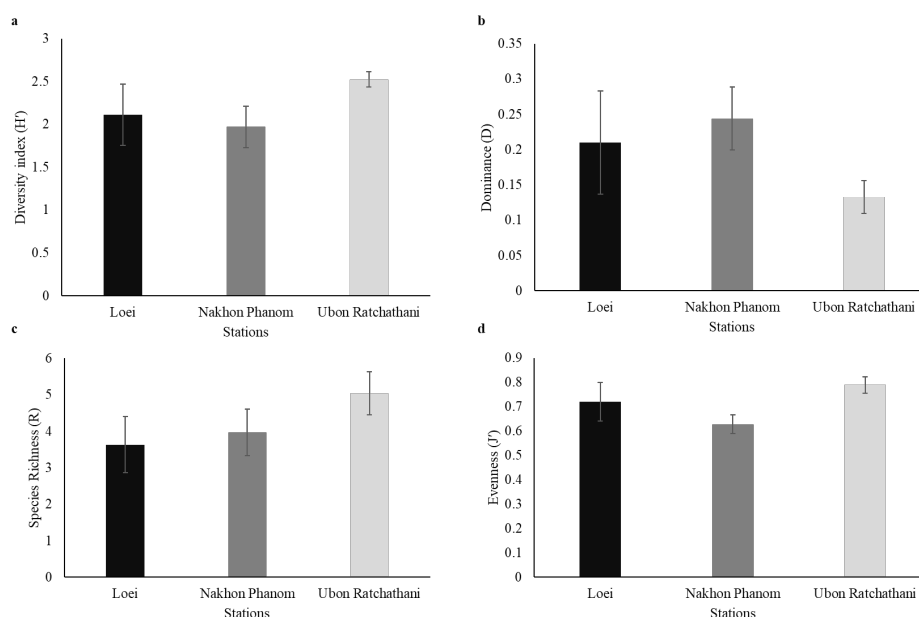


Figure 3. Fish community structure indices at three sampling sites in the Lower Mekong River, northeastern Thailand (mean $\pm$ SE): (a) diversity index (H'), (b) dominance index (D), (c) species richness (R), and (d) evenness index (J').

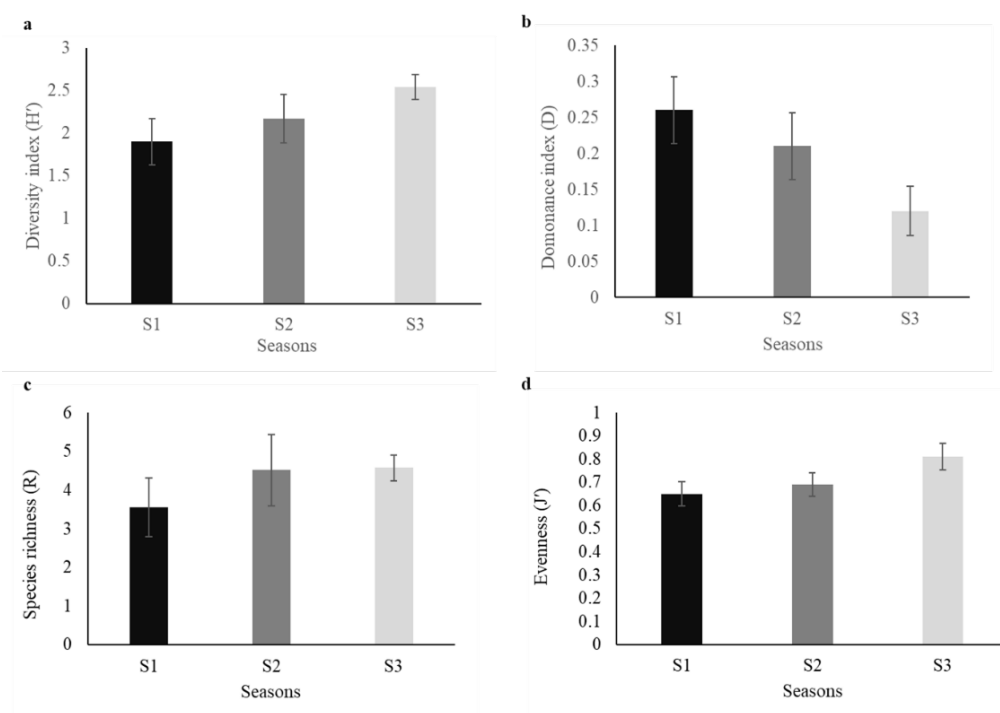


Figure 4. Fish community structure indices across three hydrological seasons in the Lower Mekong River, northeastern Thailand (mean±SE), including (a) diversity index (H'), (b) dominance index (D), (c) species richness (R), and (d) evenness index (J'). S1 = peak flood season (September–October), S2 = receding flood season (November–December), and S3 = low-water season (January–February).

Fish species were further classified into ecological functional guilds based on migration behavior and habitat use following the criteria of the Mekong River Commission. A total of six functional groups were identified. These comprised rhithron residents (G1) with nine species, main channel residents or long-distance white fishes (G2) with five species, main channel spawners or short distance white fishes (G3) with 29 species, floodplain spawners or grey fishes (G4) with 19 species, eurytopic or generalist fishes (G5) with 15 species, and floodplain residents or black fishes (G6) with two species. In addition, five species were classified as non-native or exotic species (G11). Most species were assigned to the main channel spawners group (G3). Representative species in this group included *Clupeichthys aesarnensis*, *Hypsibarbus malcolmi*, and *Phalacrodon micronemus*. The second most common group was floodplain spawners (G4), which included species such as *Barbonymus altus*, *Bagrichthys majusculus*, and *Toxotes mekongensis* (Table 1).

Fish species recorded in this study were also classified according to their conservation status based on the IUCN Red List of Threatened Species.

The results indicated that one species was classified as Endangered (EN), six species as Vulnerable (VU), two species as Near Threatened (NT), 65 species as Least Concern (LC), five species as Data Deficient (DD), and one species as Not Evaluated (NE) (Figure 5).

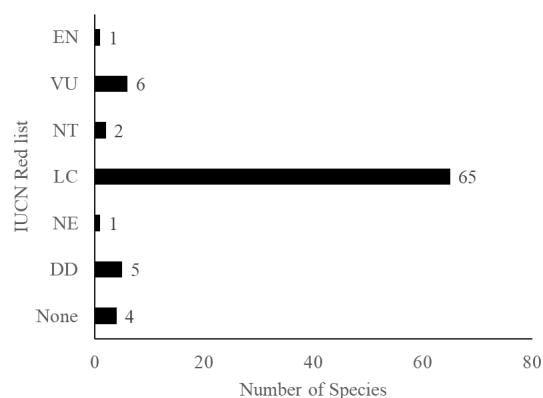


Figure 5. Conservation status of fish species recorded from the Lower Mekong River in northeastern Thailand (based on IUCN Red List categories).

Table 1. Checklist, conservation status (based on IUCN Red List), and ecological guilds of fish species recorded at each sampling station in the Mekong River, northeastern Thailand (Loei, Nakhon Phanom, and Ubon Ratchathani).

Family / Scientific Name	Guild of MRC	IUCN Red list	LEI	NPM	UBN	N
Family Notopteridae						
<i>Chitala ornata</i> (Gray, 1831)	G5	LC		+		7
<i>Notopterus notopterus</i> (Pallas, 1769)	G5	LC	+	+		13
Family Sundasalangidae						
<i>Sundasalanx mekongensis</i> (Britz & Kottelat, 1999)	G3	LC			+	1
Family Salangidae						
<i>Neosalanx brevirostris</i> (Pellegrin, 1923)	G11	DD	+		+	10
Family Clupeidae						
<i>Clupeichthys aesarnensis</i> Wongratana, 1983	G3	LC	+		+	38
<i>Tenualosa thibaudeau</i> (Durand, 1940)	G3	VU		+	+	15
Family Cyprinidae						
<i>Amblyrhynchichthys micracanthus</i> Ng & Kottelat, 2004	G3	LC		+		1
<i>A. truncatus</i> (Bleeker, 1850)	G3	LC		+		1
<i>Barbichthys laevis</i> (Valenciennes, 1842)	G3	LC			+	7
<i>Barbonymus altus</i> (Günther, 1868)	G4	LC	+	+	+	346
<i>B. gonionotus</i> (Bleeker, 1849)	G5	LC	+	+	+	5
<i>B. schwanefeldii</i> (Bleeker, 1854)	G4	LC	+			5
<i>Cirrhinus molitorella</i> (Valenciennes, 1844)	G3	NT			+	1
<i>Cosmochilus harmandi</i> Sauvage, 1878	G2	LC	+	+		51
<i>Cyclocheilichthys armatus</i> (Valenciennes, 1842)	G4	LC	+			1
<i>C. enoplos</i> (Bleeker, 1849)	G2	LC	+	+		71
<i>C. repasson</i> (Bleeker, 1853)	G4	LC	+	+		11
<i>Cyprinus carpio</i> (Linnaeus, 1758)	G11	-		+	+	5
<i>Discherodontus ashmeadi</i> (Fowler, 1937)	G1	LC	+			1
<i>Epalzeorhynchus munensis</i> (Smith, 1934)	G1	VU			+	5
<i>Esomus metallicus</i> (Ahl, 1923)	G6	LC			+	4
<i>Hampala dispar</i> (Smith, 1934)	G5	LC	+		+	4
<i>H. macrolepidota</i> (Kuhl & Van Hasselt, 1823)	G5	LC		+	+	6
<i>Henicorhynchus entmema</i> (Fowler, 1934)	G5	LC	+			1
<i>H. siamensis</i> (Sauvage, 1881)	G5	LC	+	+	+	6
<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844)	G11	-			+	1
<i>Hypsibarbus lagleri</i> (Rainboth, 1996)	G3	VU		+	+	3
<i>H. malcolmi</i> (Smith, 1945)	G3	LC		+	+	34
<i>H. vernayi</i> (Norman, 1925)	G3	LC	+	+		27
<i>H. wetmorei</i> (Smith, 1931)	G3	LC	+	+		20
<i>Labeo rohita</i> (Hamilton, 1822)	G11	-			+	1
<i>Labiobarbus siamensis</i> (Sauvage, 1881)	G5	LC			+	12
<i>Mystacoleucus obtusirostris</i> (Valenciennes, 1842)	G1	LC	+	+	+	105
<i>Opsarius koratensis</i> (Smith, 1931)	G1	LC	+		+	7
<i>Osteochilus microcephalus</i> (Valenciennes, 1842)	G5	LC			+	1
<i>Paralauca barroni</i> (Fowler, 1934)	G4	LC	+			1
<i>P. riveroi</i> (Fowler, 1935)	G4	LC			+	16
<i>Probarbus jullieni</i> (Sauvage, 1880)	G2	EN	+	+	+	5

Table 1. Checklist, conservation status (based on IUCN Red List), and ecological guilds of fish species recorded at each sampling station in the Mekong River, northeastern Thailand (Loei, Nakhon Phanom, and Ubon Ratchathani).

Family / Scientific Name	Guild of MRC	IUCN Red list	LEI	NPM	UBN	N
<i>Puntioplites falcifer</i> Smith, 1929	G3	LC		+	+	21
<i>P. proctozystron</i> (Bleeker, 1865)	G3	LC	+	+	+	219
<i>P. waandersi</i> (Bleeker, 1859)	G3	LC			+	1
<i>Raiamas guttatus</i> (Day, 1870)	G1	LC	+	+	+	55
<i>Rasbora argyrotaenia</i> (Bleeker, 1849)	G4	LC	+			13
<i>R. aurotaenia</i> Tirant, 1885	G4	LC			+	4
<i>R. borapetensis</i> Smith, 1934	G4	LC	+		+	8
<i>R. myersi</i> Brittan, 1954	G4	LC			+	1
<i>Scaphognathops bandanensis</i> Boonyaratpalin & Srirungroj, 1971	G3	VU		+	+	19
<i>Sikukia gudgeri</i> (Smith, 1934)	G4	DD	+	+	+	105
<i>Systemus rubripinnis</i> (Valenciennes, 1842)	G3	DD		+		8
<i>Thynnichthys thynnoides</i> (Bleeker, 1852)	G4	LC			+	2
<i>Tor sinensis</i> Wu, 1977	G1	VU			+	9
Family Cobitidae						
<i>Acantopsis rungthipae</i> Boyd, Nithirojpakdee & Page, 2017	G3	NE	+	+	+	7
<i>Aperioptus delphax</i> Siebert, 1991	G3	LC		+	+	4
<i>Yasuhikotakia lecontei</i> (Fowler, 1937)	G1	LC			+	2
Family Bagridae						
<i>Bagrichthys majusculus</i> Ng, 2002	G4	DD		+		3
<i>Hemibagrus spilopterus</i> Ng & Rainboth, 1999	G3	LC	+	+	+	12
<i>Mystus bocourti</i> (Bleeker, 1864)	G4	VU		+		7
<i>M. mysticetus</i> Roberts, 1992	G4	LC		+		1
<i>M. singaringan</i> (Bleeker, 1846)	G4	LC		+		3
<i>Pseudomystus siamensis</i> (Regan, 1913)	G3	LC		+		1
Family Siluridae						
<i>Belodontichthys truncatus</i> Kottelat & Ng, 1999	G3	LC		+	+	8
<i>Kryptopterus cheveyi</i> Durand, 1940	G3	LC		+	+	3
<i>Phalacronotus apogon</i> (Bleeker, 1851)	G3	LC		+	+	6
<i>P. bleekeri</i> (Günther, 1864)	G3	LC			+	1
<i>P. micronemus</i> (Bleeker, 1846)	G3	LC		+		15
<i>Wallago attu</i> (Bloch & Schneider, 1801)	G3	NT		+		1
Family Pangasiidae						
<i>Helicophagus leptorhynchus</i> Ng & Kottelat, 2000	G3	DD		+		2
<i>Pangasius bocourti</i> Sauvage, 1880	G2	LC		+		1
<i>P. conchophilus</i> Roberts & Vidthayanon, 1991	G2	LC		+		1
<i>P. macronema</i> Bleeker, 1850	G3	LC	+		+	7
<i>Pseudolais pleurotaenia</i> (Sauvage, 1878)	G3	LC		+		1
Family Hemiramphidae						
<i>Dermogenys siamensis</i> Fowler, 1934	G4	LC	+			6

Table 1. Checklist, conservation status (based on IUCN Red List), and ecological guilds of fish species recorded at each sampling station in the Mekong River, northeastern Thailand (Loei, Nakhon Phanom, and Ubon Ratchathani).

