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3. To be a medium for the exchange of
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researchers and students of Khon Kaen
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บทความหรือข้อคิดเห็นใดๆ ในวารสารนี้เป็นของผู้เขียน ไม่จำเป็นต้องสอดคล้องกับทัศนะของกองบรรณาธิการ

Preface

This special issue of the KCU Science Journal is based on a selection of papers that were presented at the First International Conference on Science and Technology for Sustainable Development of the Greater Mekong Sub-region organized by the Faculty of Science, Khon Kaen University, Khon Kaen, Thailand during 15-16th August 2006. The conference aimed to provide an excellent opportunity for researchers to share scientific and professional experience. It successfully attracted interest of more than three hundred researchers from China, Cambodia, Lao PDR, Vietnam and Thailand.

Of forty six selected papers from a large number submitted subsequently to the conference, twenty articles have been published in the special volume of KCU Research Journal. The remaining twenty six are published in this special issue of KCU Science Journal. A broad range of topics have been included, including articles in the fields of biological, physical, computational and environmental sciences to engineering. All papers published in this volume have been peer-reviewed carefully to reach scientific accuracy. I would like to express my deepest thanks to all reviewers for their hard work in the reviewing process. Their suggestions have been valuable. Also, I must express my big thanks to all the authors for their contributions to the knowledge gathered and shared in this special issue.

Finally, KCU Science Journal is proud to be part of the concluding chapter of success of the conference as represented by this special issue. Therefore the supports given to the conference organization from Khon Kaen University, the Science Society of Thailand Northeastern Branch, the Applied Taxonomic Research Center for Geo-informatics for the Development of Northeast Thailand and the Integrated Nanotechnology Research Center are due to thank very much.

Narumon Sangpradub, Ph.D

Editor, KCU Science Journal

มหาวิทยาลัยขอนแก่น

Antibacterial and Phytochemical Studies of 20 Thai Medicinal Plants against Catfish-Infectious Bacteria, *Aeromonas caviae*

การยับยั้งเชื้อแบคทีเรียและสารเคมีออกฤทธิ์ของพืชสมุนไพรไทย 20 ชนิดต่อ แบคทีเรีย *Aeromonas caviae* ที่ก่อโรคในปลาดุก

Sineenat Siri¹*, Paweena Wadbua², Wirach Wongphathanakul³,
Nilubon Kitancharoen⁴ and Pranom Chantaranothai⁵

Abstract

Many studies have been conducted on the antibacterial activity of Thai medicinal plants against human pathogens, however, little has been done on fish pathogens. Therefore, we are interested on screening the antibacterial activity of 20 Thai folkloric medicinal plant extracts against the isolated catfish-infectious bacterial strain, *Aeromonas caviae*. Nine samples extracted with water or ethanol from 6 plant species exhibited anti-*A. caviae* activity by the agar well diffusion assay. The water extract derived from *Punica granatum* showed the highest antibacterial activity with the minimal inhibitory concentration (MIC) and minimal bactericidal inhibitory activity (MBC) values of 3.125 and 6.250 mg/ml at 24 h, respectively. The bacterial growth inhibition was detected as early as 16 h of incubation with 6.250 mg/ml *P. granatum* extract. Thin layer chromatography (TLC) of phytochemical (phenolic/flavonoid compounds, sterols, proanthocyanidins, cardiac glycosides, and coumarins) and bioautographic results indicated that the antibacterial zones of *P. granatum* extract on TLC plate were positive to phenolic/flavonoid compounds, sterols, and proanthocyanidins. Therefore, this result suggested that *P. granatum* extract possessed anti- *A. caviae* activity and the extract would be a potential source for antibacterial agents in controlling *A. caviae* in a catfish farming.

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บทคัดย่อ

การศึกษาผลของพืชสมุนไพรไทยในการยับยั้งเชื้อแบคทีเรียส่วนใหญ่เป็นการศึกษาต่อแบคทีเรียก่อโรคในคน แต่มีการศึกษาผลของพืชสมุนไพรไทยเหล่านี้ต่อแบคทีเรียก่อโรคในปลาน้อยมาก ดังนั้นงานวิจัยนี้จึงสนใจที่จะศึกษาผลการยับยั้งเชื้อแบคทีเรีย *Aeromonas caviae* ซึ่งก่อโรคในปลาดุกของสารสกัดหยาบจากพืชสมุนไพรท้องถิ่นจำนวน 20 ชนิด พบว่าสารสกัดหยาบด้วยน้ำหรือเอทานอลจำนวน 9 ตัวอย่างที่สกัดจากพืช 6 ชนิด มีฤทธิ์ในการยับยั้งแบคทีเรียเมื่อวิเคราะห์ด้วยวิธีการแพร่ของสารละลายในวุ้นอะการ์ (agar well diffusion assay) สารสกัดหยาบด้วยน้ำของทับทิม (*Punica granatum*) สามารถยับยั้งแบคทีเรียได้ดีที่สุดโดยมีค่าความเข้มข้นต่ำสุดในการยับยั้งแบคทีเรีย (minimal inhibitory concentration, MIC) และค่าความเข้มข้นต่ำสุดในการฆ่าแบคทีเรียเท่ากับ 3.125 และ 6.250 มก./มล. ที่ 24 ชั่วโมงหลังการบ่มตามลำดับ นอกจากนี้ยังพบว่าสารสกัดหยาบของทับทิมที่ความเข้มข้น 6.250 มก./มล. สามารถยับยั้งการเจริญของแบคทีเรียได้ตั้งแต่วันที่ 16 หลังการบ่ม ผลการศึกษาสารออกฤทธิ์ยับยั้งแบคทีเรียด้วยวิธีโครมาโตกราฟีแบบบาง (Thin layer chromatography, TLC) ร่วมกับการตรวจสอบด้วยสารเคมี (ต่อ phenolic/flavonoid compounds, sterols, proanthocyanin, cardiac glycoside, coumarin) และการตรวจผลการยับยั้งการเจริญของแบคทีเรีย พบว่าสารสกัดจากทับทิมที่แยกบนแผ่น TLC ซึ่งยับยั้งการเจริญของเชื้อแบคทีเรียให้ผลบวกกับ phenolic/flavonoid compounds, sterols และ proanthocyanin ผลการศึกษาที่ได้แสดงให้เห็นว่าสารสกัดจากทับทิมสามารถยับยั้งการเจริญของแบคทีเรีย *Aeromonas caviae* ได้และน่าจะสามารถใช้ประโยชน์ในการควบคุมโรคที่เกิดจากแบคทีเรียดังกล่าวในการเพาะเลี้ยงปลาดุกต่อไปได้

Keywords : antibacterial, phytochemical, *Punica granatum*, catfish infectious bacteria

คำสำคัญ : การยับยั้งแบคทีเรีย, สารเคมีในพืช, ทับทิม, แบคทีเรียก่อโรคในปลาดุก

INTRODUCTION

Catfish is one of the most important economic freshwater fish of Thailand. Although, catfish aquaculture industry has grown increasingly, one threatening problem worrying most catfish farmers is bacterial infection, which can cause substantial financial loss. The application of antibiotics has been widely practiced by farmers to control catfish bacterial diseases and is quite effective. However, in recent years ineffective results of antibacterial usages have been reported, which are due to appearance of antibiotic-resistant bacterial strains. In 1995, a survey report of antibiotic resistant bacterial diseases in Thailand showed that most *Aeromonas hydrophila* and *A. sobria* isolated from diseased hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*)

were resistant to oxytetracycline, erythromycin, and sulfadiazine, which are frequently used for treatment and prevention of bacterial diseases by farmers (Somsiri, 1995). These ineffective treatments cause farmers to increase dosage or find new imported drugs to use, raising the cost of production. Therefore, finding antibacterial agents from nature to control catfish bacterial diseases has become our topic of interest, especially since the fact that there are many Thai medicinal plants having the potential of being resources of antibacterial agents. Although, antibacterial properties of these plants have been investigated, but most were focused on human pathogens and very few were on fish pathogenic bacteria. Therefore, this work had aimed to screen some local medicinal plants that possessed antibacterial

activity against catfish-infectious bacteria, *A. caviae*. In addition, the primary phytochemical study on their active components was investigated.

MATERIAL AND METHODS

1. Plant collection

Twenty local medicinal plants, which have been documented in Thai folkloric medicine, were collected from Khon Kaen province of Thailand

from October through December 2005. Voucher specimens are preserved in the herbarium of Faculty of Science, Khon Kaen University. The uses of selected plants were shown in Table 1. These plants were used alone or as mixtures for treatment of human bacterial infectious diseases, especially for skin lesions and diarrhea.

Table 1. Traditional uses of species selected for antibacterial investigation (Payorm, 1978; Runrawee, 1999).

Plant species	Common name	Part of use	Indication
<i>Acacia catechu</i>	Catechu tree	Leaves	Diarrhea and skin infection
<i>Ageratum conyzoides</i> L.	Tropical Ageratum	Whole plant	Fever and dysentery
<i>Alstonia scholaris</i>	White cheese wood	Bark	Diarrheic and skin infection
<i>Annona squamosa</i> Linn.	Sugar apple	Leaves	Chloasma
<i>Argyreia nervosa</i> Boj.	Silver morningglory	Leaves	Skin infection and decreasing inflammation
<i>Artocarpus heterophyllus</i> Lam.	Jackfruit tree	Leaves	Skin infection
<i>Azadirachta indica</i> A. Juss.	Siamese neemtree	Leaves, Bark	Fever and dysentery
<i>Cassia alata</i> Linn.	Candelabra	Leaves	Skin infection
<i>Curcuma long</i> Linn.	Turmeric	Bulb	Decreasing inflammation
<i>Ixora lobbii</i> Loud.	West Indian jasmine	Flower	Dysentery
<i>Lonicera japonica</i> Thunb.	Japanese Honey-sukle	Leaves, Stem	Diarrhea
<i>Malientha suavis</i> Pierre.	Pakwanban	Leaves	Decrease toxins
<i>Morus alba</i> L.	Mulberry tree	Leaves	Healing infected digestive systems
<i>Peltophorum pterocarpum</i>	Yellow Flumboyant	Bark	Diarrhea
<i>Piper aurantuacum</i>	Variegatum	Leaves	Gas in stomach
<i>Pithecolobium dulce</i>	Manila tamarind	Bark	Healing infected wounds
<i>Punica granatum</i> Linn.	Pomegranate	Fruit bark	Anti-bacterial activity
<i>Rhinacanthus nasutus</i> Kurz.	Thongpanchang	Leaves	Ringworm
<i>Sebania grandiflora</i> Linn.	Cork wood tree	Bark	Diarrhea
<i>Streblus asper</i> Lour.	Siamese rough brush	Fark	Diarrhea and skin infection

2. Plant material extraction

Crude plant extracts were prepared for in vitro screening by grinding 25 g of fresh samples and extracting with 100 ml of both water and 75 % ethanol at room temperature. The crude extracts were filtered through Whatman no.1 filter paper. For ethanol extracted samples, ethanol was completely evaporated at 40°C. The water- and ethanol-extracts were then lyophilized and kept in desiccators until use. The lyophilized samples of water and ethanol extracts were solubilized in distilled water and dimethyl sulfoxide (DMSO), respectively, and were passed through 0.22 µm sterilized filters before used.

3. Antibacterial screening

The antibacterial activity of the plant extracts was determined by modified agar well diffusion method (Owais et al., 2005). Luria-Bertani (LB) agar was seeded with *A. caviae* (cell density equivalent to 0.5 McFarland standard) by spreading the bacterial inoculums on the LB agar. Wells of 4.7 mm diameter were punched in the agar and filled with 30 µl of 100 mg/ml of lyophilized plant extracts. Water or DMSO, which was used to solubilize the extracts, was also used as negative control. The diameter of inhibition zones were determined after incubation at 30°C for 24 h.

4. Minimal inhibitory and minimal bactericidal concentrations

Minimal inhibitory concentrations (MIC) of the plant extracts were determined by the standard broth dilution method (NCCLS, 2002). One hundred microliters of *A. caviae* at the cell concentration equivalent to McFarland scale 0.5 were incu-

bated with the plant extractions of 50 - 0.049 mg/ml (two-fold serial dilution) for 24 h at 30°C. MIC₁₀₀ was defined as the lowest concentration in liquid medium of crude plant extract at which the visible growth of bacteria was completely inhibited (no turbidity in wells). Minimal bactericidal concentration (MBC) was defined as the lowest concentration of crude extract in which less than 0.01 % of the initial inoculum survived after 24 h. Medium from wells with no visible growth was plated on agar and colonies were counted. The value of MBC/MIC₁₀₀ determined whether an extract was bactericidal (MBC/MIC₁₀₀ < 4) or bacteriostatic (MBC/MIC₁₀₀ > 4).