Family / Scientific Name	Guild of MRC	IUCN Red list	LEI	NPM	UBN	N
Family Belontiidae						
<i>Xenentodon canciloides</i> (Bleeker, 1854)	G5	LC	+	+	+	16
Family Mastacembelidae						
<i>Mastacembelus armatus</i> (Lacepède, 1800)	G5	LC	+	+	+	6
Family Ambassidae						
<i>Parambassis siamensis</i> (Fowler, 1937)	G5	LC	+	+	+	86
Family Tetraodontidae						
<i>Toxotes mekongensis</i> Kottelat & Tan, 2018	G4	LC		+	+	24
Family Pristolepididae						
<i>Pristolepis fasciatus</i> (Bleeker, 1851)	G4	LC		+		1
Family Cichlidae						
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	G11	-	+	+		110
Family Eleotridae						
<i>Oxyeleotris marmorata</i> (Bleeker, 1852)	G5	LC	+			1
Family Gobiidae						
<i>Papuligobius ocellatus</i> (Fowler, 1937)	G5	LC	+		+	15
Family Osphronemidae						
<i>Trichopodus pectoralis</i> Regan, 1910	G6	LC		+		1
Family Soleidae						
<i>Brachirus harmandi</i> (Sauvage, 1878)	G5	LC	+	+	+	16
Family Tetraodontidae						
<i>Pao suvattii</i> (Sontirat & Soonthornsatit, 1985)	G1	LC	+			7
<i>Pao turgidus</i> (Kottelat, 2000)	G1	LC			+	1
Total						1,690

Notes:

G1	Rhithron residents	G2	Main-channel residents/ long-distance white fishes	G3	Main-channel spawners/ short-distance white fishes
G4	Floodplain spawners/ grey fishes	G5	Eurytopic/ generalist fishes	G6	Floodplain residents/ black fishes
G11	Non-Native/ Exotic / Alien Species	EN	Endangered	VU	Vulnerable
NT	Near Threatened	LC	Least Concern	DD	Data Deficient
NE	Not Evaluated	-	None	N	Number of individuals
LEI	Loei	NPM	Nakhon Phanom	UBN	Ubon Ratchathani

Most species recorded in the present study were classified as Least Concern (LC), comprising 65 species. Representative species in this category included *Cyclocheilichthys enoplos*, *Hypsibarbus malcolmi*, *Cosmochilus harmandi*, *Mystacoleucus obtusirostris*, *Raiamas guttatus*, *Acantopsis runghthipae*, and *Pseudolais pleurotaeni*. Only one species was classified as Endangered (EN), namely

Probarbus jullieni, which was recorded in all three provinces. Six species were classified as Vulnerable (VU), including *Tenuialosa thibaudeaui*, *Epalzeorhynchus munensis*, *Hypsibarbus lagleri*, *Scaphognathops bandanensis*, *Tor sinensis*, and *Mystus bocourti*. Two species were classified as Near Threatened (NT), namely *Cirrhinus molitorella* and *Wallago attu*. In addition, five alien species were

recorded in the study area, including *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Neosalanx brevirostris*, and *Oreochromis niloticus*. These species were found across the three surveyed provinces.

Water quality analysis across the Loei, Nakhon Phanom, and Ubon Ratchathani stations during all three seasons (S1–S3) revealed that pH levels remained consistently within the slightly alkaline range. The highest variability in pH was observed at the Ubon Ratchathani station, ranging from 6.76 (S1) to 8.68 (S3). Electrical conductivity (EC) showed a distinct upward trend at all stations during S3, with the maximum value recorded at the Loei station ($167.37 \pm 0.25 \mu\text{S}/\text{cm}$). Regarding dissolved oxygen (DO), the highest concentration found during S2 (10.96–12.73 mg/L), while the lowest concentration was recorded at the Ubon Ratchathani station during S1 ($6.80 \pm 0.27 \text{ mg/L}$). Water temperature fluctuated according to seasonal factors, peaking at the Loei station during S2 ($28.96 \pm 0.81^\circ\text{C}$) and reaching its minimum at the Nakhon Phanom station during S3 ($22.33 \pm 0.06^\circ\text{C}$). Overall, these fluctuations significantly demonstrate the influence of both spatial (station location) and temporal (seasonal) factors on water quality.

DISCUSSION

The study recorded a total of 84 fish species from 21 families in the Mekong River within northeastern Thailand. Species richness was highest in the family *Cyprinidae*, consistent with surveys conducted by the Mekong River Commission (2021), which reported cyprinids as the dominant group, representing 45% of all recorded species. A similar pattern was observed in the Songkhram River Basin, where cyprinids were the most abundant family among all species recorded (Buanuak *et al.*, 2004).

Analysis of species composition indicated that *Barbonymus altus* and *Puntioplites proctozystron* were the most abundant species. These species inhabit main river channels and undertake short-distance migrations for spawning and feeding (Poulsen *et al.*, 2002). They play a central role in shaping fish community structure in the Mekong River and constitute important resources for local fisheries (Baran *et al.*, 2005). In contrast, rarely observed species, such as *Puntioplites waandersi*, *Rasbora myersi*, and *Wallago attu*, likely reflect the influence of environmental factors, including water level, seasonal variation, and habitat structure, on species distribution across different areas (Chan *et*

al., 2020). Spatial analysis indicated that Ubon Ratchathani province exhibited the highest species richness, followed by Nakhon Phanom and Loei. These differences likely reflect variations in topography and habitat complexity. The lower reaches of the river typically encompass a mosaic of habitats, including rapids, sandbanks, and floodplain areas, which provide suitable conditions for a wide range of fish species (Poulsen *et al.*, 2002). Kruskal–Wallis tests revealed no significant differences in fish abundance among the provinces, suggesting that the Mekong River ecosystem in the study area maintains good habitat connectivity, allowing broad dispersal of fish populations (Baran *et al.*, 2006).

Seasonal analysis revealed that species richness was higher during the receding flood season (S2) and low-water season (S3) compared with the peak flood season (S1). This pattern reflects the hydrodynamic regime of the Mekong River, which varies with water levels. This seasonal fluctuation is consistent with the flood-pulse concept, which suggests that the predictable advance and retreat of water over the floodplain drive biological productivity and the movement of organisms (Junk *et al.*, 1989). As floodwaters recede, floodplain areas gradually dry, prompting many fish species to move back into the main river channels, resulting in increased density and higher observed species richness (Poulsen *et al.*, 2002). Conversely, during the flood season, fish disperse extensively into inundated floodplain areas, reducing detectability in the main channels (Baran, 2006). This phenomenon underscores the importance of lateral connectivity, where the unimpeded exchange of nutrients and organisms between the river channel and its floodplain is crucial for maintaining biodiversity (Ward and Stanford, 1995).

The diversity index (H') ranged from 1.90 to 2.54, indicating that fish communities exhibited moderate species diversity. Evenness index (J') ranged from 0.56 to 0.88, reflecting a moderate balance in species distribution across the study sites. During the low water period (S3), evenness was higher, indicating a more equitable distribution of individuals among species. Dominance index (D) ranged from 0.1 to 0.2 during the same period, demonstrating that no single species dominated the community (Magurran, 2004). The relatively high evenness during S3 may be attributed to the concentration of diverse taxa within the main-channel refugia, where stable low-flow conditions support a heterogeneous community structure before the next flood cycle begins.

Classification of ecological functional groups indicated that most species belonged to the main-channel spawners, or short-distance white fishes (G3). This pattern reflects the characteristics of the Mekong River as a large river system, where many fish undertake migrations for spawning and feeding (Mekong River Commission, 2019). From the perspective of fish migration ecology, these white fish species are highly sensitive to changes in hydrological cues and longitudinal connectivity (Winemiller and Jepsen, 1998). Species in this group play a key role in sustaining fisheries, as the life cycles of many species depend on movements between the main channel and adjacent floodplain areas (Mekong River Commission, 2023).

In terms of conservation, the Endangered species *Probarbus jullieni* was recorded in Loei, Nakhon Phanom, and Ubon Ratchathani provinces. This large migratory species undertakes long-distance movements along the main channel of the Mekong River and is of considerable economic importance. Spawning occurs during the dry season, from December to February (Poulsen *et al.*, 2004). Populations have declined sharply across the Mekong Basin due to overfishing, dam construction, and habitat degradation (Chevalier *et al.*, 2023; Sor *et al.*, 2023). To support conservation efforts, the Department of Fisheries has implemented hatchery propagation and restocking programs in several areas along the Mekong River. However, these measures alone may be insufficient to ensure long-term population recovery without basin-scale management. Effective strategies should include the protection of key spawning grounds and migration corridors, the implementation of seasonal fishing closures, the regulation of fishing gear to reduce juvenile capture, and the restoration of riparian and floodplain habitats that function as nursery areas. In addition, community participation and co-management are essential for enhancing the effectiveness of conservation measures and promoting sustainable fisheries management (Coates, 2001).

Another notable finding was the presence of five alien fish species, including *Oreochromis niloticus* and *Cyprinus carpio*, which were introduced for aquaculture or fisheries purposes. These species are classified as noninvasive alien species (Sahiranwong, 2020). Nevertheless, the establishment of alien species in the Mekong River ecosystem may impact native fishes through competition for food and habitat and could potentially drive long-term changes in fish community structure (Strayer, 2010).

The results of this study demonstrate that spatial and temporal variation in water quality plays an important role in shaping species richness and fish assemblage structure within the Mekong River system. During the receding flood season (S2), higher dissolved oxygen (DO) levels and favorable temperatures are likely to enhance biological activity and promote a greater concentration of fish within the main channel, resulting in increased species richness.

This pattern is consistent with the flood pulse concept proposed by Wolfgang Junk, which suggests that, as water levels decline, fish move from floodplain habitats back into the main channel, accompanied by a transfer of resources (Junk *et al.*, 1989). In contrast, the peak flood season (S1) is associated with lower DO levels in certain areas, which may limit oxygen availability. Under such conditions, some species may avoid or disperse from the main channel, leading to lower observed species richness despite the expansion of available habitat (Baran *et al.*, 2001).

Elevated EC during the low water season (S3) may indicate higher dissolved ion concentrations, potentially favor tolerant species and subtly alter fish assemblages. Nevertheless, EC likely plays a secondary, site-specific role rather than acting as a primary driver of community structure. In contrast, relatively stable pH conditions suggest limited direct constraint on fish communities, although spatial variability in pH may still reflect underlying habitat heterogeneity (Nuon *et al.*, 2025).

In this study, the widespread distribution of the Cyprinidae family, particularly *Barbonymus altus*, across all stations and seasons highlights the role of generalist species in tolerating low dissolved oxygen (DO) during peak flood season (Ngor *et al.*, 2023). This pattern indicates adaptive capacity under seasonal hydrological stress. The findings further emphasize river connectivity and water quality dynamics as key drivers of fish diversity and community composition in the Mekong River ecosystem.

The use of trawl nets alone may not fully capture the complete fish species diversity in the Mekong River. Therefore, future studies should employ multiple sampling methods to obtain a more comprehensive understanding of fish community composition. The findings of this study provide essential baseline information for supporting sustainable fisheries management and the conservation of fish resources in the face of increasing pressures on the Mekong River ecosystem.