5. Growth inhibition and minimal effective time of bactericidal activity

One hundred microliters of *A. caviae* culture at the cell concentration equivalent to McFarland scale 0.5 were incubated with the plant extractions of 50 - 0.049 mg/ml (two-fold serial dilution) at 30°C. Culture turbidity was measured every 2 h for 24 h. Ten microliters of incubated culture with 0.391, 3.125, 12.5 and 50.0 mg/ml of each plant extract were taken and the concentrations of bactericidal activity were determined at 2, 4, 8, 16 and 24 h.

6. Thin layer chromatography (TLC)-phytochemical assay

The plant extracts were spotted onto a silica gel TLC plate (Kieselogel 60 F254 0.2 mm, Merck). The plates were developed in ascending direction with toluene:acetone:methanol (1:1:3) and CHCl₃:acetone:methanol:H₂O (1:7:4:5) as mobile phase. Spots were visualized by UV irradiation at 366 nm after spraying with AlCl₃ reagent (Harbone, 1973; Wagner et al., 1984) for phenolic/flavonoid

compounds, acidic iodine-potassium iodide for alkaloids, perchloric acid for sterols (Hara, 1963), Benedict's reagent for coumarins and flavonoids, vanillin-HCl reagent for proanthocyanidins (Ribereau-Gayon, 1972), and antimony (III) chloride reagent for cardiac glycoside (Wagner et al., 1984).

7. TLC-bioautography assay

Bioautographic assay (Zampini et al., 2005) was used to locate antibacterial compounds. Developed TLC plates were dried overnight in a sterile room. Then, the plates were covered with soft medium (Mueller-Hinton broth (MHB) with 0.8 % agar) containing 10^6 colony forming units (CFU) of *A. caviae*, incubated at 30°C for 24 h, and sprayed with a 2 mg/ml iodinitrotetrazolium chloride solution. Plates were incubated at 30°C for 1h in darkness for color development. Growth inhibition areas on pink background were compared with the retention factor (R_f) of the related spots on the TLC plates of phytochemical study.

8. Statistical analysis

The data was analyzed by the two-way ANOVA test. A p value of < 0.05 was considered statistically significant.

species exhibited inhibitory activity against *A. caviae* with the average inhibition zones ranging from 25.2 ± 0.1 to 8.8 ± 0.1 mm in diameter (Figures 1 - 2). Four active crude extracts were the water extracted samples of *Punica granatum*, *Sesbania grandiflora*, *Pithecellobium dulce*, and *Argyrea nervosa*. Five active crude extracts were ethanol extracted samples derived from *Punica granatum*, *Sesbania grandiflora*, *Pithecellobium dulce*, *Peltophorum pterocarpum*, and *Ixora coccinea*. Interestingly, these medicinal plants were traditionally used by immersing the plants in water, suggesting that their active compounds would probably be found in water extracted samples. Interestingly, the extracts of *Punica granatum*, *Sesbania grandiflora*, *Pithecellobium dulce*, and *Argyrea nervosa* showed anti-*A. caviae* activity in both water and ethanol extracts but more effectively in water extracted samples, which corresponded well with traditional uses. In contrast, although *Peltophorum pterocarpum* and *Ixora coccinea* were traditionally used by soaking in water, however, their water extracts showed no anti-*A. caviae* activity. Instead, their ethanol extracts were active against the bacteria tested, so it could be assumed that their active compounds in water extracts might effective against certain bacteria and less effective against *A. caviae*.

RESULTS AND DISCUSSION

The antibacterial activity of the plant extracts

The antibacterial activity of 40 samples derived from 20 plant species extracted by water and ethanol was screened using agar well diffusion assay. Negative control wells containing either water or DMSO showed no inhibition zone. On the other hand, nine out of twenty crude extracts from 6 plant

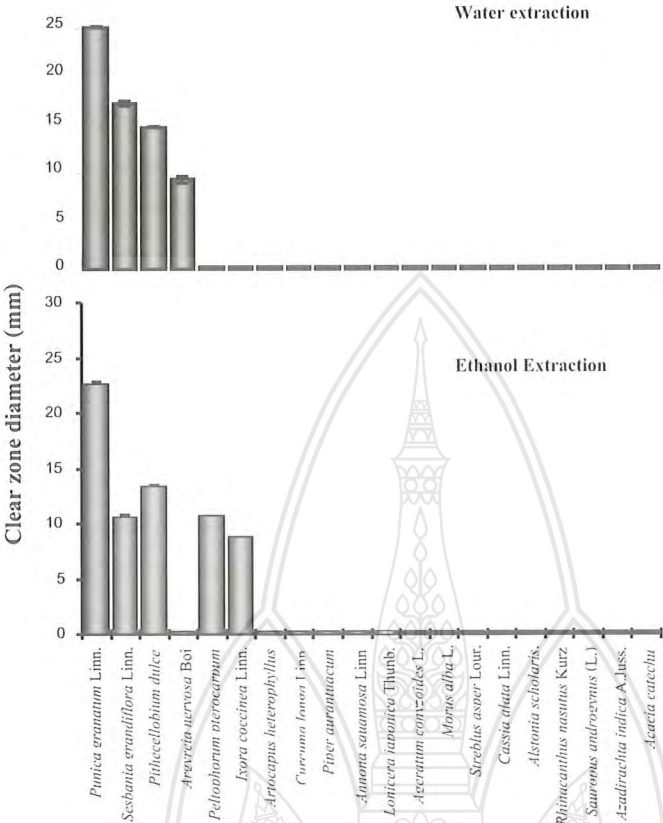


Figure 1. Antibacterial activity of water and ethanol plant extracts against *A. caviae*. The average inhibition zones of each plant extracts in water (upper) and ethanol (lower) were compared. (n = 3)



Figure 2. Inhibition zones of plant extracts against *A. caviae*. The extract of *Curcuma longa* Linn. were filled in well 1 - 3 and the extract of *Pithecellobium dulce* were filled in well 4 - 6. Well Ctrl was filled with water as negative control.

The three most effective samples with water extraction derived from *Punica granatum*, *Sebania grandiflora*, and *Pithecolobium dulce* were further analyzed for MIC and MBC values. The MIC values of the extracts of *Punica granatum*, *Sebania grandiflora*, and *Pithecolobium dulce* against *A. caviae* were 3.125, 25 and 12.5 mg/ml, respectively, while their MBC values were 6.25, 25 and 25 mg/ml, respectively (Table 2). The MBC values of all plant extracts were 1 - 2 folds of their MIC values, indicating the bactericidal ($MBC/MIC_{100} \leq 4$), not the bacterostatic ($MBC/MIC_{100} > 4$) activity of the extracts. The extract of *Punica granatum*, which exhibited the highest antibacterial activity in this experiment, was reported as anti-human pathogenic bacteria, *Bacillus anthracis*, *B. subtilis*, *Vibrio cholerae* (Trivedi and Kazmi, 1979), *Helicobacter pylori*

(Voravuthikunchai et al., 2004), *Escherichia coli* 0157:H7 (Voravuthikunchai et al., 2005), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Anesini and Perez, 1993), but not as anti-fish pathogens. Thus, this was the first report on its activity against fish pathogenic bacteria, *A. caviae*. Inhibition of bacterial growth corresponded to various concentrations of *Punica granatum* extract was also determined every 2 h in a time course of 24 h. The *Punica granatum* extract exhibited the inhibition of cell growth in a dose dependent manner (Figure 3). Cells treated with the extract at MIC value showed no significant growth change after 6 h of incubation, while the extract at MBC value decreased the absorbance reading values after 8 h of incubation and no cells were grown on the MH agar plates.

Table 2. Values of minimal inhibitory and minimal bactericidal concentrations of active plant extracts. (n = 4)

Plant species	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
<i>Punica granatum</i> Linn	3.125	6.25	2
<i>Sebania grandiflora</i> Linn	25	25	1
<i>Pithecolobium dulce</i>	12.5	25	2

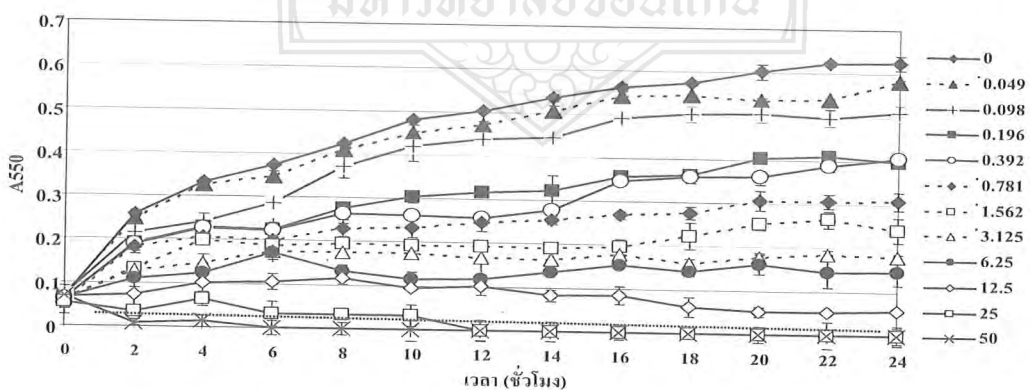


Figure 3. The *A. caviae* growth inhibition curve in response to *Punica granatum* extract. The growth of bacterial cells treated with the extract of *Punica granatum* (0 - 50 mg/ml) were inhibited in a dose dependent manner. (n = 3)

The phytochemical analysis of the extracts with anti-*A. caviae* activity

The results of TLC-autobiographic and phytochemical assays were compared to determine the phytochemical groups of the anti-*A. caviae* components. The results showed that the antibacterial components of *Punica granatum* extract were tested positive to phenolic/flavonoid compounds ($R_f = 0.79$), sterols ($R_f = 0.78$), and proanthocyanidins ($R_f = 0.81$) (Figure 4). Our results were similar to the previous reports of phytochemical studies of antibacterial components in *Punica granatum* that antibacterial components of *Punica granatum* extract were chemically tested positive to flavonoids, ste-

rols, and tannins (Voravuthikunchai et al., 2005).

The antibacterial components of *Sebania grandiflora* extract were in the groups of sterols ($R_f = 0.55$) and proanthocyanidins ($R_f = 0.67$), and those of *Pithecolobium dulce* was also identified as the groups of sterols ($R_f = 0.76$) and proanthocyanidins ($R_f = 0.74$). According to our knowledge, no antibacterial activity and phytochemical property of both plant extracts were reported. It was interesting to point out that all antibacterial zones of three plant extracts were identified as different compounds according to either different reactivity to the tested chemicals or different R_f value.

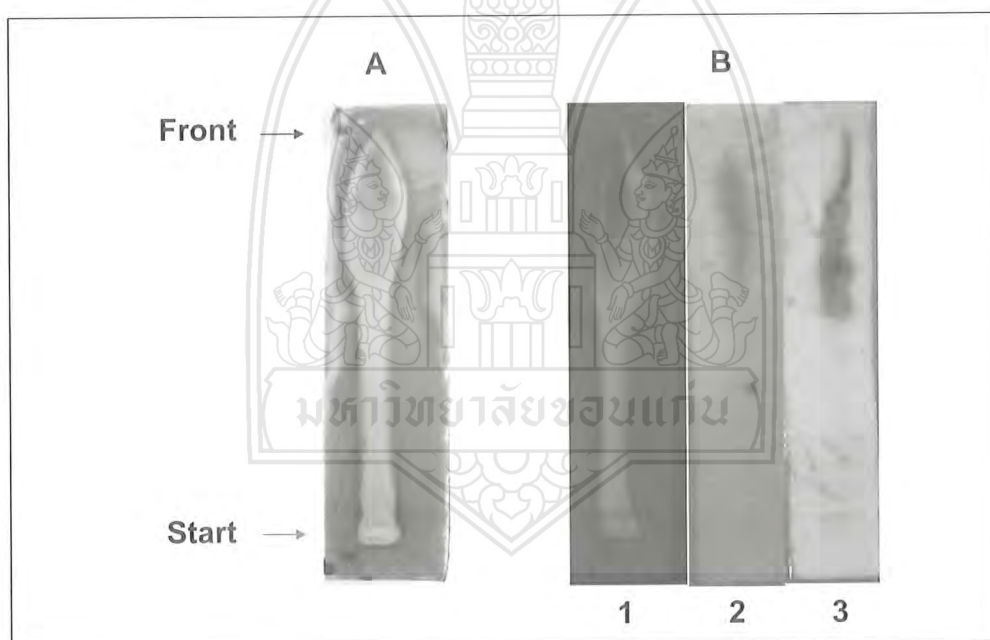


Figure 4. The phytochemical study of antibacterial zone. A) TLC bioautographic result showed the anti-*A. caviae* pattern of *Punica granatum* extract. B) TLC phytochemical results indicated that the active components were positive to phenolic/flavonoid compounds (1), sterols (2), and proanthocyanidins (3).