CONCLUSIONS

This study shows that the Mekong River in northeastern Thailand continues to support a high diversity of freshwater fishes, with 84 species representing 61 genera and 21 families. The family Cyprinidae exhibited the highest species richness. Dominant species, such as *Barbonymus altus* and *Puntioptilites proctozystron*, underscore both the ecological importance and fisheries value of the river in the region. Fish diversity varied across spatial and seasonal scales. The highest diversity was observed during the receding flood season (S2) and low-water season (S3), reflecting hydrological fluctuations and migratory patterns. Although most species are classified as least concern, the presence of the endangered species *Probarbus jullieni* and several non-native species indicates potential ecological risks. These findings highlight the importance of continuous monitoring of fish diversity and community structure to inform sustainable fisheries management and the conservation of freshwater biodiversity in the Mekong River.

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Case Study: Assessment of Microbial Quantification of Butchered Pork in Fresh Markets and Fresh Pork Shops in Nakhon Pathom, Thailand Activities

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ABSTRACT

In Thailand, finishing pigs are delivered to standard slaughterhouses after reaching market weight for slaughtering and carcass trimming, and are then delivered to fresh markets for consumers. For consumer safety, hygiene, and sanitation in fresh markets, measures should be taken to protect customer health, prevent unexpected foodborne diseases, and minimize economic losses. The objective of this study was to quantify the levels of microorganisms in pork chops sold at fresh markets and temperature-controlled shops in Nakhon Pathom province, Thailand. The results can be applied as guidelines for improving hygiene and sanitation in pork shops. We collected 5 samples from 2 fresh markets and 5 samples from temperature-controlled pork shops. Each sample had duplicate. The quantified microorganisms were as follows: total aerobic bacteria, coliforms, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. Unsurprisingly, some samples collected from fresh markets contained microorganisms exceeding the standard values. Similarly, certain samples from temperature-controlled pork shops also exceeded the standard. Therefore, the storage temperature at temperature-controlled pork shops has to be monitored and consistently maintained. Although cooking generally destroys most microorganisms in raw pork chops. However, one sample collected from fresh markets contained *Staph. aureus* 400 MPN/gram, can grow and produce enterotoxin at fresh markets. Enterotoxin is heat-stable, required only a low dose to cause illness, and resistant to typical cooking temperatures. We recommend strict control of enterotoxin production, *Staph. aureus* source, personal hygiene. Since temperature is considered, the main extrinsic factor influencing the growth of microorganisms in foods, The installation of temperature-controlled systems in fresh markets is one method to inhibit *Staph. aureus* growth. Due to humans as a *Staph. aureus* source, food handlers should be qualified as non-*Staph. aureus* carriers. Furthermore, because the human nasal cavity is a frequent habitat for *Staph. aureus*, food handlers must adhere to Standard Operating Procedures (SOPs), including washing hands frequently, not picking and blowing their noses.

Keywords: ACFS 6000-2004, fresh market, microbial contamination, pork retail establishments

INTRODUCTION

Most pork entrepreneur, therefore, focus on the production process, have surveillance, and maintain safety from upstream to downstream (Office of Agricultural Economics, 2022). Although there is farm biosecurity, hygiene at carcasses-trimming areas in the markets needs to be corrected. In other words, hygiene at carcass-trimming areas in the markets is improper. The good pork trimmings start from receiving live pigs for slaughter and verifying related documents. Pigs must arrive at slaughterhouses at least 2 hours before slaughter, and must not fast for more than 12 consecutive hours

(Thai Agricultural Standard 9004, 2024). Then pigs are then stunned by electric shock, cut at the main artery, and hung for 3–5 minutes to drain blood. To open pores at pork skin and facilitate hair removal, carcasses are scalded and dehaired using hot water at 60–65 °C. They are subsequently singed to eliminate remaining down and eviscerated for organ removal. After slaughter, lesions and tissue are examined. Finally, carcasses are washed and stored at 0–4 °C. During cutting and packing of pork pieces, pork must have a temperature lower than 7 °C. Also, pork must be delivered to temperature-controlled pork shops by temperature-controlled trucks. Another method of selling pork is not cutting and packing of pork pieces.

However, warm carcasses are delivered and trimmed at fresh markets.

Food safety is one of considered issues of consumers and includes the absence of microorganisms in food. Foodborne diseases cannot only be fatal, but they can also cause large economic losses. Since microorganisms are tiny, traditional pour plate or spread plate methods are alternative ways to observe living microorganisms. The advantage of spread plate method is to avoid the thermal shock to psychrophiles. Compared to spread plate method, the Most Probable Number (MPN) method is suitable for enumerating low numbers of viable microorganisms in food samples. The MPN method consists of 3, 4, or 5 inoculating replicate tubes of an appropriate liquid medium with each set prepared using three different sample dilutions. The MPN results are obtained by referring to the MPN table. Under specific circumstances, coliforms, including most strains of *Escherichia coli*, serve as indicators of fecal contamination and sanitation. In contrast, most *Salmonella* strains are recognized as human pathogens and represent a major concern in food safety. *Salmonellas* are now established as one of the most important causes of foodborne illness worldwide (Adams and Moss, 2008). Acute diarrheal disease remains a major public health issue. *Salmonella* spp. infection is one of the leading causes of acute diarrheal disease despite the preventive measures implemented (Popa and Papa, 2021).

In Thailand, *Salmonella* is a major cause of gastroenteritis in pig-based products, and we still lack information regarding sources of *Salmonella* contamination sources (Saenkankam et al., 2025). For example, a 4 years old Japanese man developed enteritis caused by *Salmonella* serovar Kedougou without bacteremia after returning from Chiang Mai, Thailand (Okumura et al., 2025). Due to *Staph. aureus* enterotoxin production is relatively mild and short-lived type of illness, staphylococcal food poisoning is perhaps more likely to be under-reported than others. However, *Staph. aureus* still causes foodborne outbreaks and cross-contamination in Thailand. As reported, one survey found *Staph. aureus* contamination in 110 pork samples from 12 fresh meat markets in Nongchok District, Bangkok, Thailand (Kanungpean et al., 2021).

The number of registered and operating pig slaughterhouses in Thailand is approximately 1,546. Most of them are located in Central Thailand. Nakhon Pathom Province has 17 licensed pig slaughterhouses and pork processing plants in several districts such as Mueang Nakhon Pathom, Kamphaeng Saen, Bang Len, and Sam Phran

(Nakhon Pathom Provincial Statistical Office, 2025). For pork production in Nakhon Pathom Province, living pigs from farms meeting Good Agricultural Practices (GAP) standards are delivered to slaughterhouses. Finally, carcasses are cut in half and trimming in Good Manufacturing Practice (GMP) certified cutting factories. Pork is sold at temperature-controlled pork shops.

In the metropolitan area, Nakhon Pathom Province is considered the largest pork market in the Central and Western regions (Nakhon Pathom Provincial Office of Agriculture and Cooperatives, 2019). Nakhon Pathom Province has certified under Good Manufacturing Practices (GMP) pig slaughterhouses, cutting plants, and producers selling pork at large fresh markets such as Thung Phra Meru Market and Sriwichai Market. Although many fresh markets are located in Nakhon Pathom Province, there is a lack of hygienic data on pork sold at these markets.

The objective of this study was to quantify microorganism amounts in pork chops sold in fresh markets and temperature-controlled pork shops in Nakhon Pathom Province, Thailand. The results in this study show that hygiene and sanitation at fresh markets and temperature-controlled pork shops had to be corrected. Particular concern is given to *Staph. aureus*, as it produces heat-resistant enterotoxins. Chill temperature, qualified food handlers as non-*Staph. aureus* carriers, and good personal hygiene are the *Staph. aureus* controlling methods in pork chops.

MATERIALS AND METHODS

This study is quantitative data research. The data will be used to develop guidelines for good hygiene practices for food handlers at both fresh markets and temperature-controlled pork shops, with the ultimate goal of enhancing food safety standards.

Pork samples at Thung Phra Meru Market and Sriwichai Market

Duplicate pork samples were collected from five fresh pork shops located at Thung Phra Meru Market and Sriwichai Market between 07:00 and 13:00 (Figure 1). Each sample consisted of 300 g of pork sirloin. All samples were aseptically packed into sterile, sealed plastic bags and transported under refrigerated conditions ($\leq 4^{\circ}\text{C}$), as summarized in Table 1. Microbiological quality analyses were performed, and results were evaluated in accordance with the National Bureau of Agricultural Commodity and Food Standards 6000-2004.

Pork samples collected from temperature-controlled pork shops at Mueang Nakhon Pathom District

Pork samples were also collected from five temperature-controlled pork shops in Mueang Nakhon Pathom District during the same sampling period (07:00–13:00) (Figure 2). Each sample consisted of 300 g of pork sirloin and was collected

in duplicate. Samples were aseptically packaged in sterile, sealed plastic bags and transported at temperatures not exceeding 4 °C (Table 1). Microbiological analyses were conducted, and the results were assessed according to the National Bureau of Agricultural Commodity and Food Standards 6000-2004.



Figure 1. Pork chops sold at Thung Phra Meru Market (a) and Sriwichai Market (b).



Figure 2. Pork chops sold at temperature-controlled pork shops in Mueang Nakhon Pathom District

Table 1. Sources of pork chops collected from fresh markets and temperature-controlled pork shops.

The type of selling pork chops	Sources of selling pork chops	Sample code
Fresh markets	Thung Phra Meru Market (L-1)	L-1-1
		L-1-2
	Thung Phra Meru Market (L-2)	L-2-1
		L-2-2
	Thung Phra Meru Market (L-3)	L-3-1
		L-3-2
	Sriwichai Market (L-4)	L-4-1
		L-4-2
	Sriwichai Market (L-5)	L-5-1
		L-5-2
Temperature-controlled pork shops	LG shop (S-1)	S-1-1
		S-1-2
	BC shop (S-2)	S-2-1
		S-2-2
	BG shop (S-3)	S-3-1
		S-3-2
	CM shop (S-4)	S-4-1
		S-4-2
	SM shop (S-5)	S-5-1
		S-5-2

The tools used in this study

All samples were carried out by the Veterinary Research and Development Center (Western Region), Department of Livestock Development, Ministry of Agriculture and Cooperatives. Each sample was analyzed in duplicate for Total aerobic bacteria, coliforms, *E. coli*, *Salmonella spp.*, and *Staph. aureus* were quantified using AOAC (2000) Official Method 966.23C, AOAC (2000) Official Method 966.24, AOAC (2000) Official Method 967.26, and AOAC (2000) Official Method 975.55, as summarized in Table 2. The means were reported and compared with the National Bureau of Agricultural Commodity and Food Standards 6000-2004.

Statistical analysis

All data were analyzed for One-sample t-test to determine whether the observed mean values of microbial counts significantly deviated from the established limits set by the National Bureau of Agricultural Commodity and Food Standards (TAS 6000-2004). Statistical significance was evaluated at a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Fresh markets selling pork and temperature-controlled pork shops are regularly monitored and are surveillance from government agencies. In order to improve good pig carcass management in fresh markets and temperature-controlled pork shops in Nakhon Pathom Province, we required microorganism analysis of pork samples. Food safety means not only slaughterhouse standards but also pork sold at both fresh pork shops and temperature-controlled pork shops. According to National Bureau of Agricultural Commodity and Food Standards 6000-2004, microorganism analysis includes total aerobic bacteria, coliforms, *E. coli*, *Salmonella spp.*, and *Staph. aureus*. Table 2 presents the microorganism amounts detected in all pork samples and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives).

Table 2. The average of microorganism amounts found in pork chops collected from fresh markets and temperature-controlled pork shops

Microorganism	The quantified microorganism amounts		Standard ¹
	Fresh markets	Temperature-controlled pork shops	TACFS. 6000-2004
Total aerobic bacteria	6.10x10 ⁵ (2/5) ²	4.13x10 ⁴ (0/5)	≤5x10 ⁵ cfu/gram
Coliforms	9.50x10 ³ (3/5)	2.77x10 ² (0/5)	≤5x10 ³ MPN/ gram
<i>E. coli</i>	8.52x10 ³ (3/5)	6.10x10 ¹ (1/5)	≤10 ² cfu/ gram
<i>Salmonella</i> spp.	All detected	detected	Undetected in 25 grams of sample
<i>Staph. aureus</i>	55 (1/5)	10 (0/5)	≤10 ² MPN/ gram

¹Reference: Department of Livestock Development, Ministry of Agriculture and Cooperatives. 2008.

²The numbers in parenthesis show the numbers of pork chops having microorganisms exceeded the standard.

Total aerobic bacteria

Based on Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives), total aerobic bacteria should not be than 5x10⁵ cfu/gram. Here, the findings show that two out of five pork samples collected from fresh pork shops at Thung Phra Meru Market and Sriwichai Market had total aerobic bacteria exceed than Livestock and Livestock Products Standards. Total aerobic bacterial amounts in the two samples were 1.1x10⁶ and 3.1x10⁶ cfu/gram. On the other hand, all samples collected from temperature-controlled pork shops at Mueang Nakhon Pathom District had total aerobic bacteria less than Livestock and Livestock Products Standards.