CONCLUSION

The present study demonstrated the results of screening 20 species of Thai folkloric medicinal plants against infected-catfish bacteria, *A. caviae*. Nine out of twenty extracts were active against *A. caviae*. The extract of *Punica granatum* was the most effective against *A. caviae* with bactericidal action. The growth inhibition study showed a dose dependant response to various concentrations of *Punica granatum* extract. Furthermore, the antibacterial components of *Punica granatum* were tested positive to phenolic/flavonoid compounds, sterols, and proanthocyanidins. The result of high antibacterial activity against fish pathogen of *Punica granatum* extract, thus, suggested that it could have potential uses as an agent to control bacterial fish diseases caused by *Aeromonas* spp.

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Estrogenic Activity of Wan-chak-mot-luk (*Curcuma xanthorrhiza* Roxb) Evaluated by Yeast Two Hybrid System
เอสโตรเจนแอกติวิตีของว่านชักมดลูก (*Curcuma xanthorrhiza* Roxb) ตรวจวัดด้วยระบบยีสต์ทูไฮบริด

Sineenat Siri^{1*} and Kritsana Incamnoi²

Abstract

One of the many natural compounds of plant origin, termed phytoestrogens, can mimic estrogenic functions due to its binding capability to the estrogen receptor. Thai medicinal plants traditionally used for management of menopausal symptoms, therefore, are hypothesized of having estrogenic activity. In this research, Wan-chak-mot-luk (*Curcuma xanthorrhiza* Roxb), a plant used in ancient Thai medicines for inducing normal menstruation, was evaluated for estrogenic activity by using the constructed yeast two hybrid system with the plasmids expressing two fusion proteins (Gal4BD-ER and Gal4AD-TIF2) and the chromosomal integrated lacZ reporter gene. This system could detect the estrogenic activity of 10^{-8} - 10^{-5} M standard 17β -estradiol in a dose dependent manner with EC₅₀ at 4.07×10^{-7} M. The estrogenic activity of *C. xanthorrhiza* Roxb extracts of 250, 150 and 50 mg/ml was evaluated by the yeast two hybrid system and the extract of 250 mg/ml was active with the estrogenic activity of 1.73 ± 0.09 fold compared to that of the control DMSO. In contrast, the control negative plant (white radish) extracts of 250, 150, and 50 mg/ml showed no estrogenic activity compared to that of the control DMSO. Therefore, it is interesting to further investigate on the estrogenic compounds of *C. xanthorrhiza* Roxb and the application of the constructed yeast two hybrid system to screen other Thai medicinal plants with estrogenic activity.

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บทคัดย่อ

สารธรรมชาติจากพืชที่เรียกว่าไฟโตเอสโตรเจนสามารถทำหน้าที่คล้ายฮอร์โมนเอสโตรเจนได้โดยการเข้าจับกับเอสโตรเจนรีเซพเตอร์ พืชสมุนไพรไทยหลายชนิดที่ใช้ในการรักษาอาการผิดปกติหลังหมดประจำเดือนจึงถูกสันนิษฐานว่าอาจมีเอสโตรเจนแอคติวิตี ดังนั้นในงานวิจัยนี้จึงต้องการตรวจวัดเอสโตรเจนแอคติวิตีของว่านชักมดลูก (*Curcuma xanthorrhiza* Roxb) ซึ่งมีสรรพคุณเกี่ยวกับการรักษาอาการประจำเดือนมาไม่ปกติโดยใช้ระบบยีสต์ทูไฮบริด โดยยีสต์ทูไฮบริดที่ใช้เป็นเซลล์ยีสต์ที่ดัดแปลงพันธุกรรมให้มีพลาสมิดสำหรับสร้างโปรตีนสายผสม 2 ชนิด คือ Gal4BD-ER และ Gal4AD-TIF2 และมีจีนรายงาน lacZ ในโครโมโซม ระบบยีสต์ทูไฮบริดที่สร้างขึ้นสามารถวัดเอสโตรเจนแอคติวิตีของสารมาตรฐาน 17β -estradiol ได้สัมพันธ์กับความเข้มข้นในช่วง 10^{-8} - 10^{-5} โมลาร์ และมีค่า EC₅₀ เท่ากับ 4.07×10^{-7} โมลาร์ เมื่อใช้ระบบยีสต์ทูไฮบริดในการวัดเอสโตรเจนแอคติวิตีของสารสกัดหยาบของว่านชักมดลูกที่ความเข้มข้น 250, 150 และ 50 มก./มล. พบว่าเฉพาะสารสกัดที่ความเข้มข้น 250 มก./มล. มีค่าเอสโตรเจนแอคติวิตีเป็น 1.73 ± 0.09 เท่าเมื่อเทียบกับชุดควบคุม DMSO ในขณะที่สารสกัดหยาบจากหัวผักกาดขาวที่ความเข้มข้น 250, 150 และ 50 มก./มล. ซึ่งใช้เป็นชุดควบคุมแบบลบไม่พบเอสโตรเจนแอคติวิตีเมื่อเทียบกับชุดควบคุม DMSO ดังนั้นการศึกษาชนิดของสารไฟโตเอสโตรเจนจากว่านชักมดลูกและการใช้ระบบยีสต์ทูไฮบริดในการศึกษาสารไฟโตเอสโตรเจนจากพืชสมุนไพรไทยจึงเป็นหัวข้อที่น่าสนใจสำหรับการศึกษาต่อไป

Keywords : estrogenic activity, yeast two hybrid, *Curcuma xanthorrhiza* Roxb

คำสำคัญ : เอสโตรเจนแอคติวิตี, ยีสต์ทูไฮบริด, ว่านชักมดลูก

INTRODUCTION

Many compounds in the environment, including phytoestrogens, pharmaceuticals and industrial chemicals, have been found to mimic estrogen activity by binding with estrogen receptors and influencing estrogen-signaling pathways (Legler, 2002). The most interesting estrogen mimics are phytoestrogen, a term of plant derived chemicals that exerts estrogenic activity (Mueller, 2002). In traditional Thai medicinal books, many plant species used for managing menopausal symptoms are hypothesized of having estrogenic activity. One species of interest is *Curcuma xanthorrhiza* Roxb, which is locally known as Wan-chak-mot-luk. The rhizome of *C. xanthorrhiza* Roxb was used to relieve uterus pain, manage normal functions of the

uterus after child delivery, and relieve abnormal menstruation cycle symptoms. Thus, it was the species of interest to investigate on whether it possessed estrogenic activity.

In vivo and in vitro assays have been developed to determine estrogenic substances. Although, in vivo assay was suitable for analyzing estrogenic substances and their derivatives after being digested in the body, it had many limitations, including unsuitable for screening on a large-scale, high cost, and poor sensitivity. In contrast, in vitro assay could screen large numbers of samples simultaneously, is inexpensive, and can determine more definitive end points (Zacharewski, 1997). One of the in vitro assay with high efficiency is the yeast two-hybrid assay, because its principle was based on the bind-

ing of estrogenic substances to a fusion protein of human estrogen receptor-Gal4 binding domain (Figure 1). This complex recruits the other fusion protein of coactivator TIF2-Gal activation domain, resulting in expression of the reporter gene, β -galactosidase (Torchia et al., 1997). The estrogenic activity of the substances can be determined from the β -galactosidase activity to hydrolyze the substrate (ONPG) to the product of yellow color (o-nitro phenol) that was measured by a spectrophotometer at the wavelength of 420 nm (Nishikawa et al., 1999).

In this study, the yeast two hybrid system was constructed by making two recombinant plasmids that could express Gal4 BD-ER and Gal4 AD-TIF2 fusion proteins and transforming these plasmids into the yeast strain YRG-2 that contained the chromosomal integrated *lacZ* reporter gene. The system was then used to evaluate the estrogenic activity of the extract of *C. xanthorrhiza* Roxb.

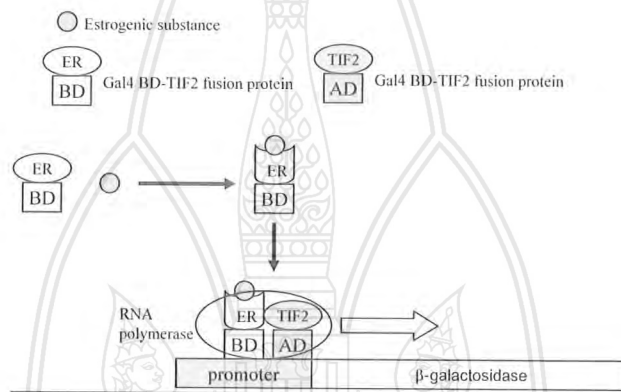


Figure 1. The yeast two hybrid system scheme. The binding between the estrogenic substance and Gal4 AD-ER fusion protein, resulting in the interaction of the Gal4 BD-TIF2 fusion protein which specifically binds to the Gal4 specific binding sequence on the promoter. The protein complex can induce the transcription of the reporter gene, β -galactosidase.

MATERIAL AND METHODS

1. Chemicals and Biological Material

All reagents used in this experiment are of analytical grade. Plasmid vectors, pAD-Gal4-2.1 and pBD-Gal-Cam, are kind gifts from Assistant Professor Dr. Preeya Wangsomnuk, Faculty of Science, Khon Kaen University. MCF-7 cells are the gift from the National Center of Genetic Engineering and Biotechnology (BIOTECH), Pathumthani. Me-

dicinal plant samples were obtained from the group of medicinal plants of Khamdanpattana village, Nong Khai and white radish was bought from a local market.

2. Preparation of plant extracts

The minced plant samples were extracted in 75 % ethanol for 24 h at room temperature by using the ratio of 100 g/800 ml. After filtering,

ethanol was evaporated under vacuum in a rotary evaporator at 55 °C. The sample was then lyophilized and stored in a desiccator until use.

3. Construction of yeast two hybrid system

The ligand binding domain (LBD) of the hER α (codons 252-600; Nakano et al., 2002) and the receptor interaction domains (RID) of TIF2 (codons 624-1287; Voegel et al., 1996) were amplified by RT-PCR using total RNA from MCF-7 cells. The reverse transcription (RT) reaction contained 2 μ g total RNA, 100 ng oligo dT, 0.5 mM dNTP, 10 mM DTT, 1x reverse transcription buffer, and 200 U Superscript III reverse transcriptase (Invitrogen, USA). Subsequently, the cDNA was used as the template in PCR reaction, which contained 2 μ l of cDNA from RT-reaction, 0.2 mM dNTP, 2.5 mM MgCl₂, 1x PCR buffer, 150 ng of each specific primers, and 1.25 U Taq DNA polymerase. The DNA was amplified by the following program parameters: the pre-denaturing cycle of 94 °C for 2 min, the 30 cycles of 94 °C for 30s, 55 °C for 30s, 72 °C for 45s, and the last cycle of 72 °C for 5 min.

pAD-Gal4-2.1 and TIF2 PCR product were digested with XbaI and BamHI enzymes. The pBD-Gal4-Cam and ER-PCR product were digested with SalI and EcoRI enzymes. Each reaction contained 5 μ g plasmid DNA or 1 μ g insert DNA, 50 U of each restriction enzymes, and 1x enzyme buffer. The reactions were incubated at 37 °C for 24 h. The digested plasmid was separated on an agarose gel and target bands were purified from gel using PerfectPrep gel clean up kit (Eppendorf, USA). The digested PCR-products were purified using a Qiaquick PCR purification kit (Qiagen, USA). Digested TIF2 DNA was ligated to digest pAD-Gal4-2.1 (referred

as pAD-TIF2), while digested ER-DNA was ligated to digested pBD-Gal4-Cam (referred as pBD-ER). The ligation reaction contained various molar ratios between vector and insert DNA, 400 U T₄ DNA ligase and 1x T₄ ligation buffer. After incubation at 4 °C overnight, the recombinant plasmids were transformed into XL1-Blue (Stratagene, USA) using the standard method (Sambrook et al., 1989). The positive bacterial clones were selected on LB agar containing appropriated antibiotics, and subsequently confirmed by PCR. The insert DNA sequence was sequenced by the BioService Unit (Thailand). Subsequently, the recombinant plasmids were transformed into yeast cells, YRG-2 (Stratagene, USA) using a lithium acetate method (Stratagene, USA). The positive yeast clones were selected on SD agar lacking tryptophan and leucine and later confirmed the presence of plasmids by PCR method. The positive yeast clones were then screened for the most effective clones to measure estrogenic activity of 10⁻⁵ M 17 β -estradiol.

4. Determining estrogen activity of samples by yeast two hybrid system

The β -galactosidase enzyme activity in response to the samples were determined by the constructed yeast clones according to the Clontech protocol. Yeast cells were cultured in 1.5 ml SD until OD₆₀₀ reached 0.5. The culture was incubated with 19 μ l of either 17 β -estradiol, DMSO, or plant extracts for 5 h at 30 °C. The cells were collected by centrifugation at 12,000 rpm for 30 sec and washed twice in Z buffer (0.1 M sodium phosphate [pH 7.0], 10 mM KCl, and 1 mM MgSO₄). The cells were broken with freeze and thaw cycles in liquid nitrogen. The lysate was incubated with 160

μ l of 4 mg/ml 2-nitrophenyl- β -D-galactoside (ONPG) for 30 min at 30 °C. The reaction was stopped by adding 100 μ l of 1 M Na_2CO_3 and measured the absorbency at 420 nm. The β -galactosidase activity is calculated as following;

$$\beta\text{-galactosidase units} = 1000 \times \text{OD}_{420} / (t \times V \times \text{OD}_{600})$$

Where: t is the incubation time in min.

V is 0.1 ml x concentration factor

OD_{420} nm is the absorbance by o-nitrophenol

OD_{600} nm is the cell density at the start of the assay

RESULTS AND DISCUSSION

1. Construction of yeast two hybrid system

Amplified DNA fragments of partial TIF2 and ER genes were obtained with the expected sizes, which were 1, 811 bp and 1,060 bp, respectively (Figure 2). TIF2 DNA was ligated to pAD-Gal4-2.1 and ER DNA was ligated to pBD-Gal-Cam, yielding the recombinant plasmids of pAD-TIF2 and pBD-ER, respectively. The insert sequences were checked by DNA sequencing in both directions. The sequences were aligned to TIF2 (accession number X97674) and ER (accession number NM_000125) genes using the NCBI blast 2 sequence program. Both sequences showed 100 % similarity compared to TIF2 and ER genes. Subsequently, both recombinant plasmids were

transformed into YRG2 yeasts. Positive clones were selected on SD agar lacking tryptophan and leucine, then the presence of both plasmids was confirmed by PCR (Figure 3). To characterize the ability of the genetically engineered yeast cells, the yeast cells were assayed for β -galactosidase activity in response to various concentrations of 17 β -estradiol (10 folds serial dilution), ranging from 10^{-10} - 10^{-4} M. The results showed that the yeast clone could significantly measure the β -galactosidase activity at all concentrations of 17 β -estradiol compared to that of vehicle DMSO. The enzyme activities were increased in response to the concentrations of 10^{-10} - 10^{-5} M standard estradiol, except at 10^{-4} M estradiol (Figure 4). From a graph, the significant dose response was found at the concentrations of 10^{-8} - 10^{-5} M of 17 β -estradiol. The median effective concentration (EC50) was 4.07×10^{-7} M or 407 nM. Although, the EC50 value of the constructed yeast clone was lower than those of the previous reported clones in a range of approximately 100 - 700 folds (Zhang et al., 2005a; Zhang et al., 2005b; Wang et al., 2003; Jung et al., 2004), the constructed yeast clone could significantly determine estrogenic activity as low as 10^{-8} M of 17 β -estradiol. So there was potential to evaluate estrogenic activity of interested plant samples.

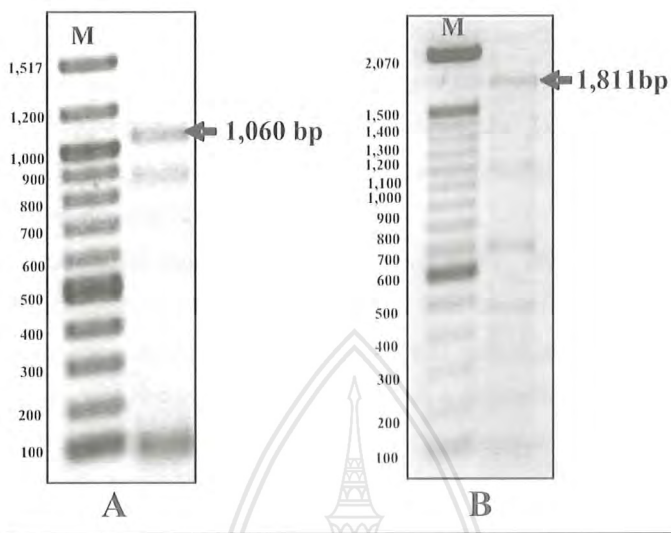


Figure 2. ER and TIF2 amplified products from RT-PCR. A) The ER amplified DNA of 1,061 bp. B) The TIF2 amplified DNA of 1,811 bp. The amplified products were separated on 1.2% agarose gel using 100 volts for 50 min. Lane M was the 100 DNA ladder markers.

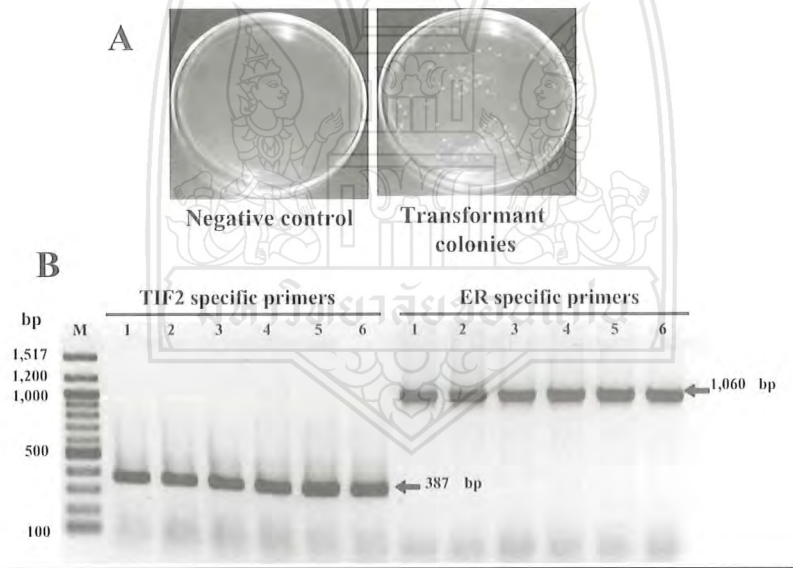


Figure 3. The screening of positive yeast clones. A) The positive transformants were first screened by SD medium lacking tryptophan and leucine. B) The presences of both recombinant plasmids in each clone (1-6) were confirmed by PCR method using specific primers to TIF2 and ER. The specific PCR products to TIF2 and ER were 387 bp and 1,060 bp, respectively (arrows).

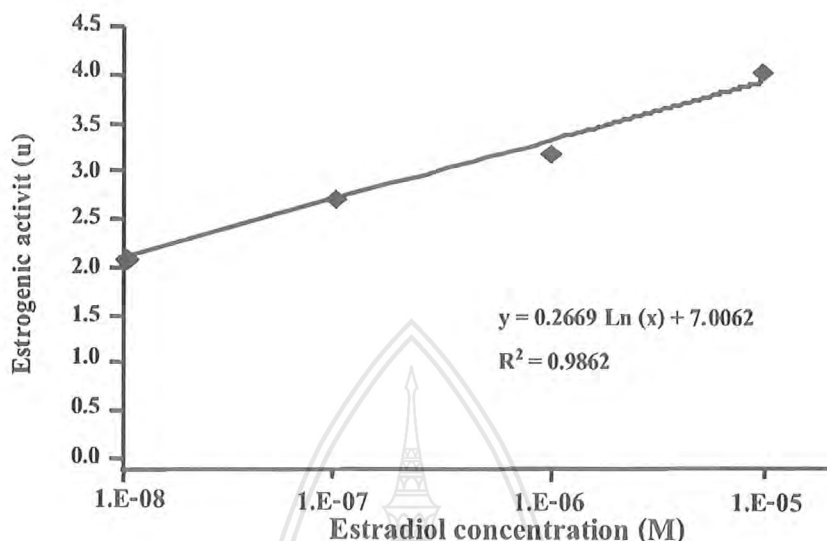


Figure 4. Standard curve of estrogenic activity of 17β -estradiol evaluated by the yeast two hybrid system. The curve showed the estrogenic activity values of 10^{-8} - 10^{-5} M 17β -estradiol, which was dose dependent ($n = 4$). The EC_{50} was calculated from x in equation: $y = (y_{max} + y_{min})/2$.

2. Estrogen activity of *C. xanthorrhiza* Roxb evaluated by yeast two hybrid system

The estrogenic activity of the extracts of 250, 150, and 50 mg/ml *C. xanthorrhiza* Roxb (Wanchuk-mot-luk) was evaluated by the constructed yeast clone. The genetically modified yeast clone could significantly measure the estrogenic activity of *C. xanthorrhiza* Roxb only at the concentration of 250 mg/ml, while negative control of white radish extract of 250 mg/ml showed no estrogenic activity (Figure 5). In addition, *Pueraria mirifica* Roxb (white Kwao-kruae), which was reported of its phytoestrogens and derivatives including miroestrol, daidzin, daidzein,

coumestrol, genistin, puerarin, mirificin, kwakhurin, and mirificoumestan (Chansakaow et al., 2000), was also used as positive control in this experiment. The extracts of *P. mirifica* showed estrogenic activity at all used concentrations: 250, 150 and 50 mg/ml. There was a report on estrogenic activity of *C. xanthorrhiza* Roxb analyzed by the uterotrophic effect in mice (Piyachaturawat et al., 1995), however, there was no report on active estrogenic compounds. Therefore, its estrogenic components and molecular action of *C. xanthorrhiza* Roxb are interesting topics for future investigation.

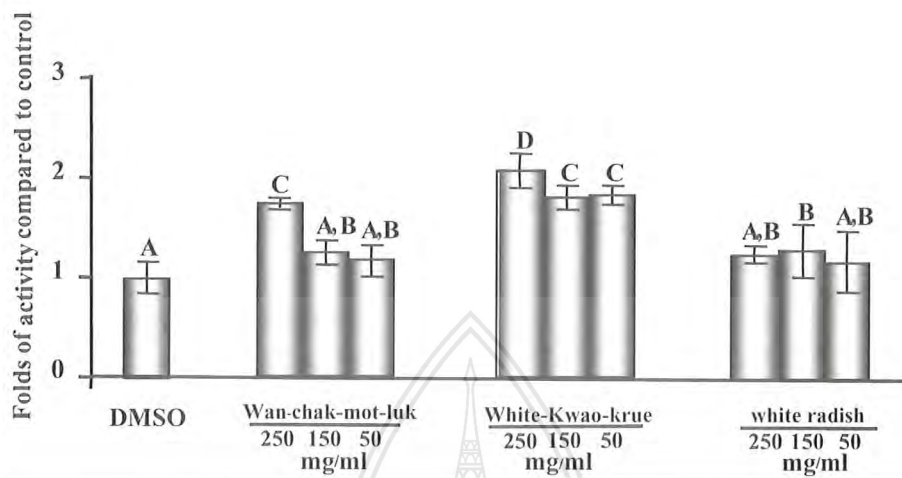


Figure 5. The estrogenic activity of Wan-chak-mot-luk extract. The estrogenic activity of the crude extract of Wan-chak-mot-luk was compared to that of white Kwao-krue (positive control plant), white radish (negative control plant), and DMSO (vehicle) determined by the constructed yeast two-hybrid system (n=4).