Coliforms

According to Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives), coliforms in pork sample should be less than 5x10³ MPN/gram. Three out of five pork samples collected from fresh pork shops at Thung Phra Meru Market and Sriwichai Market had coliforms exceed than 5x10³ MPN/gram. Coliforms in the three samples were 9.2x10³, 1.5x10⁴, and 7x10⁴ MPN/gram. Unlike pork samples collected from fresh pork shops at Thung Phra Meru Market and Sriwichai Market, none sample gathering from temperature-controlled pork shops at Mueang Nakhon Pathom District had coliforms exceed than 5x10³ MPN/gram.

E. coli

Pork should match with Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives), so pork chops should have *E. coli* less than 100 cfu/gram. Three out of five pork samples collected from pork fresh shops at Thung Phra Meru Market and Sriwichai Market had *E. coli* less than 100 cfu/gram. *E. coli* in three pork chops were 1.3x10², 1.5x10⁴, 7x10⁴ cfu/gram. Surprisingly, one out of five pork samples collected from temperature-controlled pork shops at Mueang Nakhon Pathom District having *E. coli* exceed than 100 cfu/gram. The number of *E. coli* in pork samples collected from temperature-controlled pork shops at Mueang Nakhon Pathom District was 4.6x10¹ cfu/gram.

Salmonella spp.

Unlike other Livestock and Livestock Products Standards, *Salmonella* spp. should not be found in 25-gram sample. The results demonstrate that the numbers of 25-gram samples collected from fresh pork shops in Thung Phra Meru Market and Sriwichai Market and temperature-controlled pork shops in Mueang Nakhon Pathom District having *Salmonella* spp. were five and three, respectively. In other words, the percentages of pork samples collected from fresh pork shops in Thung Phra Meru Market and Sriwichai Market and temperature-controlled pork shops in Mueang Nakhon Pathom District having *Salmonella* spp. were 100 and 60, respectively.

Staph. aureus

According to Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives), pork should have *Staph. aureus* less than 100 MPN/gram. Although the mean MPN/gram of *Staph. aureus* found in five pork samples collected from fresh pork shops in Thung Phra Meru Market and Sriwichai Market was 55, the MPN/gram of *Staph. aureus* in one pork sample was 400. In contrast, none pork sample collected from temperature-controlled pork shops in Mueang Nakhon Pathom District had *Staph. aureus* exceed than 100 MPN/gram.

In this study, we quantified microorganism amounts of total aerobic bacteria, coliforms, *E. coli*, *Salmonella* spp., and *Staph. aureus* for improving hygiene of pork-selling shops and food handlers to match Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives). Generally, poor hygiene is originated from dust, insects, dirt, storage temperature abuse, and cross-contamination such as knives and cutting boards. Pork selling shops should be monitored microbial inspection regularly. If pork selling shops have cleaning systems, hygiene controlling systems according to standards, cold rooms with proper temperature control, clearly separated areas, the shops can certify and comply selling pork with food safety standards.

In this study, we compared microorganism amounts between pork samples collected from Thung Phra Meru Market and Sriwichai Market with temperature-controlled pork shops located in Mueang Nakhon Pathom District. The results clearly show that pork sold at Thung Phra Meru Market and Sriwichai Market had higher food safety risks, compared to pork sold in temperature-controlled pork shops in Mueang Nakhon Pathom District. Many pork chop samples show the numbers of microorganisms including total aerobic bacteria, coliforms, *E. coli*, *Salmonella* spp., and *Staph. aureus* exceeding than Livestock and Livestock Products Standards (Department of Livestock Development, Ministry of Agriculture and Cooperatives). Therefore, it is necessary to improve hygiene of the involved personal hygiene, trimming areas, equipment used for trimming such as knives and cutting boards, transportation, storage temperature in fresh markets to meet standards similar to certified slaughterhouses to enhance food safety, reduce the risks of foodborne diseases, and protect consumer health. Pork shops should meet standards, have

strictly control system in order to get food safety and high-quality pork (Sangthongpinij, 2022).

Temperature is the primary extrinsic factor affecting the growth and survival of microorganisms in foods, and most pathogens multiply rapidly between 4 and 60 °C (Erkmen *et al.*, 2014). Temperature-controlled system requires higher cost, so fresh markets do not have this system in order to sell inexpensive pork chops. Based on the survey of microorganism contamination in pork sold at fresh market and temperature-controlled pork shops in Nakhon Pathom Province, most pork samples collected from fresh markets had microorganism contamination exceed than Livestock and Livestock Products Standards, compared to pork sold in temperature-controlled pork shops. Unsurprisingly, the normal temperature of Thailand is 27 °C due to one of tropical countries. The optimal temperature of these microorganisms is 30-40 °C, so they grow quickly at fresh markets.

Generally, coliforms are recognized as the indicator of sanitation. According to coliforms survey, fresh markets and temperature-controlled pork shops needed to improve personal hygiene and sanitation. Also, storage temperature at both places had to be corrected. Most salmonellas are regarded as human pathogens and are one of the most important causes of foodborne illness worldwide. In addition to foodborne pathogens, some strains are originated from pigs such as *S. enterica*. *Salmonella* spp. can grow from temperatures just above 5.1 up to 47.1 °C. The results show that most temperature-controlled pork shops at Mueang Nakhon Pathom District did not chill enough to control *Salmonella* spp. growth. Finally, the principal habitat of the *staphylococci* is the skin, skin glands and the mucous membranes of warm-blooded animals, for example pigs. Also, about 30–50% of healthy people carry out *Staph. aureus* in their nose and nasal cavity (Erkmen and Bozoglu, 2016). Although *Staph. aureus* associated-outbreaks were less than *Salmonella* spp., *Staph. aureus* requiring only 4 hours at warm temperature produces heat-stable enterotoxin, and only 10 ng toxin per gram of contaminated food is sufficient to cause symptom. (Seo and Bohach, 2014) (Adams, A.R. and Moss, M.O., 2008) In addition to low dose of heat-stable enterotoxin production, normal cooking temperature of foods cannot destroy Staphylococcal enterotoxin (Erkmen and Bozoglu, 2016).

Based on this study, the following recommendations are proposed as guidelines for managing pork carcass fabrication in fresh markets and temperature-controlled pork shops:

1.) Good sanitation plays a critical role in eliminating microbial reservoirs and preventing cross-contamination throughout the supply chain. Aligning with Thailand's Food Management Strategy (National Food Committee, 2017), retailers must prioritize consumer safety by implementing rigorous cleaning protocols for equipment and food-contact surfaces. Furthermore, establishing adequate infrastructure for hand and tool washing, alongside systematic waste management, is essential for maintaining the microbiological quality of pork products.

2.) Personal hygiene and food handlers are a primary vector for pathogens including *Staphy. aureus* (Erkmen, and Bozoglu, 2016). stringent personal hygiene is imperative. Handlers should undergo mandatory annual physical examinations to ensure they are not asymptomatic carriers. Additionally, the consistent use of protective gear and the avoidance of high-risk behaviors, such as unprotected coughing or wearing jewelry during operation, must be strictly enforced.

3.) Chill storage refers to the preservation of food, particularly fresh pork, at temperatures between 0–5 °C (Adams and Moss, 2008). Traditional fresh markets in Thailand generally lack the infrastructure required for temperature maintenance. To sustain temperatures below 5 °C, these markets must install temperature-control systems. Furthermore, temperature-controlled pork retail shops should strictly monitor and calibrate their storage temperatures, as microbial loads in some samples were found to exceed established safety standards.

4.) Standard Operating Procedures (SOPs) and Sanitation Standard Operating Procedures (SSOPs) should be formalized to ensure continuous sanitary conditions. These protocols must encompass comprehensive hand-washing techniques, the use of complete protective attire (hairnets, masks, and disinfected boots), and the systematic cleaning and storage of equipment after each use to mitigate contamination risks. (Adams and Moss, 2008).

5.) Hazard Analysis and Critical Control Points (HACCP) is a systematic approach designed to ensure food safety. By identifying potential hazards during production and distribution, establishing preventive parameters, and validating corrective actions, stakeholders can manage safety risks more effectively than through end-product testing alone (Buchanan and Williams, 2014).

Conclusions

The microorganism amounts in pork chops sold at fresh markets and temperature-controlled pork shops have to be monitored regularly since some samples did not match with the standard. Due to warm temperature at fresh markets in Thailand, microorganisms can grow rapidly and some of them are foodborne pathogens. We concerned *Staph. aureus* since it can produce heat-stable enterotoxin and healthy human can be the *Staph. aureus* carriers. We suggest to install the temperature-controlled system at fresh markets. Food handlers should be qualified as non- *Staph. aureus* carriers, have annual physical exam, and follow Standard Operating Procedure (SOP) such as wash hands frequently, not pick and blow their noses.

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Comparison of Plant Propagation Portions and the Effectiveness of Azolla with Chemical Fertilizer on the Growth of *Episcia cupreata*

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ABSTRACT

This research aimed to compare plant propagation portions and the efficiency of Azolla on growth and development of *Episcia cupreata*. This research was conducted using a factorial design in a CRD with of 2 factors: 1) plant propagation portions: mother plant and stolon, 2) nutrient source: Osmocote (chemical fertilizer; 13-13-13) ratio 6 g per plant, dried Azolla (biofertilizer) at 30 g per 1 kg of medium; Osmocote 6 g and dried Azolla 30 g), and no-fertilizer (control treatment). The results showed that both plant propagation portions, the mother plant and the stolon, were not significantly different in canopy width, plant height, number of leaves per plant, number of stolons per plant, number of flowers per plant, flower width, and tube length. This indicated that both plant propagation portions can use as plant propagation material. Factor of nutrients source, it was found that the Osmocote affected canopy width (33.37 cm), plant height (6.50 cm), number of leave per plant (87.0 leaves), number of stolons per plant (16.17 stolon), number of flowers per plant (5.25 flowers), flower width (1.93 cm), and tube length (2.34 cm) which is significantly greater than the other treatments. However, this treatment was not significantly different from canopy width, number of leaves per plant, flower width, and tube length when compared to the treatment that use Osmocote + Azolla. The interaction between two factors, stolon with Osmocote treatment or stolon with Osmocote + Azolla that affected canopy width, number of leaves per plant, number of stolons per plant, flower width, and tube length better than other treatments. Further studies is recommended to optimize the ratio of Azolla combined with Osmocote fertilizer in order to reduce the use of chemical fertilizer in *Episcia cupreata* production in the future.

Keywords: Flame Violet; Azolla; osmocote; chemical fertilizer; biofertilizer

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบส่วนขยายพันธุ์และประสิทธิภาพของแหนแดงต่อการเจริญเติบโตของพรมกำมะหยี่ วางแผนการทดลองแบบปัจจัยร่วมในสุ่มสมบูรณ์ ประกอบด้วย 2 ปัจจัย 1) ส่วนขยายพันธุ์พืช: ลำต้นแม่ และไหล 2) แหล่งธาตุอาหาร :ปุ๋ยออสโมคอต (ปุ๋ยเคมี) สูตร 13-13-13 อัตรา 6 กรัม แหนแดงแห้ง (ปุ๋ยชีวภาพ) 30 กรัมต่อวัสดุปลูก 1 กิโลกรัม ปุ๋ยออสโมคอต+แหนแดงแห้ง (ปุ๋ยออสโมคอต 6 กรัม และแหนแดงแห้ง 30 กรัม) และไม่ใส่ปุ๋ย (กรรมวิธีควบคุม) ผลการทดลอง พบว่า ส่วนขยายพันธุ์ทั้งลำต้นแม่ และไหลไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในด้านความกว้างทรงพุ่ม ความสูงต้น จำนวนใบต่อต้น จำนวนต้นไหลต่อต้น จำนวนดอกต่อต้น ความกว้างดอก และความยาวหลอดดอก ซึ่งชี้ให้เห็นว่าทั้งสองส่วนสามารถใช้เป็นส่วนขยายพันธุ์ได้ดี ปัจจัยแหล่งธาตุอาหารพบว่า ปุ๋ยออสโมคอต ส่งผลให้ความกว้างทรงพุ่ม (33.37 เซนติเมตร) ความสูงต้น (6.50 เซนติเมตร) จำนวนใบต่อต้น (87.0 ใบ) จำนวนไหล (16.17 ไหล) จำนวนดอกต่อต้น (5.25 ดอก) ความกว้างดอก (1.93 เซนติเมตร) ความยาวหลอดดอก (2.34 เซนติเมตร) มากกว่ากรรมวิธีอื่นๆอย่างมีนัยสำคัญ แต่อย่างไรก็ตามกรรมวิธีนี้ไม่มีความแตกต่างอย่างมีนัยสำคัญในด้านความกว้างทรงพุ่ม จำนวนใบต่อต้น ความกว้างดอก และความยาวหลอดดอกเมื่อเปรียบเทียบกับกรรมวิธีที่ใช้ปุ๋ยออสโมคอตร่วมกับแหนแดง ผลของปฏิสัมพันธ์ระหว่างสองปัจจัย พบว่า กรรมวิธีที่ใช้ไหลและให้ปุ๋ยออสโมคอต หรือไหลและให้ปุ๋ยออสโมคอต+แหนแดง ส่งผลให้มีความกว้างทรงพุ่ม จำนวนใบต่อต้น จำนวนไหลต่อต้น ความกว้างดอก และความยาวหลอดดอกมีการเจริญเติบโตที่ดีเมื่อเปรียบเทียบกับกรรมวิธีอื่นๆ และอาจมีการศึกษาทดลองการปรับสัดส่วนของแหนแดงร่วมกับปุ๋ยออสโมคอตให้มีความเหมาะสมยิ่งขึ้นเพื่อลดปริมาณการใช้ปุ๋ยเคมีในการผลิตพรมกำมะหยี่ในอนาคต