CONCLUSION

The present study showed the successful construction of a yeast two hybrid system that could measure the estrogenic activity of 10^{-8} - 10^{-5} M 17β -estradiol in a dose dependent manner. In this experiment, the system could measure the estrogenic activity of the crude extracts of *C. xanthorrhiza* Roxb and *P. mirifica* at the minimal concentration of 50 mg/ml and 250 mg/ml, respectively. In contrast, negative control of white radish extract showed no estrogenic activity. Thus, the constructed yeast two hybrid system could be used to screen phytoestrogens in other Thai medicinal plants with the sensitivity of at least equivalent to 50 mg/ml of *P. mirifica* or 250 mg/ml of *C. xanthorrhiza* Roxb.

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Susceptibility of *Pseudomonas aeruginosa* to Lytic Phage Infection

ความไวของแบคทีเรีย *Pseudomonas aeruginosa* ต่อการติดเชื้อด้วย Lytic Phage

Wilailak Siripornadulsil^{1*} and Saowapa Kiengarm²

Abstract

The *Pseudomonas aeruginosa* (PA) bacteria including 65 clinical and 8 environmental isolates were tested for their susceptibility to PA lytic phage infection using plaque assay and determining by bacterial cell lysis. Bacterial cultures containing 106 CFU/mL were inoculated with 4×10^3 PFU/mL of PA phage to achieve the multiplication of infection (MOI) at the ratio of 1 PA phage particle to 250 bacterial cells. The results showed that 14 clinical isolates and 2 environmental isolates were susceptible to phage infection and exhibited a clear or lytic plaque phenotype on LB agar. All 73 isolates of *P. aeruginosa* were also tested for the biofilm development on 96 well polystyrene microtiter plate by staining with crystal violet dye. Interestingly, 6 of 65 clinical isolates but none of environmental isolates could produce biofilm known as a virulence factor of *P. aeruginosa* and all biofilm producing isolates were resistant to lytic phage. It suggests that biofilm may help protect bacterial cells from PA phage infection. Thus, the direct use of PA lytic phage or in a combination with other treatments could offer an effective approach for controlling the non-biofilm producing or biofilm producing *P. aeruginosa*, respectively.

บทคัดย่อ

จากการศึกษาความไวของแบคทีเรีย *Pseudomonas aeruginosa* (PA) จำนวน 65 ไอโซเลตที่แยกจากผู้ป่วยและ 8 ไอโซเลตที่แยกจากสิ่งแวดล้อม ต่อการติดเชื้อด้วย PA lytic phage โดยทดสอบแบคทีเรีย 10^6 CFU/มล. ด้วย PA lytic phage 4×10^3 PFU/มล. หรือมีค่า multiplication of infection (MOI) ที่มีปริมาณ PA lytic phage 1 อนุภาคต่อแบคทีเรีย 250 เซลล์ ด้วยวิธี plaque assay พบว่าแบคทีเรียที่แยกจากผู้ป่วย 14 ไอโซเลต

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และที่แยกจากสิ่งแวดล้อม 2 ไอโซเลต ทำให้เกิดวงใสของแบคทีเรียหรือ lytic plaque บนอาหารเลี้ยงเชื้อ LB ในการทดสอบการสร้าง biofilm ซึ่งเป็นปัจจัยหนึ่งในการก่อโรคของแบคทีเรีย *P. aeruginosa* โดยศึกษารูปแบบการเจริญของแบคทีเรียบน 96 well polystyrene microtiter plate และย้อมเซลล์ด้วยสี crystal violet พบว่าแบคทีเรียที่แยกจากผู้ป่วย จำนวน 6 ไอโซเลตเท่านั้น ที่มีการสร้าง biofilm และเป็นแบคทีเรียที่ไม่มีความไวต่อการติดเชื้อด้วย PA lytic phage ซึ่งแสดงให้เห็นว่า biofilm อาจทำหน้าที่เป็นเกราะในการป้องกันแบคทีเรียจากการถูกทำลายด้วย PA lytic phage ดังนั้น PA lytic phage จึงมีศักยภาพในการนำไปศึกษาเพื่อใช้ควบคุมแบคทีเรีย *P. aeruginosa* ต่อไป

Keywords: *Pseudomonas aeruginosa*, lytic phage, biofilm

คำสำคัญ: *Pseudomonas aeruginosa*, ไวรัสที่ทำให้เซลล์แบคทีเรียแตก, แผ่นจุลินทรีย์

INTRODUCTION

Pseudomonas aeruginosa (PA) is a ubiquitous Gram negative bacterium found throughout the environment. It is an opportunistic pathogen that infects a wide variety of eukaryotes including humans, animals, insects, and plants. It causes serious and often life-threatening infections in immunocompromized humans such as hospitalized patients and those who are suffering from severe burns, cystic fibrosis, AIDS, undergoing cancer therapy, or recovering from major surgery (Brooun et al., 2000; Hanlon et al., 2001; Whitchurch et al., 2004). The rise of its resistance to a broad range of antibiotics has created an urgent need for alternative treatments for such a serious bacterial infection. *P. aeruginosa* can grow as a biofilm of considerable thickness (> 200 mm) and can secrete large quantities of alginate exopolysaccharide (Hanlon et al., 2001). Biofilm is a complex of bacterial communities adhered together to a substratum surface (Figure 3). In nature, most bacteria are thought to live predominantly in biofilms and *P. aeruginosa* is also a representative model for study of bacterial biofilm (O'Toole and Kolter, 1998; Budzik et al.,

2004). Resistance to antimicrobial agents is a general feature of all biofilms and this structure might be responsible for up to 65 % of all bacterial infections (Brooun et al., 2000; Hentzer et al., 2001). When grown in the biofilm profile, *P. aeruginosa* may resist biocides up to 100 times greater than the planktonic or free-swimming cells typically studied in the laboratory (Brooun et al., 2000).

As an alternative to the use of antibiotics, there are some attempts to use bacteriophages (phages) as antibacterial agents to combat bacterial infection, and this therapeutic strategy has had some success (Barrow and Soothill, 1997; Hagens et al., 2004). Phages were able to migrate through *P. aeruginosa* biofilm by reduction of alginate viscosity to reach the bacterial surface (Hanlon et al., 2001). It has been suggested that the advantage for using phages to control infection is the phage specificity to their bacterial hosts and thus are harmless to other organisms (Hanlon et al., 2001).

In this study, we described an investigation into the susceptibility of both clinical and environmental isolates of *P. aeruginosa* to lytic phage infection. Bacterial biofilm development that may

affect the phage ability in infection was also observed.

MATERIAL AND METHODS

Bacteria

Sixty five clinical isolates of *P. aeruginosa* were isolated from the various clinical specimens of patients in Songklanagarind hospital and they were all resistant to imipenem antibiotic (Tunyapanit, 2004). All 8 isolates of environmental *P. aeruginosa* were isolated from seawater samples.

Isolation of PA phage

PA phage was isolated using plaque assay. Briefly, seawater samples were filtered through 0.22 μm millipore filter. The supernatant was mixed with ~ 106 CFU/mL of each tested bacterial strains including *P. aeruginosa* PSU, *Escherichia coli*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio hollisae*. The mixture was inoculated into 0.7 % Luria Bertani (LB) soft agar, mixed, incubated at room temperature (RT) for 2 minutes and poured onto the pre-poured LB agar plate. Plates were then incubated at 37°C, 24 hrs.

Susceptibility of *P. aeruginosa* to phage infection

PA phage at the titer of 4×10^3 PFU/mL was tested against 10^6 CFU/mL of all 73 *P. aeruginosa* isolates using plaque assay as described previously. Each bacterial isolates were tested in 4 replicates.

Biofilm development of *P. aeruginosa*

P. aeruginosa isolates were inoculated into LB broth and incubated at 37 °C, 18 hrs. Cultures were diluted to 1:10 and 100 μL was added into 96 well polystyrene microtiter plate. The microtiter plate was covered with a sterile lid and placed into a closed container with a water-soaked cotton to maintain the moisture and incubated at 37°C, 24 hrs. After incubation, 100 μL of 1 % filtered crystal violet was added into each well and incubated at RT for 5 minutes. The suspension was discarded and washed 3 times with 200 μL of sterile distilled water. Then, 100 μL of 95 % ethanol was added, mixed gently and measured for the absorbance at 595 nm using microtiter plate reader.

RESULTS AND DISCUSSION

Isolation of PA phage

The PA phage was isolated from the seawater sample using plaque assay. Infection of *P. aeruginosa* PSU by PA phage exhibited the clear plaques on the surface of LB agar stained with crystal violet dye (Figure 1A). The complete clear plaque under the microscope at 400X magnification indicated that PA phage infects *P. aeruginosa* PSU via the lytic cycle (Figure 1B). The PA phage is also specific to only *P. aeruginosa* since it was not able to infect other tested bacteria including *E. coli*, *V. harveyi*, *V. parahaemolyticus* and *V. hollisae* (data not shown).

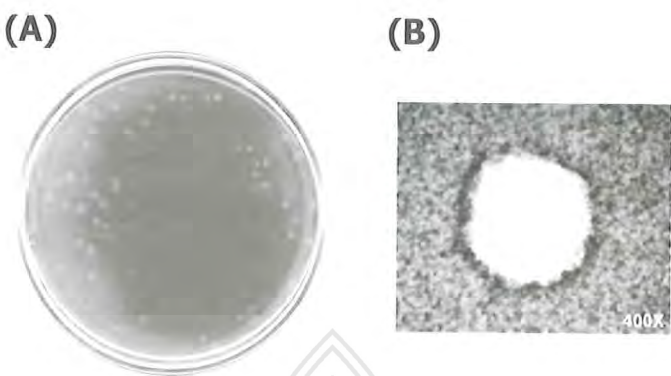


Figure 1. Plaque assay (A) Infection of *P. aeruginosa* PSU by PA phage on LB agar surface stained with crystal violet dye. (B) The complete clear plaque of PA phage under a microscope.

Susceptibility of *P. aeruginosa* to PA phage infection

From plaque assay, 14 of 65 (21 %) clinical and 2 of 8 (25 %) environmental *P. aeruginosa* isolates were susceptible to PA lytic phage infection. The plaque diameter of infected clinical and environmental isolates were various at ranges of 0.5 - 5.0 mm and 0.2 - 0.5 mm, respectively (Table 1 and data not shown). The sensitivity to PA phage is relatively high since only one particle of phage could infect 250 bacterial cells (MOI = 0.004). None of PA phage sensitive isolates were able to produce biofilm and their colonies were either greenish or non-pigmented (Table 1). Thus, the bacterial cells without protection from biofilm may be effectively infected by phage.

Table 1. Properties of PA lytic phage including ability to infect 14 isolates of clinical *P. aeruginosa*, plaque size, greenish pigment formation and biofilm development.

Clinical <i>P. aeruginosa</i>	Plaque diameter (mm)	Pigment	Biofilm
PA1, PA2, PA4, PA10	0.5 – 1.0	+	-
PA65	0.5 – 1.0	-	-
PA14, PA46, PA62	1.0 – 2.0	+	-
PA34	1.0 – 2.0	-	-
PA25	3.0 – 4.0	+	-
PA42, PA50	3.0 – 4.0	-	-

Resistance of *P. aeruginosa* to PA phage infection

Fifty-one isolates of clinical *P. aeruginosa* were resistant to PA phage infection. However, only 6 PA phage resistant isolates showed the development of biofilm and 2 isolates produced the greenish pigment (Table 2 and Figure 2). Thus, biofilm matrix may help protect bacterial cells from phage infection and pigment is unlikely related to phage infection.

Biofilm development of *P. aeruginosa*

Six clinical *P. aeruginosa* isolates were able to develop a biofilm on the surface of a polystyrene microtiter plate (Figure 2). The biofilm development was determined by the quantification of crystal

violet dye bound to the bacterial biofilm. After solubilizing with ethanol, the dye was measured for the absorbance at 595 nm. The control was the dye bound to LB medium on polystyrene plate in the absence of bacteria.

Table 2. Resistance of 6 clinical *P. aeruginosa* isolates to PA lytic phage infection. These isolates are able to develop the biofilm on polystyrene microtiter plate and two isolates produced a greenish pigment on LB agar.