คำสำคัญ: พรมญี่ปุ่น; แหนแดง; ปุ๋ยออสโมคอต; ปุ๋ยเคมี; ปุ๋ยชีวภาพ

บทนำ

พรมก้ามหยา หรือพรมญี่ปุ่น (*Episcia cupreata* (Hook.) Hanst. จัดอยู่ในวงศ์ Gesneriaceae เป็นไม้ประดับล้มลุก เจริญขึ้น (Li et al., 2025) มีอายุหลายปี ลำต้นเดี่ยวทอดยาวไปตาม แนวระนาบกับพื้น และสามารถสร้างส่วนขยายพันธุ์ที่เรียกว่าไหล แตกออกจากต้นแม่ได้ การขยายพันธุ์มักใช้ลำต้นแม่และไหลในการ ปักชำแต่ยังไม่มีข้อมูลที่แน่ชัดว่าส่วนใดที่มีศักยภาพในการ เจริญเติบโตที่ดีกว่า ซึ่งความพิเศษของพรมก้ามหยา คือ ใบที่มีสีส้ม และสลดลายที่หลากหลายสะดุดตา ใบเป็นใบเดี่ยว เรียงสลับตรงกัน ข้าม รูปไข่ ปลายใบแหลม ขอบหยัก ผิวใบหนาย่น คล้ายพรม ก้ามหยา อีกทั้งดอกยังมีขนาดกะทัดรัด เป็นดอกเดี่ยว มีกลีบเลี้ยง บริเวณโคนดอกเชื่อมติดกันเป็นหลอดรูปแตร และปลายดอกจะแยก ออกเป็น 5 แฉก (เกษตรดิจิทัล, 2564; ชัยพรและคณะ, 2563) และมีสีส้มสวยงามขึ้นกับสายพันธุ์ เช่น พรมก้ามหยา *Episcia cupreata* (Hook.) Hanst. 'Frosty' จะมีแผ่นใบสีขาวอมเทา ขอบ ใบสีเขียวอ่อน ดอกมีสีแดง ภายในหลอดสีเหลือง พรมก้ามหยา *Episcia cupreata* (Hook.) Hanst. 'Silver Skies' มีแผ่นใบมีสี เขียวอมเทา ขอบใบสีเขียวเข้ม โคนใบสีม่วง ดอกมีสีแดงอมส้ม ภายในหลอดสีเหลือง และ *Episcia cupreata* Easterbrook 'Cleopatra' แผ่นใบตรงกลางมีสีเขียวอ่อน มีรูปทรงคล้ายใบโอ๊ค ล้อมรอบด้วยสีขาวบางๆ ขอบใบมีสีชมพูอ่อน ดอกมีสีแดงสดและมี ขน เป็นต้น (เกษตรดิจิทัล, 2564; Natural parks, 2022) เนื่องจาก ความสวยงามของใบและดอกของพรมก้ามหยา จึงนิยมผลิตเป็นไม้ กระถางประดับตกแต่งภายในอาคาร เพราะสามารถอยู่รอดได้ใน สภาพแสงน้อย (Harbaugh et al., 1981) รวมถึงปลูกในลักษณะไม้ กระถางแขวนด้วย สามารถสร้างบรรยากาศภายในให้รู้สึกสดชื่น สบายตา เต็มเต็มความต้องการทางจิตวิทยา เสริมสร้าง สภาพแวดล้อมภายในบ้าน (Chen et al., 2005) และที่สำคัญยังสามารถบำบัดฝุ่น PM 2.5 ได้ดีอีกด้วย (เทคโนโลยีชาวบ้าน, 2564) การปลูกพรมก้ามหยาโดยทั่วไปจะปลูกในวัสดุปลูกที่ระบายน้ำและ เก็บความชื้นได้ดี ไม่ชอบสภาพน้ำแฉะ แสงแดดรำไร (บ้านและสวน, 2567) การจัดการธาตุอาหารควรมีการใส่ปุ๋ยอย่างเพียงพอ เนื่องจาก มีการออกดอกอยู่เสมอ ในปัจจุบันการผลิตพรมก้ามหยาใน ประเทศไทย มักใช้ปุ๋ยละลายช้าเป็นแหล่งธาตุอาหารเพื่อส่งเสริมการ เจริญเติบโตของใบและดอกของพรมก้ามหยา (ชัยพร และคณะ, 2563) หรือที่เรียกว่าปุ๋ยออสโมโค้ท ออสโมโค้ทเป็นปุ๋ยที่ ประกอบด้วยธาตุอาหารที่จำเป็นต่อการเจริญเติบโตของพืช มี คุณสมบัติเฉพาะคือ จะค่อยๆปลดปล่อยธาตุอาหารออกมา โดยมี น้ำซึมผ่านชั้นเคลือบเรซินที่ห่อหุ้มเม็ดปุ๋ยเพื่อละลายธาตุอาหารที่อยู่ ภายใน จากนั้นธาตุอาหารจะค่อยๆ ซึมผ่านชั้นเคลือบออกมาด้วย

กระบวนการออสโมซิสที่ละน้อย จึงทำให้พืชได้รับธาตุอาหารอย่าง ต่อเนื่อง สม่าเสมอ และไม่เป็นอันตรายต่อรากพืช (โชติส, ม.ป.ป.) จึง เป็นที่นิยมในการผลิตไม้ดอกไม้ประดับกระถาง แต่อย่างไรก็ตามปุ๋ย ออสโมโค้ทที่มีราคาค่อนข้างสูง

แทนแดง (*Azolla* spp.) นับเป็นปุ๋ยชีวภาพทางเลือกชนิด หนึ่งที่ย่อยสลายและปลดปล่อยธาตุอาหารได้เร็ว โดยเฉพาะธาตุ ไนโตรเจน เนื่องจากโพรไบของแทนแดงมีสาหร่ายสีเขียวแกมน้ำเงิน (Cyanobacteria) อาศัยอยู่ ซึ่งเป็นแบคทีเรียที่สามารถตรึงไนโตรเจน จากอากาศได้ (สำนักงานเกษตรและสหกรณ์ จังหวัดชุมพร, 2567) โดยแทนแดง (แห้ง) มีองค์ประกอบทางเคมีที่สำคัญ ได้แก่ ปริมาณ ไนโตรเจนทั้งหมด เท่ากับ 4.58% ปริมาณฟอสฟอรัสทั้งหมด เท่ากับ 0.64% ปริมาณโพแทสเซียมทั้งหมด เท่ากับ 5.08% ธาตุอาหารรอง ได้แก่ ปริมาณแคลเซียมทั้งหมด เท่ากับ 2.59% ปริมาณแมกนีเซียม ทั้งหมด เท่ากับ 0.39% (ศิริลักษณ์ และคณะ, 2563) เห็นได้ว่าแทน แแดงนอกจากจะเป็นแหล่งธาตุไนโตรเจนที่สำคัญแล้ว ยังเป็นแหล่ง ฟอสฟอรัส โพแทสเซียม และอื่นๆที่สำคัญ ที่ใช้เป็นแหล่งธาตุอาหาร แก่ไม้ดอกไม้ประดับได้อย่างดี จากการทดลองของศศิกันต์ และ ชูตินันท์ (2566) ได้ทำการศึกษาผลของวัสดุปลูกที่มีส่วนผสมของ แทนแดงต่อการเจริญเติบโตของดาวเรือง ผลการทดลองพบว่า กรรมวิธีที่ใช้แทนแดงแห้ง หน้าดิน และปุ๋ยคอก ในอัตราส่วน 1:1:1 โดยปริมาตร ส่งผลให้ดาวเรืองมีอัตราการเจริญเติบโตมากที่สุด ชี้ให้เห็นว่าแทนแดงแห้งมีศักยภาพในการเป็นวัสดุปลูก

ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบส่วน ขยายพันธุ์ของพรมก้ามหยาที่เหมาะสมและนำแทนแดงมาประยุกต์ เป็นแหล่งธาตุอาหารในการผลิตพรมก้ามหยาให้มีคุณภาพมากขึ้น และลดการใช้ปุ๋ยเคมี อีกทั้งข้อมูลการใช้แทนแดงในการผลิตไม้ ดอกไม้ประดับมีการศึกษาค่อนข้างน้อย จึงทำให้คณะวิจัยมีความ สนใจทำการทดลองนี้

วิธีการ

การวางแผนการทดลอง

วางแผนแบบปัจจัยร่วมแบบกลุ่มสมบูรณ์ (Factorial in CRD) ประกอบด้วย 2 ปัจจัย ได้แก่

ปัจจัยที่ 1: ส่วนขยายพันธุ์พืช ได้แก่ ลำต้นแม่ และไหล

ปัจจัยที่ 2: แหล่งธาตุอาหาร ได้แก่ 1) ปุ๋ยออสโมโค้ท (ปุ๋ยเคมี) สูตร 13-13-13 อัตรา 6 กรัม อัตราตามคำแนะนำ (โชติส, ม.ป.ป.) ใส่เพียง 1 ครั้ง ตลอดการทดลอง โดยใส่หลังจากย้ายต้นกล้า หลังการปักชำลงในกระถางพลาสติกขนาด 6 นิ้ว 2) แทนแดงแห้ง (ปุ๋ยชีวภาพ) 30 กรัม ต่อวัสดุปลูก 1 กิโลกรัม อัตราตามคำแนะนำ 3) ปุ๋ยออสโมโค้ทร่วมกับแทนแดงแห้ง (ปุ๋ยออสโมโค้ท 6 กรัม และ แทน แแดงแห้ง 30 กรัม ต่อวัสดุปลูก 1 กิโลกรัม) และ 4) ไม่ใส่ปุ๋ย

มีทั้งหมด 8 กรรมวิธี กรรมวิธีละ 4 ซ้ำ ดังนี้

1. ลำต้นแม่ x ปุ๋ยออสโมโค้ท
2. ลำต้นแม่ x แหนแดงแห้ง
3. ลำต้นแม่ x ปุ๋ยออสโมโค้ท+แหนแดงแห้ง
4. ลำต้นแม่ x ไม่ใส่ปุ๋ย
5. ไหล x ปุ๋ยออสโมโค้ท
6. ไหล x แหนแดงแห้ง
7. ไหล x ปุ๋ยออสโมโค้ท+แหนแดงแห้ง
8. ไหล x ไม่ใส่ปุ๋ย

การดำเนินงาน

การปักชำ

คัดเลือกส่วนขยายพันธุ์พืช ได้แก่ ลำต้นแม่และไหล ที่มี ความยาวประมาณใกล้เคียงกัน คือ 3.4-3.8 เซนติเมตร และมี 2 คูใบ โดยนำส่วนขยายพันธุ์ไปปักชำในทราย คลุมด้วยพลาสติกและมัดปาก ถัง เพื่อชักนำการเกิดราก รดน้ำทุกๆ 3 วัน เป็นเวลา 2 สัปดาห์

การปลูกและการดูแล

เริ่มดำเนินการผสมวัสดุปลูก ได้แก่ ขุยมะพร้าวและกาบ มะพร้าวสับ อัตราส่วน 1:1 โดยปริมาตร และใส่ในกระถางพลาสติก ขนาด 6 นิ้ว โดยทุกกรรมวิธีจะมีวัสดุปลูกน้ำหนัก 1 กิโลกรัม จากนั้น นำส่วนขยายพันธุ์พืช ได้แก่ ลำต้นหลักและไหล ปลูกในกระถาง และ เติมหาตุอาหารตามในแต่ละกรรมวิธี ทุกกรรมวิธีเลี้ยงภายใต้โรงเรือน พรางแสง เป็นหลังคาที่คลุมด้วยสแลนสีดาพรางแสง 70 เปอร์เซ็นต์ ให้น้ำวันเว้นวัน (ปริมาตร 400 มิลลิลิตรต่อต้น) และทำการกำจัด วัชพืชที่ขึ้นในกระถาง