Clinical <i>P. aeruginosa</i>	Phage infection	Pigment	Biofilm
PA16	-	+	+
PA17	-	-	+
PA20	-	+	+
PA41	-	-	+
PA44	-	-	+
PA52	-	-	+

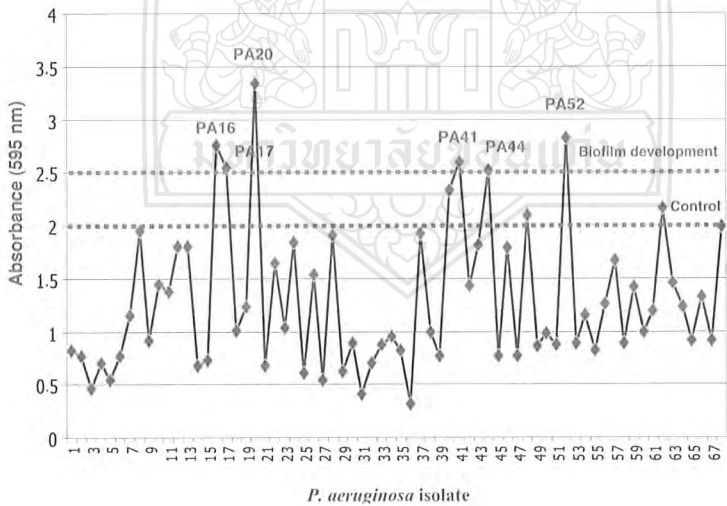


Figure 2. Biofilm development of 6 clinical *P. aeruginosa* isolates including PA16, PA17, PA20, PA41, PA44 and PA52. The biofilm formation was determined by quantification of the absorbance (595 nm) at the value 0.5 higher than that of control.

A model for bacterial biofilm development and phage resistance

P. aeruginosa are thought to live in the biofilm complex (O'Toole and Kolter, 1998; Budzik et al., 2004). The model of biofilm formation and its role in phage resistance is proposed. Planktonic cells swim by flagella-mediated motility and adhere to the substratum surface and bacterial community living together in the biofilm is formed and developed by type IV pili-mediated motility (O'Toole

and Kolter, 1998; Davey and O'Toole, 2000). The planktonic cells are free living and thus the individual cells are easy to expose to phage contact and infection. However, the bacterial cells within the biofilm are clumped together and surrounded by alginate exopolysaccharide matrix. This biofilm structure may prevent the PA phage from penetrating through the bacterial cells and thus, make it resistant to phage infection (Figure 3).

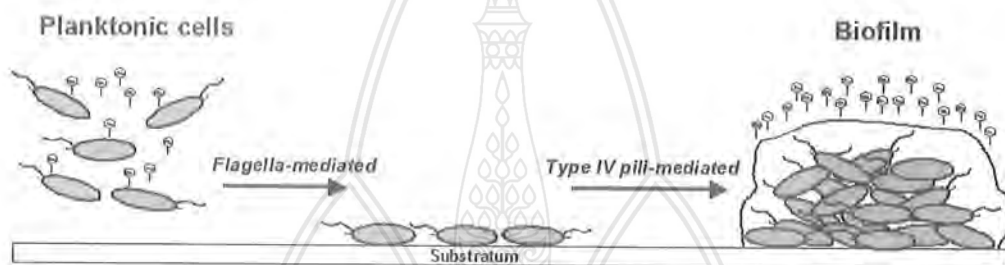


Figure 3. A model for bacterial biofilm development and phage resistance (adapted from O'Toole and Kolter, 1998; Davey and O'Toole, 2000).

CONCLUSIONS

We have demonstrated that the PA lytic phage is able to infect both clinical and environmental isolates of *P. aeruginosa*. Susceptibility of *P. aeruginosa* to phage infection is not correlated to pigment production. However, the efficiency of phage infection is affected by biofilm in which its densely packed nature may inhibit phage from reaching the bacterial cell surface. Thus, the direct use of PA lytic phage or in a combination with other treatments could offer an effective approach for controlling the non-biofilm producing or biofilm producing *P. aeruginosa*, respectively. There is evidence suggesting that the type IV pili of

P. aeruginosa is involved in biofilm and/or phage attachment (Craig et al., 2004). The functional role of *P. aeruginosa* type IV pili is currently under investigation.

ACKNOWLEDGMENTS

We thank Ms. Wanutsanun Tunyapanit for kindly providing all clinical isolates of *P. aeruginosa*. This work was supported by a grant from the Prince of Songkhla University.

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The Efficiency of Effective Microorganisms (EM) on Oil and Grease Treatment of Food Debris Wastewater

ประสิทธิภาพของจุลินทรีย์อีเอ็มในการบำบัดน้ำมันและไขมัน ในน้ำทิ้งเศษอาหาร

Surasak Siripornadulsil^{1*} and Wimonnuch Labteephanao²

Abstract

The aim of this study is to characterize the efficiency of effective microorganisms (EM) in removing oil and grease from food debris wastewater. The results showed that the amount of oil and grease were reduced 99.36, 91.41, 87.80 and 85.91 % after being treated with 10 % (v/v) commercial EM of the following trademarks; EX-M, Nongbour-Ubon, Q-Se and Podo-6, respectively. In contrast, the amount of oil and grease of wastewater without EM or control was also reduced 31.83 %. Interestingly, the numbers of lipase producing bacteria of all treated-EM were 10 - 50 times higher than the control. In addition, the lipase activities of isolated bacteria from all treated-EM are 20 % higher than the isolated bacteria from the control. All selected isolates of lipase producing bacteria were identified by biochemical tests as *Bacillus* spp.

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของจุลินทรีย์ EM ในการบำบัดน้ำมันและไขมันในน้ำทิ้งตามบ้านเรือน ผลการศึกษาพบว่า ปริมาณน้ำมันและไขมันลดลงร้อยละ 99.36, 91.41, 87.8 และ 85.91 จากการบำบัดด้วยจุลินทรีย์ EM ที่มีชื่อทางการค้า หนองบัวอุบล กิวนช พด. 6 และ EX-M ตามลำดับ ในขณะที่ชุดควบคุมที่ไม่ได้เติม EM มีปริมาณน้ำมันและไขมันลดลงเพียงร้อยละ 31.83 นอกจากนี้ปริมาณของเชื้อแบคทีเรียที่สามารถสร้างเอนไซม์ไลเปสได้ถึงหมักที่เติมจุลินทรีย์ EM มีค่าสูงกว่าถึงหมักควบคุม ประมาณ 10 - 50 เท่า เมื่อคัดเลือกแบคทีเรียที่สร้างเอนไซม์ไลเปสจำนวน 10 ไอโซเลต จากถังหมักที่เติม EM มาทำการวัดกิจกรรมของเอนไซม์ไลเปสพบว่า แบคทีเรียที่แยกได้จากถังหมักที่เติมจุลินทรีย์ EM ทั้ง 4 ชนิด มีกิจกรรมของเอนไซม์ไลเปสที่ใกล้เคียงกัน แต่สูงกว่าแบคทีเรียจากถังหมักควบคุมประมาณ 20% จากการทดสอบโดยวิธีทางชีวเคมีพบว่าแบคทีเรียทุกไอโซเลตที่สามารถผลิตเอนไซม์ไลเปสได้ จัดอยู่ในจีนัสบาซิลลัส

Keywords: Effective microorganisms (EM), food debris wastewater, lipase producing bacteria

คำสำคัญ: จุลินทรีย์ที่มีประสิทธิภาพ, น้ำทิ้งเศษอาหาร, แบคทีเรียที่สร้างไลเปส

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INTRODUCTION

Food waste has increased annually. Oil, fat, and grease are important components in food waste which creates severe damage on the environment. They can form oil films on water surfaces preventing oxygen diffusion from air into water, resulting in death of aquatic organisms. Improperly managed oil and grease from houses and restaurants has become a significant problem for wastewater collection and treatment systems. Fat, oil, and grease coats accumulate in pipes, pumps, and equipment; leading to the costly and hazardous flow of waste grease into drain lines.

Recently, the bioremediation of lipid-rich wastes has been studied by using a variety of microorganisms (Brahimi-horn et al., 1991; Borja et al., 1994; Zinebi et al., 1994; Stams and Elferink, 1997; Dharmsthiti and Kuhasuntisuk, 1998; Mongkolthanaruk and Dharmsthiti, 2002). The technology of Effective Microorganisms (commonly termed EM Technology) was developed in the 1970's by Professor Teruo Higa (Higa, 1991; Higa and Wididana, 1991). EM contains selected species of microorganisms, which can be commonly found in many ecosystems, including lactic acid bacteria (LAB), yeasts, actinomycetes photosynthetic bacteria, and other types of organisms. These were blended in a molasses or sugar medium and maintained at a low pH under ambient conditions.

In this study, we have investigated the potential of EM to lower the lipid content of lipid-rich food debris wastewater.

MATERIAL AND METHODS

Growth of EM Organisms

The EM organisms used in this study were derived from four different commercial EMs in trademarks of EX-M, Nongbour-Ubon, Q-Se and Podo-6. The growth of EM organisms during inoculant preparation and food wastewater treatment were performed at room temperature in static condition. For inoculant preparation, the cells were grown in 10 % (v/v) molasses for 5 days in static condition. The EM organisms' growth was determined by viable plate count. Total bacteria, lactic acid bacteria (LAB), and yeast were counted on Plate Count Agar (PCA: 0.5 % tryptone, 0.25 % yeast extract, 0.1 % dextrose, and 1.5 % agar, pH 7.0), MRS agar (1 % casein peptone, 1 % meat extract, 0.5 % yeast extract, 2 % glucose, 0.1 % Tween-80, 0.2 % K₂HPO₄, 0.5 % Na-acetate, 0.2 % (NH₄)₂ citrate, 0.02 % MgSO₄·7H₂O, 0.005 % MnSO₄·H₂O and 1.5 % agar, pH 6.5) and YPD agar (1 % yeast extract, 2 % peptone, 2 % dextrose and 1.5 % agar, pH 7.0) respectively.

Lipid-rich food debris wastewater treatment

The lipid-rich food debris wastewater was collected from the food center at Khon Kaen University (KKU) (Figure 1A). The preparation of each EM inoculant was performed according to the commercial instructions. Treatment experiments were performed in 1.5 L EM bucket with 10 % (v/v) inoculum, initial cell count approximately $2.0 - 7.0 \times 10^8$ cells/mL for 15 days at room temperature with static condition (Figure 1B). The control did not add EM. The population of total bacteria, lactic acid bacteria and yeast were counted by total plate count everyday for 8 days as shown in Figure 2.

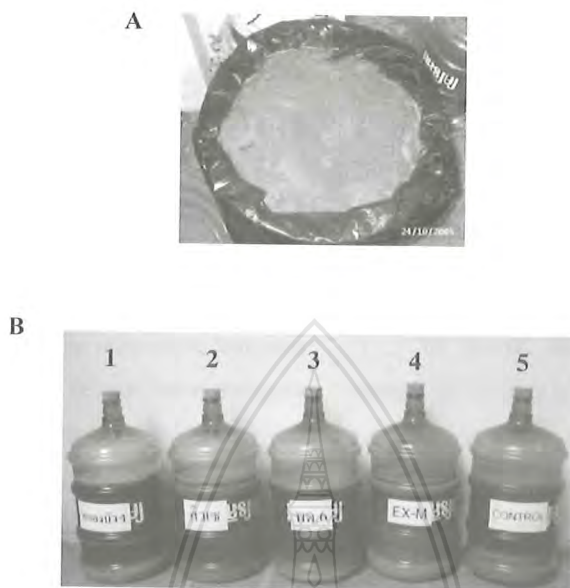


Figure 1. (A) The lipid-rich food debris wastewater. (B) Fermentation of food debris wastewater: 1, Nongbour-Ubon; 2, Q-Se; 3, PoDo-6; 4, EX-M and 5, control.

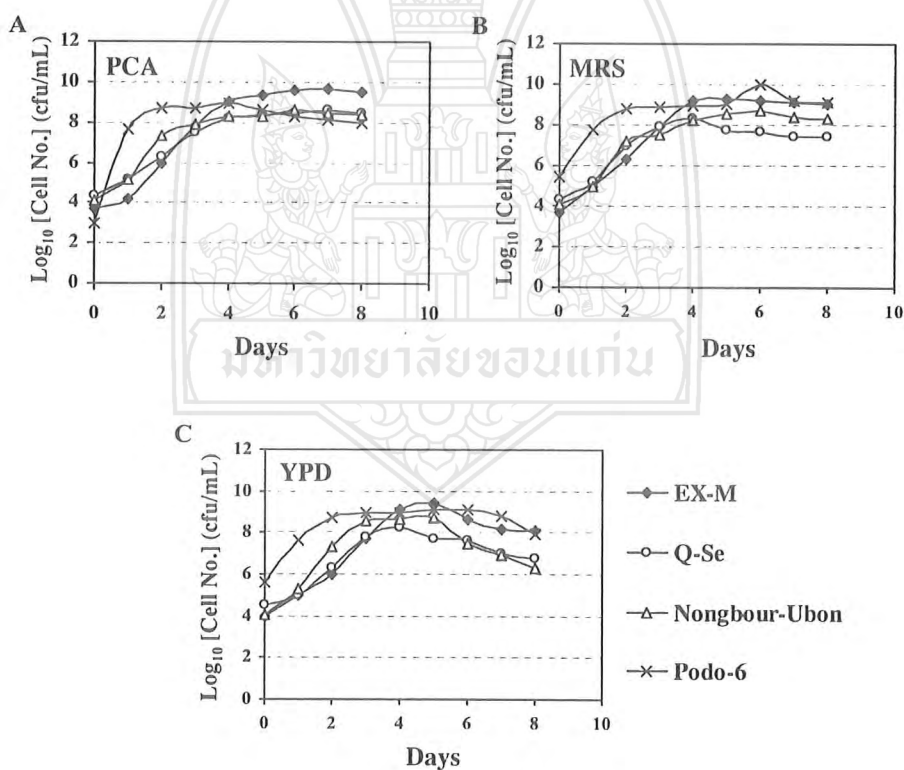


Figure 2. Growth of EM organisms during inoculant preparation at room temperature with static condition. (A) Total bacteria on PCA medium, (B) LAB on MRS medium and (C) yeast on YPD medium.

Determination of lipid content

Lipid content was determined by Soxhlet extraction method (Environmental Engineering Association of Thailand, 2004). A 50 mL acidic sample, adjusted with HCl to pH (2.0) was used for each assay. Then the solution was filtered through 3 layers of muslin. Lipid in food debris wastewater was extracted repeatedly with 20 mL of 1, 1, 2-trichloro-trifluoroethane (freon) at 47 °C by Soxhlet extractor (Buchi B-811). The extracted lipid was oven-dried at 103 °C for 10 min. The remaining dry weight indicated the presence of oil, fat, and grease in the sample. Relative lipid content percentage was measured as a following equation; (Remaining lipid dry weight/Initial lipid dry weight) X 100

Isolation and identification of EM Organisms from EM Product

Lipase-producing bacteria from lipid-rich food debris wastewater treatment of each EM product (including EX-M, Nongbour-Ubon, Q-Se, and Podo-6) and control were isolated and selected for high lipase activity. The bacteria were further identified by biochemical tests.

Lipase Assay

The selected lipase-producing bacteria were grown in minimal medium (0.03 % yeast extract, 0.1 % Tween 80, 0.05 % K_2HPO_4 , 0.5 % $(NH_4)_2SO_4$, 0.03 % $MgSO_4 \cdot 7H_2O$, 0.05 % $CaCO_3$, 0.01 % gum arabic and 1.5 % agar, pH 7.0) containing 2 % coconut or palm oil for 48 hrs. The bacterial cells were removed by centrifugation at 6,000 rpm for 10 min at 4 °C. The supernatant was determined for lipase activity and determined by colorimetric method based on the activity in cleavage of *p*-nitrobutyrate

(pNPB) (Margesin et al., 2002). Lipase activity was expressed as μg pNPB (mL of enzyme solution X 10 min).

RESULTS

Growth of EM organisms

The growth of EM organisms during inoculant preparation and lipid-rich food wastewater treatment were showed in Figures 2 and 3, respectively. For the inoculant preparation, the growth of total bacteria, LAB, and yeast of all EM trademarks was similar (Figure 2). In contrast, the lipid-rich food wastewater treated with Podo-6 showed the lowest numbers of total bacteria, LAB and yeast (Figure 3A, 3B, 3C). The numbers of lipase-producing bacteria were highest for wastewater treated with EX-M and followed by Q-Se, Nongbour Ubon, Podo-6 when grown on the minimal medium containing coconut oil (Figure 3D). However, the numbers of lipase-producing bacteria were quite similar for all EM treatments when grown on minimal medium containing palm oil (Figure 3E).

Determination of Lipid Content

Lipid content of lipid-rich food wastewater during the treatment was shown in Figure 4. The results showed that EX-M was the most effective EM in decreasing the lipid content within 6 days followed by Nongbour-Ubon, Q-Se and Podo-6.

Lipase Assay

Lipase activity of isolated bacteria from lipid-rich wastewater treatment were investigated. The ability of lipase to degrade coconut oil and palm oil was shown in Figures 5 and 6, respectively. The results showed that the lipase

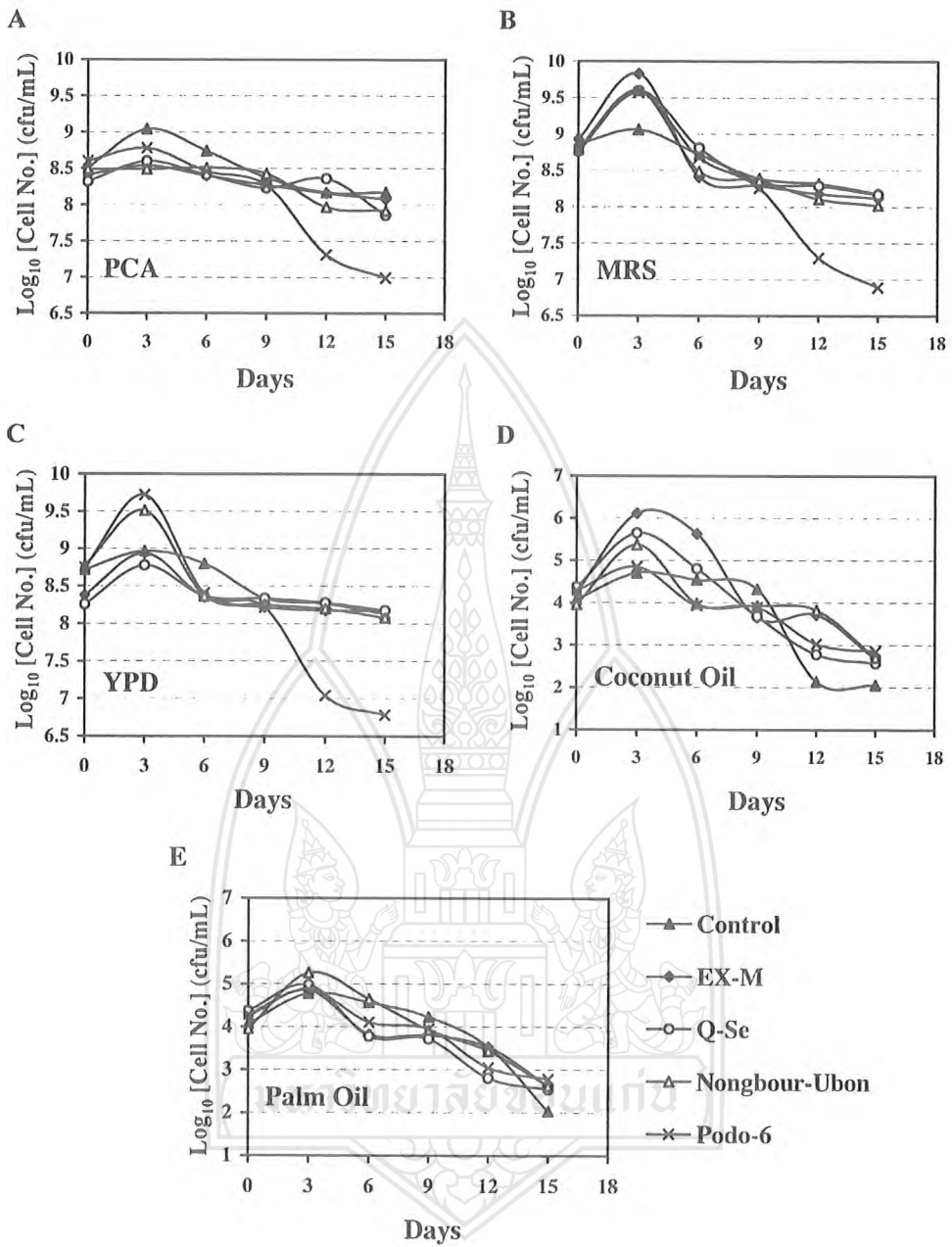


Figure 3. Growth of EM organisms during lipid-rich food wastewater treatment. (A) Total bacteria on PCA medium, (B) LAB on MRS medium, (C) yeast on YPD medium, (D) and (E) lipase-producing bacteria grown on the minimal medium containing coconut and palm oil, respectively.

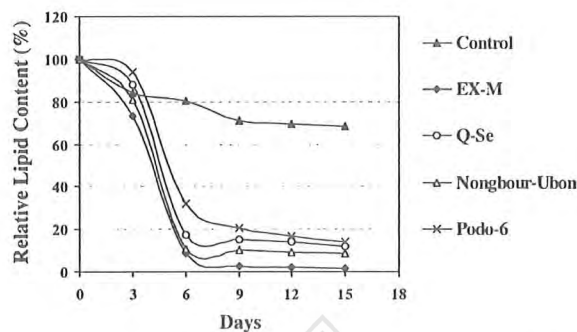


Figure 4. Relative lipid content of lipid-rich food debris wastewater after treatment with EM.

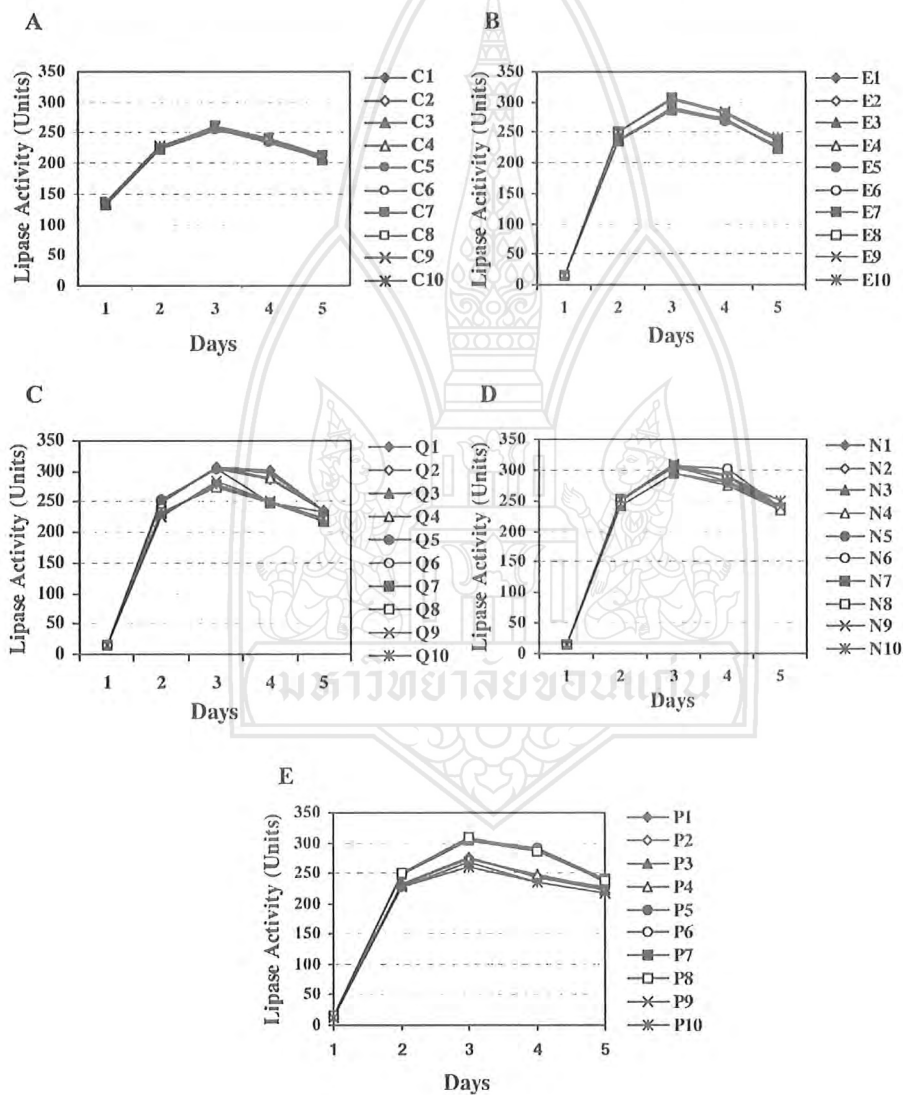


Figure 5. Lipase activity of bacteria isolated from lipid-rich food debris wastewater. The activity was studied on the ability to degrade coconut oil. (A) Control; (B) EX-M; (C) Q-Se; (D) Nongbour-Ubon; (E) Podo-6.