การบันทึกข้อมูล

เริ่มบันทึกข้อมูลหลังย้ายปลูก 1 สัปดาห์ สัปดาห์ละ 1 ครั้ง นาน 13 สัปดาห์ บันทึกข้อมูลดังนี้ 1. ความกว้างทรงพุ่ม (เซนติเมตร) วัดเส้นผ่าศูนย์กลางทรงพุ่มจากซ้ายไปขวา 2. ความสูง ต้น (เซนติเมตร) วัดจากโคนต้นจนถึงปลายยอด 3. จำนวนใบ (ใบ) นับจำนวนใบที่เกิดขึ้นทั้งหมด 4. จำนวนไหล (ไหล) นับจำนวนไหลที่เกิดขึ้นทั้งหมด 5. จำนวนดอก (ดอก) นับจำนวนดอกที่เกิดขึ้นทั้งหมด 6. ความกว้างดอก (เซนติเมตร) วัดเส้นผ่าศูนย์กลางของดอก และ 7. ความยาวหลอดของดอก (เซนติเมตร) วัดตั้งแต่โคนหลอดดอกถึง ฐานกลีบดอก

การวิเคราะห์ผลทางสถิติ

วิเคราะห์ข้อมูลทางสถิติโดยใช้โปรแกรม STATISTIX โดย วิเคราะห์แบบ Least Significant Difference (LSD) ที่ระดับความ เชื่อมั่น 95%

สถานที่ทำการทดลอง

ณ โรงเรือนสาขาศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลล้านนา ลำปาง

ช่วงเวลาดำเนินการ

ระหว่างเดือนมกราคม 2568 – เดือนพฤษภาคม 2568

ผลการทดลองและอภิปราย

การเจริญเติบโตทางด้านลำต้น

จากการทดลองผลของปัจจัยส่วนขยายพันธุ์ทั้งลำต้นแม่ และไหล ในด้านความกว้างทรงพุ่มเฉลี่ย ความสูงต้นเฉลี่ย และ จำนวนไหลต่อต้นเฉลี่ย ในสัปดาห์ที่ 13 หลังการย้ายปลูก พบว่า ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ โดยส่งผลให้ความกว้าง ทรงพุ่ม ความสูงต้น และจำนวนไหลต่อต้น อยู่ในช่วง 23.76-24.18 เซนติเมตร 4.34-4.84 เซนติเมตร และ 7.77-8.75 เซนติเมตร ตามลำดับ (Table 2) แต่อย่างไรก็ตามพบว่า กรรมวิธีที่ใช้ไหลเป็นส่วนขยายพันธุ์ ส่งผลให้มีจำนวนใบเฉลี่ยทั้งหมดที่เกิดขึ้นมากกว่า การใช้ลำต้นแม่ในการขยายพันธุ์ คือ 54.50 ใบ (Table 2) จากการ รายงานของ นคร เหลืองประเสริฐ (ม.ป.ป.) กล่าวว่า โครงสร้างของ เซลล์ท่อลำเลียงน้ำและอาหารของไหลสตรอบเอรี มีการปรับตัวได้ดี ส่งผลให้การลำเลียงน้ำและธาตุอาหารเกิดขึ้นได้เป็นจำนวนมาก ซึ่ง ธาตุอาหารเหล่านี้จำเป็นอย่างยิ่งในการสร้างต้นไหล อีกทั้งไหลยังมี คุณสมบัติพิเศษ คือ สามารถเคลื่อนย้ายสารอาหารและน้ำได้อย่าง อิสระทั้งสองทิศทาง โดยต้นแม่สามารถลำเลียงน้ำและอาหารไปสู่ ต้นไหล ในทางกลับกันต้นไหลสามารถลำเลียงน้ำและสารอาหารไปสู่ ต้นแม่ได้เช่นกัน แต่อย่างไรก็ตาม ไหลที่ใช้ในการทดลอง ยังอยู่ในช่วง ระยะแรกของการเติบโต ไหลยังสามารถสังเคราะห์แสงเองได้น้อย พืชจะเกิดการลำเลียงคาร์โบไฮเดรตที่สร้างจากใบของต้นแม่มาสะสม ในไหลเพื่อใช้ในพัฒนาไหลและใบอ่อนได้อย่างมีประสิทธิภาพ (Vadera et al., 2025)

ผลของแหล่งธาตุอาหารต่อความกว้างทรงพุ่มเฉลี่ย พบว่า ตั้งแต่สัปดาห์ที่ 3 หลังการย้าย กรรมวิธีที่ใช้ปุ๋ยออสโมโค้ท ตาม อัตราตามคำแนะนำและการใช้แหนแดงร่วมกับการใช้ปุ๋ยออสโมโค้ท ส่งผลให้ความกว้างทรงพุ่มเฉลี่ยสูงกว่ากรรมวิธีอื่นๆ อย่างมีนัยสำคัญ ทางสถิติ โดยมีค่าเท่ากับ 33.37 เซนติเมตร และ 32.93 เซนติเมตร ในสัปดาห์ที่ 13 หลังการย้ายปลูก ตามลำดับ นอกจากนี้ยังพบว่า กรรมวิธีที่ได้รับแหนแดงเพียงอย่างเดียว ส่งผลให้ความกว้างทรงพุ่ม มากกว่ากรรมวิธีที่ไม่ใส่ปุ๋ยอย่างมีนัยสำคัญทางสถิติ โดยมีค่าเท่ากับ 21.12 เซนติเมตร และ 8.47 เซนติเมตร ในสัปดาห์ที่ 13 หลังการ ย้ายปลูก ตามลำดับ (Table 1) ส่วนในด้านความสูงต้นและจำนวน ไหลมีแนวโน้มในทิศทางเดียวกัน พบว่า กรรมวิธีที่ใช้ปุ๋ยออสโมโค้ท เพียงอย่างเดียว ส่งผลให้ความสูงต้นเฉลี่ย และจำนวนไหลสูงที่สุด คือ 6.50 เซนติเมตร และ 16.17 ไหล ตามลำดับ เนื่องจากการปุ๋ย ออสโมโค้ทจัดเป็นปุ๋ยเคมีชนิดหนึ่งที่จะค่อยๆปลดปล่อยธาตุอาหาร

ได้อย่างช้าๆ ซึมผ่านชั้นสารเคลือบ โดยกระบวนการออสโมซิส (osmosis) สม่าเสมอต่อเนื่องยาวนานได้ถึง 3 เดือน (livingpop, 2564) จึงสามารถส่งเสริมการเจริญเติบโตทางด้านความสูงต้นได้อย่างต่อเนื่องและมีประสิทธิภาพ และส่งผลสืบเนื่องไปจนถึงกระบวนการสร้างไหลได้อย่างมีประสิทธิภาพได้เช่นกัน รองลงมาคือกรรมวิธีที่ใช้ปุ๋ยออสโมโค้ทร่วมกับแทนแดง ที่ส่งผลให้ความสูงต้นเฉลี่ยและจำนวนไหลเท่ากับ 5.56 เซนติเมตร และ 13.83 ไหล ตามลำดับ (Table 2) สอดคล้องกับการทดลองของ Maze *et al.*, (2023) ที่ทำการทดลองนำแทนแดงมาใช้กับอัตราปุ๋ยเคมีที่แตกต่างกันต่อการเจริญเติบโตของชูกินี พบว่า กรรมวิธีที่ใช้ปุ๋ยเคมีในอัตราแนะนำ (100% of the recommended dose NPK) ร่วมกับการใส่แทนแดง ส่งผลให้ความสูงต้นชูกินีมีความสูงเฉลี่ยมากที่สุดและมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกรรมวิธีอื่นๆ และประเด็นที่น่าสนใจ คือ กรรมวิธีที่ใช้แทนแดงเพียงอย่างเดียวสามารถสร้างไหลได้ 3.04 ไหล ในขณะที่กรรมวิธีที่ไม่ใส่ปุ๋ยไม่เกิดการสร้างไหล (Table 2) ชี้ให้เห็นว่าแทนแดงมีอิทธิพลต่อการสร้างส่วนขยายพันธุ์ (ไหล) ของพรมก้ามหอยได้ เนื่องจากแทนแดงแห้งนับเป็นปุ๋ยชีวภาพ ที่หากผ่านการย่อยสลายแล้วจะสามารถปลดปล่อยธาตุอาหารโดยเฉพาะธาตุไนโตรเจนทั้งในรูปแบบแอมโมเนียมและไนเตรทได้เป็นอย่างดี โดยในช่วง 7 วันแรก แทนแดงแห้งจะมีการปลดปล่อยไนโตรเจนในรูปของแอมโมเนียมที่สูงกว่าไนเตรท หลังจากวันที่ 7 พบว่าแอมโมเนียมจะลดลง แต่ไนเตรทจะมีปริมาณสูงขึ้นอย่างต่อเนื่องตลอดการทดลองซึ่งเป็นผลดีต่อพืชอย่างมากที่พืชสามารถได้รับธาตุไนโตรเจนได้อย่างสม่ำเสมอ (154 วัน) ซึ่งธาตุไนโตรเจนนับว่าเป็นธาตุอาหารหลักสำคัญต่อการเจริญเติบโตของพืชอย่างมาก เนื่องจากธาตุไนโตรเจนองค์ประกอบของกรดอะมิโน โปรตีน รวมถึงคลอโรฟิลล์ ซึ่งเป็นรงควัตถุที่เป็นส่วนสำคัญกระบวนการสังเคราะห์ด้วยแสง เพื่อสร้างอาหารแก่พืชในการเจริญเติบโตต่อไป (สำนักงานเกษตรและสหกรณ์ จังหวัดอ่างทอง, 2563)

ส่วนในด้านจำนวนใบพบว่าการใช้ปุ๋ยออสโมโค้ทเพียงอย่างเดียว และการใส่ปุ๋ยออสโมโค้ทร่วมกับแทนแดง ส่งผลให้จำนวนใบเฉลี่ยมากที่สุดคือ 87.00 และ 81.75 ใบ ตามลำดับ รองลงมาคือการใช้แทนแดง ส่งผลให้มีจำนวนใบเท่ากับ 30.37 ใบ ส่วนกรรมวิธีที่ไม่ใส่ปุ๋ยพบว่าจำนวนใบน้อยที่สุด คือ 6.25 ใบ (Table 2) สอดคล้องกับการทดลองของ มนตรี และคณะ (2559) ได้ทำการทดสอบการใช้แทนแดงร่วมกับปุ๋ยไนโตรเจนต่อการเจริญเติบโตปาล์มน้ำมัน มีทั้งหมด 5 กรรมวิธี ได้แก่ การใช้ปุ๋ยไนโตรเจนอัตราตามคำแนะนำ 2) ใส่ปุ๋ยไนโตรเจนอัตราตามคำแนะนำร่วมกับแทนแดง 3) ใส่ปุ๋ยไนโตรเจน 0.75 เท่า ของอัตราตามคำแนะนำร่วมกับแทนแดง 4) ใส่ปุ๋ยไนโตรเจน 0.50 เท่าของอัตราตามคำแนะนำร่วมกับ

แทนแดง และ 5) ใส่ปุ๋ยไนโตรเจน 0.25 เท่าของอัตราตามคำแนะนำร่วมกับแทนแดง ผลการทดลองพบว่า การใช้ปุ๋ยไนโตรเจนอัตราตามคำแนะนำร่วมกับแทนแดง ส่งผลให้ต้นปาล์มน้ำมันมีจำนวนทางใบเพิ่มมากที่สุด คือ 18.17 ทางใบต่อ 6 เดือน ซึ่งไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกรรมวิธีการใช้ปุ๋ยไนโตรเจนอัตราตามคำแนะนำเพียงอย่างเดียว โดยส่งผลให้มีจำนวนทางใบเพิ่ม 17.41 ทางใบต่อ 6 เดือน อีกทั้งยังพบว่าอิทธิพลของการใส่แทนแดงในดินปลูกที่ผสมมูลไส้เดือน ส่งผลให้จำนวนใบของต้นกระเจียวเขียวมีจำนวนมากกว่าดินปลูกมูลไส้เดือนที่ไม่ใส่แทนแดง (Annapooma *et al.*, 2022)

ผลของปฏิสัมพันธ์ระหว่างสองปัจจัย ในด้านความกว้างทรงพุ่ม พบว่า กรรมวิธีที่ใช้ไหลร่วมกับการได้รับปุ๋ยออสโมโค้ทและแทนแดง มีแนวโน้มส่งผลให้ความกว้างทรงพุ่มเฉลี่ยตั้งแต่ลำต้นที่ 1-13 หลังการย้ายปลูก โดยมีค่าอยู่ระหว่าง 8.87-33.62 เซนติเมตร (Table 1) ส่วนในด้านความสูงต้นในลำต้นที่ 13 หลังการย้ายปลูกพบว่า กรรมวิธีที่ใช้ลำต้นแม่ร่วมกับการให้ปุ๋ยออสโมโค้ท ส่งผลให้ความสูงต้นเฉลี่ยสูงสุด คือ 6.37 เซนติเมตร รองลงมา คือการใช้ไหลร่วมกับการให้ปุ๋ยออสโมโค้ท และลำต้นแม่ร่วมกับการให้ปุ๋ยออสโมโค้ทและแทนแดง มีค่าเท่ากับ 6.62 เซนติเมตร และ 6.00 เซนติเมตร ตามลำดับ (Table 2) อาจเป็นเพราะลำต้นแม่เป็นส่วนขยายพันธุ์ที่มีการพัฒนาโครงสร้างเนื้อเยื่อต่อลำเลียงน้ำ ลำเลียงอาหารอย่างสมบูรณ์แล้วที่สามารถลำเลียงธาตุอาหารได้อย่างมีประสิทธิภาพ อีกทั้งแหล่งธาตุอาหารทั้งปุ๋ยออสโมโค้ท เป็นปุ๋ยเคมีที่สามารถปลดปล่อยธาตุอาหารออกมาได้อย่างสม่ำเสมอที่ประกอบด้วยธาตุ ไนโตรเจน โพแทสเซียม ฟอสฟอรัส ยาวนานมากถึง 3 เดือน ในด้านจำนวนใบ พบว่า กรรมวิธีที่ใช้ไหลร่วมกับการใส่ปุ๋ยออสโมโค้ท และกรรมวิธีที่ใช้ไหลร่วมกับการใส่ปุ๋ยออสโมโค้ทและแทนแดง ส่งผลให้จำนวนใบเฉลี่ยมากที่สุด คือ 92.00 ใบ และ 91.75 ใบ ตามลำดับ (Table 2) ส่วนจำนวนไหล พบว่า กรรมวิธีที่ใช้ลำต้นแม่ร่วมกับการให้ปุ๋ยออสโมโค้ท ไหลร่วมกับการให้ปุ๋ยออสโมโค้ทและไหลร่วมกับการให้ปุ๋ยออสโมโค้ทและแทนแดง ส่งผลให้จำนวนไหลเฉลี่ยมากที่สุดและมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ คือ 16.33 ไหล 16.00 ไหล และ 15.67 ไหล ตามลำดับ ในทางตรงกันข้าม กรรมวิธีที่ใช้ลำต้นแม่และไหลที่ไม่ใส่ปุ๋ย พบว่า ไม่เกิดการสร้างไหล (Table 2) ชี้ให้เห็นว่าธาตุอาหารในการผลิตพรมก้ามหอยมีส่วนสำคัญอย่างมากในการผลิตไหล เพื่อใช้ในการขยายพันธุ์ต่อไป

การเจริญเติบโตของดอก

จากการทดลอง ผลของปัจจัยส่วนขยายพันธุ์ทั้งลำต้นแม่และไหล พบว่า ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ในด้านจำนวนดอกเฉลี่ยต่อต้น ความกว้างดอกและความยาวของหลอด

ดอก อยู่ในช่วง 3.62-4.44 ดอกต่อต้น 1.44-1.47 เซนติเมตร และ 1.72-1.73 เซนติเมตร ตามลำดับ (Table 3)

ผลของแหล่งธาตุอาหารพบว่า กรรมวิธีที่ใช้แทนแฉะเพียงอย่างเดียวส่งผลให้ต่อจำนวนดอกเฉลี่ยต่อต้นมากที่สุด คือ 6.50 ดอก แต่อย่างไรก็ตามพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกรรมวิธีที่ใช้ปุ๋ยออสโมโค้ทเพียงอย่างเดียว คือ 5.25 ดอก สอดคล้องกับการทดลองของ Khair *et al.*, (2021) ที่ทำการทดสอบการใช้ปุ๋ยชีวภาพแทนแฉะร่วมกับการให้ปุ๋ยเคมีต่อการเติบโตและผลผลิตของข้าวพบว่า กรรมวิธีที่ใส่ปุ๋ยเคมี N-P-K เพียงอย่างเดียว ที่เตรียมจากแม่ปุ๋ย Urea Triple Super Phosphate (TSP) และ Muriate of Potash (MOP) (คิดเป็นปริมาณรวม 2.12-1.2-1.63 กรัมต่อกระถาง) ส่งผลให้จำนวนดอกข้าวต่อรวง (spikelet per panicle) ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบการให้แทนแฉะเพียงอย่างเดียว (50 กรัมต่อกระถาง) และเนื่องจากแทนแฉะแห้งเป็นปุ๋ยชีวภาพ ที่ไม่เพียงที่จะสามารถปลดปล่อยธาตุไนโตรเจน ได้เพียงเท่านั้น ยังสามารถปลดปล่อยธาตุฟอสฟอรัส และโพแทสเซียมที่เป็นธาตุอาหารสำคัญที่ช่วยในการพัฒนาการสร้างดอกได้ดี จากการทดลองของศิริลักษณ์ และคณะ (2563) พบว่า แทนแฉะแห้งมีคุณสมบัติที่สามารถปลดปล่อยธาตุฟอสฟอรัสที่เป็นประโยชน์สู่ดินเพิ่มขึ้นจากดินที่ไม่ใส่แทนแฉะถึง 133.8 มิลลิกรัมต่อกิโลกรัม นอกจากนี้แทนแฉะยังสามารถปลดปล่อยโพแทสเซียมที่แลกเปลี่ยนได้สูงเพิ่มขึ้นจากดินที่ไม่ใส่แทนแฉะถึง 298.9 มิลลิกรัมต่อกิโลกรัม และแทนแฉะยังสามารถปลดปล่อยโพแทสเซียมและฟอสฟอรัสได้เป็นระยะเวลานานตลอดการทดลอง (154 วัน) ดังนั้นจากข้อมูลข้างต้นชี้ให้เห็นว่าปริมาณธาตุอาหารที่แทนแฉะปลดปล่อยออกมาได้นี้เป็นปริมาณที่เพียงพอต่อสำหรับช่วงพัฒนาการสร้างดอกของพรมก้ามเหยี่ยวและในทางตรงกันข้ามกรรมวิธีที่ไม่ใส่ปุ๋ยแฉะต้นก้ามเหยี่ยวไม่เกิดการสร้างดอก (Table 3) อาจเป็นเพราะปริมาณธาตุอาหารไม่เพียงพอต่อการเจริญเติบโตและพัฒนาการของพรมก้ามเหยี่ยว

ส่วนในด้านความกว้างดอกพบว่ากรรมวิธีที่ใส่ปุ๋ยออสโมโค้ทร่วมกับแทนแฉะ ส่งผลให้ความกว้างดอกมีค่าเฉลี่ยสูงสุดคือ 1.99 เซนติเมตร แต่อย่างไรก็ตามไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกรรมวิธีที่ใส่ปุ๋ยออสโมโค้ท คือ 1.93 เซนติเมตร รองลงมาคือ กรรมวิธีที่ใส่แทนแฉะเพียงอย่างเดียว มีความดอกเฉลี่ย เท่ากับ 1.90 เซนติเมตร (Table 3) อาจเป็นผลของการส่งเสริมกันของธาตุฟอสฟอรัส และโพแทสเซียม ได้จากปุ๋ยออสโมโค้ทและแทนแฉะ จึงส่งผลให้ความกว้างดอกดีกว่าการใส่ปุ๋ยออสโมโค้ทเพียงอย่างเดียว และในด้านความยาวหลอดดอกพบว่า กรรมวิธีที่ใส่ปุ๋ยออสโมโค้ท แทนแฉะ และออสโมโค้ทร่วมกับแทนแฉะ

ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ โดยมีค่าความยาวหลอดดอกอยู่ช่วง 2.26-2.34 เซนติเมตร (Table 3)

ผลของปฏิสัมพันธ์ระหว่างสองปัจจัย ในด้านจำนวนดอกต่อต้นพบว่า กรรมวิธีที่ใส่ลำต้นแม่ร่วมกับการใช้แทนแฉะเพียงอย่างเดียว ส่งผลให้จำนวนดอกต่อต้นมากที่สุด คือ 8.75 ดอก ซึ่งมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกรรมวิธีอื่นๆ (Table 3) เนื่องจากการเกิดดอกของพรมก้ามเหยี่ยวมีจุดกำเนิดบริเวณซอกใบ (Axillary inflorescences อาจเป็นเพราะลำต้นแม่ที่ผ่านการเจริญเติบโตเต็มที่แล้ว (Mature stem) จะมีโครงสร้างหลักคือข้อ (Node) และปล้อง (Internode) ซึ่งบริเวณข้อจะเป็นจุดกำเนิดของตา (Buds) ที่สามารถพัฒนาไปเป็นใบ กิ่ง หรือดอกได้ (ทรูปลูกปัญญา, 2568) อีกทั้งประกอบกับได้รับธาตุอาหารจากแทนแฉะแห้งที่ปลดปล่อยได้ทั้งธาตุอาหารหลัก ธาตุอาหารรอง ได้แก่ ธาตุไนโตรเจนที่ปลดปล่อยได้ทั้งรูปแอมโมเนียมไอออน และไนเตรท ซึ่งเป็นรูปที่พืชดูดซึมใช้ในการเจริญเติบโต โดยช่วง 7 วันแรกไนโตรเจนในรูปแอมโมเนียมไอออนจะปลดปล่อยออกมาได้ปริมาณสูง และหลังจากนั้นจะเริ่มลดปริมาณลง แต่อย่างไรก็ตามไนโตรเจนในรูปไนเตรท ยังคงสามารถปลดปล่อยถึง 154 วัน ส่วนฟอสฟอรัสในรูปที่เป็นประโยชน์ และโพแทสเซียมที่แลกเปลี่ยนได้ สามารถปลดปล่อยออกมาจากแทนแฉะแห้งได้อย่างต่อเนื่อง โดยดินที่มีการเติมแทนแฉะแห้งจะมีฟอสฟอรัสที่เพิ่มขึ้นจากดินที่ไม่ใส่แทนแฉะเฉลี่ยเท่ากับ 133.8 มิลลิกรัมต่อกิโลกรัม ส่วนโพแทสเซียมพบว่า ดินที่ใส่แทนแฉะแห้ง มีปริมาณโพแทสเซียมในดินเพิ่มขึ้นเมื่อเทียบกับดินที่ไม่ใส่แทนแฉะเฉลี่ย เท่ากับ 298.9 มิลลิกรัมต่อกิโลกรัม และสามารถปลดปล่อยได้ต่อเนื่องนานถึง 154 วัน ส่วนธาตุอาหารรอง พบว่า แทนแฉะแห้งสามารถปลดปล่อยธาตุแคลเซียม และแมกนีเซียมที่แลกเปลี่ยนได้ได้อย่างต่อเนื่องและคงที่ โดยในดินที่ใส่แทนแฉะแห้งจะมีปริมาณแคลเซียมที่แลกเปลี่ยน เพิ่มขึ้นจากดินควบคุมเฉลี่ยเท่ากับ 314.8 มิลลิกรัมต่อกิโลกรัม ส่วนแมกนีเซียมพบว่าในของดินที่ใส่แทนแฉะแห้ง ส่งผลให้มีปริมาณแมกนีเซียมเพิ่มขึ้นจากดินที่ไม่ใส่แทนแฉะเฉลี่ย เท่ากับ 46.8 มิลลิกรัมต่อกิโลกรัม นอกจากนี้ยังพบว่า แทนแฉะแห้งส่งผลให้มีปริมาณอินทรีย์วัตถุสูงขึ้นจากดินที่ไม่ใส่แทนแฉะ จาก 2.12 เปอร์เซ็นต์ เป็น 2.86 เปอร์เซ็นต์ (ศิริลักษณ์, 2563) ดังนั้นจึงเห็นได้ว่าการใส่แทนแฉะแห้งพืชจะสามารถได้รับธาตุอาหารที่หลากหลาย อีกทั้งธาตุอาหารเหล่านี้ยังมีบทบาทสำคัญต่อการส่งเสริมการเจริญเติบโตของพืชทั้งในระยะการเจริญเติบโตทางลำต้น และระยะการเจริญเติบโตทางสืบพันธุ์หรือการสร้างดอก โดยธาตุไนโตรเจนเป็นองค์ประกอบสำคัญของโปรตีนและกรดอะมิโน ซึ่งมีส่วนช่วยในการเจริญเติบโตทางลำต้นและใบ และเป็นส่วนหนึ่งของโครงสร้างคลอโรฟิลล์ ส่งผลให้ใบมีสีเขียวและมีการสังเคราะห์แสงเพิ่มขึ้น ขณะที่ธาตุฟอสฟอรัสมีบทบาทสำคัญในการควบคุมการออก

ดอก ติดผล และการสร้างเมล็ด รวมทั้งส่งเสริมการเจริญเติบโตและการแพร่กระจายของระบบราก ส่วนธาตุโพแทสเซียมมีหน้าที่เกี่ยวข้องกับการลำเลียงน้ำตาลจากแหล่งสร้างอาหาร (source) ได้แก่ ใบ ไปยังแหล่งรับ (sink) ได้แก่ ดอก ผล เมล็ด และราก ตลอดจนมีส่วนช่วยในการสังเคราะห์น้ำตาล แป้ง โปรตีน ที่จำเป็นต่อการเจริญเติบโตของพืช ส่วนธาตุแคลเซียมมีความสำคัญต่อกระบวนการแบ่งเซลล์ และธาตุแมกนีเซียมเป็นองค์ประกอบของคลอโรฟิลล์ที่มีบทบาทสำคัญในกระบวนการสังเคราะห์ด้วยแสง รวมถึงการสังเคราะห์กรดอะมิโน เป็นต้น (สำนักสำรวจดินและวางแผนการใช้ที่ดิน, ม.ป.ป.) ดังนั้นจึงสามารถกล่าวได้ว่าธาตุอาหารเหล่านี้มีบทบาทเชื่อมโยงและส่งเสริมซึ่งกันและกัน อันเป็นปัจจัยสำคัญที่ส่งผลต่อการเจริญเติบโตและพัฒนาการของพืชทั้งด้านลำต้นและดอกได้อย่างมีประสิทธิภาพ

ส่วนในด้านความกว้างดอกและความยาวหอยดอกพบว่า การใช้ส่วนผสมขี้วัวทั้งจากลำต้นแม่หรือโหล ที่ได้รับธาตุอาหารจากออสโมโค้ทเพียงอย่างเดียว แหนแดงเพียงอย่างเดียว และ

การใช้ปุ๋ยออสโมโค้ทร่วมกับแหนแดง ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ โดยมีค่าอยู่ในช่วง 1.89-2.01 เซนติเมตร และ 2.26-2.35 เซนติเมตร ตามลำดับ (Table 3) นอกจากนี้สิ่งที่สังเกตได้จากการทดลองนี้คือ กรรมวิธีที่ใส่แหนแดงลงไปในวัสดุปลูก เพื่อใช้เป็นแหล่งอาหารพืช พบว่า กรรมวิธีเหล่านั้นจะสามารถรักษาความชื้นในวัสดุได้ดีกว่ากรรมวิธีที่ไม่ได้ใส่แหนแดง เนื่องจากแหนแดงมีความสามารถกักเก็บน้ำได้ดี (Novair *et al.*, 2020; Chanapanchai *et al.*, 2025) จึงสามารถเป็นแนวทางการลดปริมาณน้ำในการใช้ผลิตพรมก้ามหอยได้ อย่างไรก็ตามข้อเสียของการใส่แหนแดงเพียงอย่างเดียวคือ แผ่นใบเหลือง ขอบใบไม่สร้างรงควัตถุได้ตามลักษณะปกติ สีสนิมชัด ในทางกลับกันกรรมวิธีที่ใช้แหนแดงร่วมกับปุ๋ยเคมีออสโมโค้ท สามารถทำให้ลักษณะการเจริญเติบโตของพรมก้ามหอยได้ตามปกติ อาจเป็นเพราะการส่งเสริมกันของธาตุอาหารที่ปลดปล่อยออกมาจากทั้งสองแหล่ง ดังนั้นการใช้แหนแดงเพียงอย่างเดียวอาจไม่เหมาะสมต่อการผลิตพรมก้ามหอยควรมีการใส่ปุ๋ยเคมีร่วมด้วย

Table 1 Effects of plant propagation portions and the effectiveness of azolla with chemical fertilizer on canopy width (cm) of *Episcia cupreata* at 1 -13 weeks after transplant

Factors	Weeks after transplant						
	1	3	5	7	9	11	13
Plant Propagation Portions							
Mother plant	7.90	10.22 ^b	13.03	17.03	19.66	22.60	24.18
Stolon	7.68	11.43 ^a	13.70	16.86	19.80	21.67	23.76
Nutrients source							
Osmocote	7.25	11.90 ^a	15.88 ^a	22.51 ^a	27.12 ^a	30.95 ^a	33.37 ^a
Dried Azolla	8.06	11.21 ^a	13.07 ^b	15.58 ^b	17.57 ^b	19.58 ^b	21.12 ^b
Osmocote + Dried Azolla	8.19	11.98 ^a	16.13 ^a	21.32 ^a	25.85 ^a	29.53 ^a	32.93 ^a
No-fertilizer	7.69	8.21 ^b	8.37 ^c	8.38 ^c	8.38 ^c	8.47 ^c	8.47 ^c
Plant Propagation Portions x Nutrients source							
Mother plant x Osmocote	7.25	11.25 ^{bc}	15.37 ^{ab}	23.12 ^a	27.37 ^a	32.07 ^a	34.57 ^a
Mother plant x Dried Azolla	8.37	11.37 ^{bc}	13.20 ^b	15.95 ^b	18.27 ^b	20.12 ^b	21.05 ^b
Mother plant x Osmocote + Dried Azolla	7.50	9.87 ^{cd}	14.90 ^{ab}	20.37 ^a	24.32 ^a	29.32 ^a	32.25 ^a
Mother plant x No-fertilizer	7.62	8.40 ^d	8.67 ^c	8.70 ^c	8.70 ^c	8.87 ^c	8.87 ^c
Stolon x Osmocote	7.25	12.55 ^{ab}	16.40 ^a	21.90 ^a	26.87 ^a	29.82 ^a	32.17 ^a
Stolon x Dried Azolla	7.75	11.05 ^{bc}	12.95 ^b	15.22 ^b	16.87 ^b	19.05 ^b	21.20 ^b
Stolon x Osmocote + Dried Azolla	8.87	14.10 ^a	17.37 ^a	22.27 ^a	27.37 ^a	29.75 ^a	33.62 ^a
Stolon x No-fertilizer	7.75	8.02 ^d	8.07 ^c	8.07 ^c	8.07 ^c	8.07 ^c	8.07 ^c
F-test							
Plant Propagation Portions (A)	ns	*	ns	ns	ns	ns	ns
Nutrients source (B)	ns	*	*	*	*	*	*
A x B	ns	*	*	*	*	*	*
C.V. (%)	18.49	14.86	12.68	12.41	13.61	11.57	13.43

ns = not significant

Different letters in the same column show statistically significant differences when analyzed using the Least Significant Difference (LSD) method at a 95% confidence level ($P < 0.05$)

Table 2 Effects of plant propagation portions and the effectiveness of azolla with chemical fertilizer on plant height (cm), number of leaves per plant, and number of stolons per plant of *Episcia cupreata* at 13 weeks after transplant

Factors	Plant height (cm)	Number of leaves per plant	Number of stolons per plant
Plant Propagation Portions			
Mother plant	4.84	48.19 ^b	7.77
Stolon	4.34	54.50 ^a	8.75
Nutrients source			
Osmocote	6.50 ^a	87.00 ^a	16.17 ^a
Dried Azolla	3.37 ^c	30.37 ^b	3.04 ^c
Osmocote + Dried Azolla	5.56 ^b	81.75 ^a	13.83 ^b
No-fertilizer	2.94 ^c	6.25 ^c	0.00 ^d
Plant Propagation Portions x Nutrients source			
Mother plant x Osmocote	6.37 ^a	82.00 ^b	16.33 ^a
Mother plant x Dried Azolla	3.62 ^c	33.00 ^d	2.75 ^c
Mother plant x Osmocote + Dried Azolla	6.00 ^{ab}	71.75 ^c	12.00 ^b
Mother plant x No-fertilizer	3.37 ^{cd}	6.00 ^c	0.00 ^d
Stolon x Osmocote	6.62 ^a	92.00 ^a	16.00 ^a
Stolon x Dried Azolla	3.12 ^{cd}	27.75 ^d	3.33 ^c
Stolon x Osmocote + Dried Azolla	5.12 ^b	91.75 ^{ab}	15.67 ^a
Stolon x No-fertilizer	2.50 ^d	6.50 ^c	0.00 ^d
F-test			
Plant Propagation Portions (A)	ns	*	ns
Nutrients source (B)	*	*	*
A x B	*	*	*
C.V. (%)	15.19	13.16	17.55

ns = not significant

Different letters in the same column show statistically significant differences when analyzed using the Least Significant Difference (LSD) method at a 95% confidence level ($P < 0.05$)

สรุปผลการทดลอง

จากการทดลองการเปรียบเทียบส่วนขยายพันธุ์พบว่าทั้งลำต้นและไหลไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในด้านความกว้างทรงพุ่ม ความสูงต้น จำนวนใบต่อต้น จำนวนต้นไหลต่อต้น จำนวนดอกต่อต้น ความกว้างดอก และความยาวหลอดดอก แสดงให้เห็นว่าทั้งสองส่วนสามารถใช้ในการเป็นส่วนขยายพันธุ์ที่ดีของพรมก้ามหอยได้ ส่วนแหล่งธาตุอาหารพบว่า แหล่งธาตุอาหารจากปุ๋ยออสโมคอต์ ส่งผลให้ความกว้างทรงพุ่ม ความสูงต้น จำนวนใบต่อต้น จำนวนไหล จำนวนดอกต่อต้น ความกว้างดอก ความยาวหลอดดอก มากกว่ากรรมวิธีอื่นๆอย่างมีนัยสำคัญ แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติกับกรรมวิธีที่ให้ออสโมคอต์ร่วมกับແໜແດງในด้านความกว้างทรงพุ่ม จำนวนใบต่อต้น ความกว้างดอก และ

ความยาวหลอดดอก และประเด็นที่น่าสนใจคือ การใส่ແໜແດງเพียงอย่างเดียว ส่งผลให้การเจริญเติบโตในทุกพารามิเตอร์ดีกว่า กรรมวิธีที่ไม่ใส่ปุ๋ย ยกเว้นด้านความสูงต้นที่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ซึ่งเห็นว่าແໜແດງมีประสิทธิภาพที่สามารถปลดปล่อยธาตุอาหารแก่พืชได้ และผลของปฏิสัมพันธ์ระหว่างสองปัจจัย พบว่า กรรมวิธีที่ใช้ไหลในการปลูกร่วมกับการให้ปุ๋ยออสโมคอต์เพียงอย่างเดียว หรือออสโมคอต์ร่วมกับແໜແດງ ส่งผลให้มีแนวโน้มความกว้างทรงพุ่มของพรมก้ามหอยที่ดีตั้งแต่ช่วงแรกไปจนถึงช่วงท้ายของการทดลอง จำนวนใบต่อต้น จำนวนไหลต่อต้น ความกว้างดอก และความยาวหลอดดอก แต่อย่างไรก็ตามจากข้อสังเกตเพิ่มเติมพบว่า การใส่ออสโมคอต์ที่ใส่ແໜແດງร่วมไปด้วย ส่งผลความชื้นในวัสดุปลูกนั้นสูงกว่าการไม่ใส่ແໜແດງ ซึ่งสามารถเป็นแนวทางการลดการให้น้ำได้

Table 3 Effects of plant propagation parts and the effectiveness of azolla with chemical fertilizer on number of flowers per plant, flower width, and tube length of *Episcia cupreata*

Factors	Number of flowers per plant (cm)	Flower width (cm),	Tube length (cm)
Plant Propagation Portions			
Mother plant	4.44	1.47	1.72
Stolon	3.62	1.44	1.73
Nutrients source			
Osmocote	5.25 ^{ab}	1.93 ^{ab}	2.30 ^a
Dried Azolla	6.50 ^a	1.90 ^b	2.26 ^a
Osmocote + Dried Azolla	4.37 ^b	1.99 ^a	2.34 ^a
No-fertilizer	0.00 ^b	0.00 ^c	0.00 ^b
Plant Propagation Portions x Nutrients source			
Mother plant x Osmocote	5.25 ^b	1.96 ^a	2.28 ^a
Mother plant x Dried Azolla	8.75 ^a	1.91 ^a	2.27 ^a
Mother plant x Osmocote + Dried Azolla	3.75 ^b	2.01 ^a	2.34 ^a
Mother plant x No-fertilizer	0.00 ^c	0.00 ^b	0.00 ^c
Stolon x Osmocote	5.25 ^b	1.90 ^a	2.32 ^a
Stolon x Dried Azolla	4.25 ^b	1.89 ^a	2.26 ^a
Stolon x Osmocote + Dried Azolla	5.00 ^b	1.98 ^a	2.35 ^a
Stolon x No-fertilizer	0.00 ^c	0.00 ^b	0.00 ^c
F-test			
Plant Propagation Portions (A)	ns	ns	ns
Nutrients source (B)	*	*	*
A x B	*	*	*
C.V. (%)	30.46	5.99	5.14

ns = not significant

Different letters in the same column show statistically significant differences when analyzed using the Least Significant Difference (LSD) method at a 95% confidence level (P < 0.05)

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