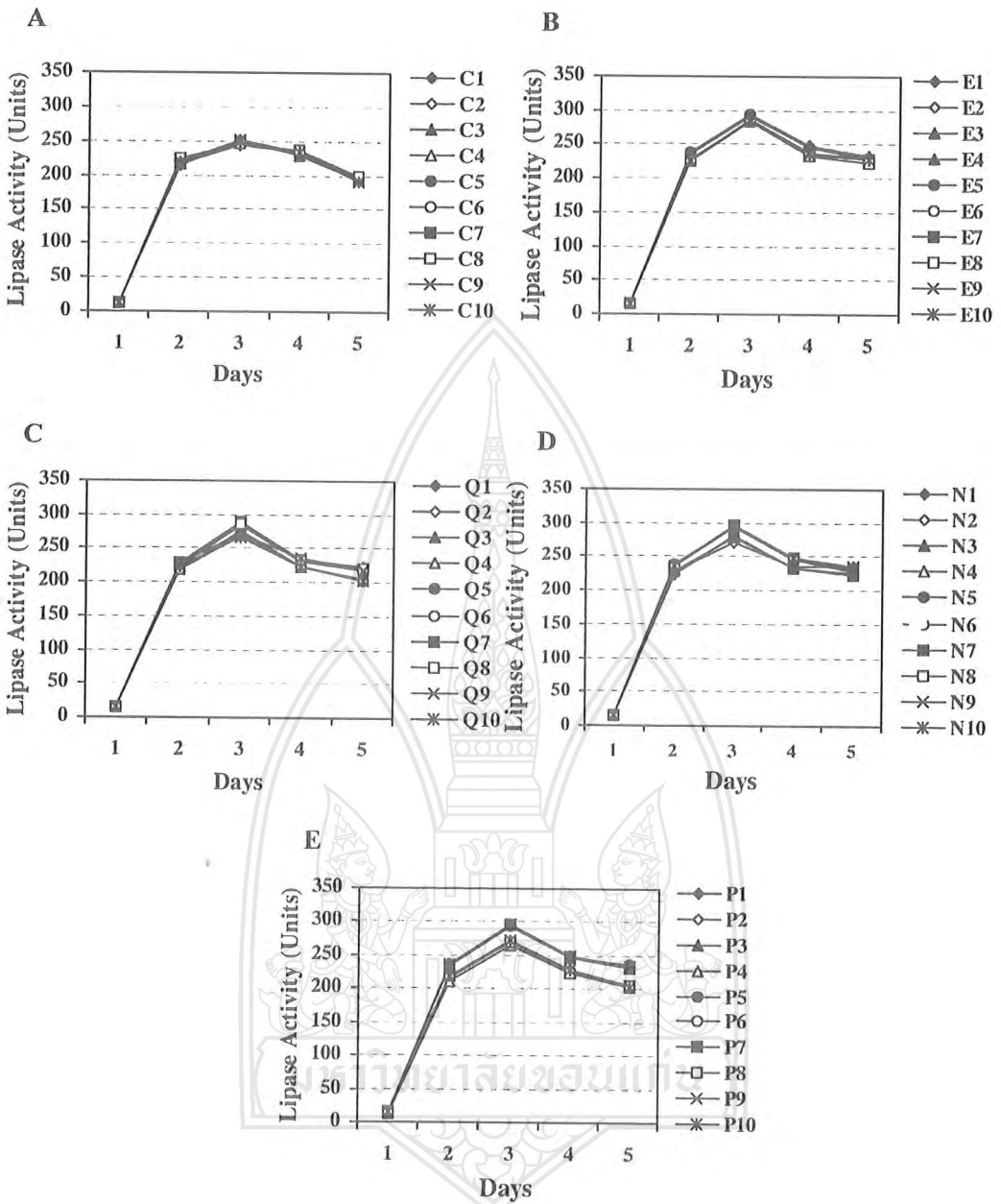


Figure 6. Lipase activity of bacteria isolated from lipid-rich food debris wastewater. The activity was studied on the ability to degrade palm oil. (A) Control; (B) EX-M; (C) Q-Se; (D) Nongbour-Ubon; (E) Podo-6.

activity in degrading coconut oil and palm oil of isolated bacteria from lipid-rich wastewater treatment was approximately 20 % higher than those isolated from the control.

DISCUSSION

We have studied and characterized the efficiency of four different commercial EMs to remove oil and grease in lipid-rich food debris wastewater collected from the food center at KKU. We found that EX-M was the most effective for increasing of the growth of coconut oil-degrading bacteria (Figure 3D). We also found that EX-M was the most effective EM for elimination of oil, fat and grease by 99.36 % within 6 days followed by Nongbour-Ubon (91.41 %), Q-Se (87.80 %) and

Podo-6 (85.91 %) (Figure 4). In contrast, the amount of oil and grease of untreated-EM or control was also reduced 31.83%. In addition, the numbers of lipase producing bacteria of all treated-EM were 10 - 50 times higher than the control. The results that all EM microorganisms might create a suitable condition for growth of lipid-producing bacteria. Furthermore, the lipase activities of bacteria isolated from all treated-EM were 20 % higher than the ones isolated from untreated control. The biochemical characteristics of all lipase-producing bacteria isolated from both treated-EM and control were identical to a member of *Bacillus* sp. (Table 1) as described by BERGY's Manual of Systematic Bacteriology (1986).

Table 1. Characteristics of lipase-producing bacteria.

Test	Characteristic
Gram's stain	Positive
Cell morphology	Rod
Motility	-
Catalase	+
Oxidase	-
Utilization of citrate	-
Deamination of phenylalanine	-
Hydrolysis of gelatin	-
Nitrate reduction	-/+
Lysine decarboxylase	-
Methyl red (MR/VP)	+/- or -/+
Triple Sugar Iron (TSI)	A/A, K/A

Note : + = positive result, - = negative result
: A = acid production, K = alkaline production

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Experimental Infection of Aquatic Fungi on Eggs of African Catfish (*Clarias gariepinus* Burch)

การทดลองการติดเชื้อราของไข่ปลาตุกรัสเซีย (*Clarias gariepinus* Burch)

Chutima Hanjavanit^{1*}, Nilubol Kitancharoen² and Charuwan Rakmanee³

Abstract

Experimental infection of 4 isolates of aquatic fungi: *Saprolegnia diclina* MHSK 01, *Achlya* sp. MHSK 06, *Achlya ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 at concentrations of 1×10^1 , 1×10^2 , 1×10^3 and 1×10^4 zoospores/mL, respectively, on eggs of African catfish (*Clarias gariepinus* Burch) were conducted. The results showed that 1×10^4 zoospores/mL could induce the highest infection that led to the highest mortality and the lowest hatching rate. In a comparison study, it showed that *A. ambisexualis* MHSK 07 was more virulent than other isolates. Histological examination of the infected eggs showed numerous pores on the egg envelopes and there were fungal hyphae penetrated and accumulated in the cytoplasm.

บทคัดย่อ

การทดลองการติดเชื้อรา 4 ชนิด คือ *Saprolegnia diclina* สายพันธุ์ MHSK01 *Achlya* sp. สายพันธุ์ MHSK 06 *Achlya ambisexualis* สายพันธุ์ MHSK 07 และ *Aphanomyces* sp. สายพันธุ์ MHSK 12 ที่ระดับความเข้มข้น 1×10^1 , 1×10^2 , 1×10^3 และ 1×10^4 สปอร์ต่อมิลลิลิตร ตามลำดับ ต่อไข่ปลาตุกรัสเซีย (*Clarias gariepinus* Burch) พบว่าเชื้อราที่ความเข้มข้น 1×10^4 สปอร์ต่อมิลลิลิตร สามารถชักนำให้ไข่ปลาตุกรัสเซียเกิดการติดเชื้อราได้สูงที่สุด ทำให้มีอัตราการตายของไข่ปลาสูงที่สุดและมีอัตราการฟักเป็นตัวต่ำที่สุด ผลการศึกษาเปรียบเทียบความรุนแรงในการติดเชื้อรา พบว่า *A. ambisexualis* สายพันธุ์ MHSK 07 เป็นสายพันธุ์ที่มีความรุนแรงมากกว่าสายพันธุ์อื่น และผลการตรวจสอบลักษณะทางจุลพยาธิวิทยาของไข่ปลาที่ติดเชื้อราพบมีรูจำนวนมากเกิดขึ้นที่เยื่อหุ้มไข่ เนื่องจากเส้นใยราแทรกผ่านทะลุเยื่อหุ้มไข่และไปสะสมที่ไซโทพลาซึม

Keywords: Aquatic fungi, Eggs of African catfish (*Clarias gariepinus* Burch), Experimental infection

คำสำคัญ: ไรน้ำ, ไข่ปลาตุกรัสเซีย (*Clarias gariepinus* Burch), การทดลองการติดเชื้อ

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INTRODUCTION

In seed production of African catfish (*Clarias gariepinus* Burch), loss during egg stage is one of the factors which decreases the number of the production. The main reduction is caused by a fungal infection (Viveen et al., 1986). Fungal infection on eggs has also been reported from many fish species (Czeczuga and Woronowicz, 1993; Czeczuga and Muszynska, 1997; Kitancharoen and Hatai, 1997; Kitancharoen et al., 1998). The objectives of this study were to test the possibility of infection of *Saprolegnia diclina* MHSK 01, *Achlya* sp. MHSK 06, *Achlya ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 on healthy eggs of African catfish by direct contact with various concentrations of zoospores to examine histopathological infected eggs.

MATERIAL AND METHOD

1. African catfish eggs

Egg samples of African catfish were obtained from Sripan farm hatchery, Yangnoi District, Amphur Kosumphisai, Maha Sarakham Province and were brought back to the Department of Biology, Faculty of Science, Khon Kaen University. Only healthy eggs were selected for experiment and they were washed 3 times with sterilized tap water (STW).

2. Fungal isolates

Four isolates of aquatic fungi: *S. diclina* MHSK 01, *Achlya* sp. MHSK 06, *A. ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 derived from infected eggs of African catfish and common carp (*Cyprinus carpio*) were used in this experiment. The fungi were cultured on Glucose Yeast Extract

Agar (GY agar), incubated at 25 °C and were sub-cultured every 2 - 4 weeks.

3. Zoospore production preparation

Edges of 2 days fungal colony were cut with a No.2 cork borer (5.5 mm in diameter) and placed in a Petri dish containing 20 mL of Glucose Yeast extract Broth (GY broth), and then incubated at 25 °C for 24 - 48 h. Only mycelia were cut, washed with STW for 3 times, then transferred into 20 mL of STW and incubated at 25 °C for 16 - 20 h for zoospore production. Zoospore emergence was observed under an inverted microscope (Nikon Phase Contrast-2 ELWD 0.3, Japan). Numbers of zoospores were determined using a haemocytometer (improved Neubauer, Erma®).

4. Experimental infection

250 to 500 eggs of African catfish were used for each control and test group. The test groups were exposed to *S. diclina* MHSK 01, *Achlya* sp. MHSK 06 and *A. ambisexualis* MHSK 07 with zoospore concentrations of 1×10^1 , 1×10^2 , 1×10^3 and 1×10^4 zoospores/mL, respectively, whereas some test groups were exposed to *Aphanomyces* sp. MHSK 12 with 1×10^1 and 1×10^2 zoospores/mL, respectively, due to *Aphanomyces* sp. could not be induced to produce at 1×10^3 and 1×10^4 zoospores/mL. Complete Randomized Design with 5 treatments was used in this experiment. Three replicates of each experiment were carried out. The control and the test groups were held separately in 200 mL plastic boxes with well-aerated, sterilized well water at 25 °C. The infection, mortality and hatching rates of fish eggs were investigated after being exposed to the zoospores for 29 - 34 h.

5. Histological preparation

The healthy and infected eggs (eggs covered with fungal mycelia) (Figure 1) were fixed in 10 % phosphate buffer formalin for 24 h and

were processed for routine paraffin embedding. The serial sections were cut at 5 μ m and stained by Haematoxylin and Eosin (H&E), Periodic acid Schiff (PAS) reagent, Giemsa and Grocott (Humason, 1972).

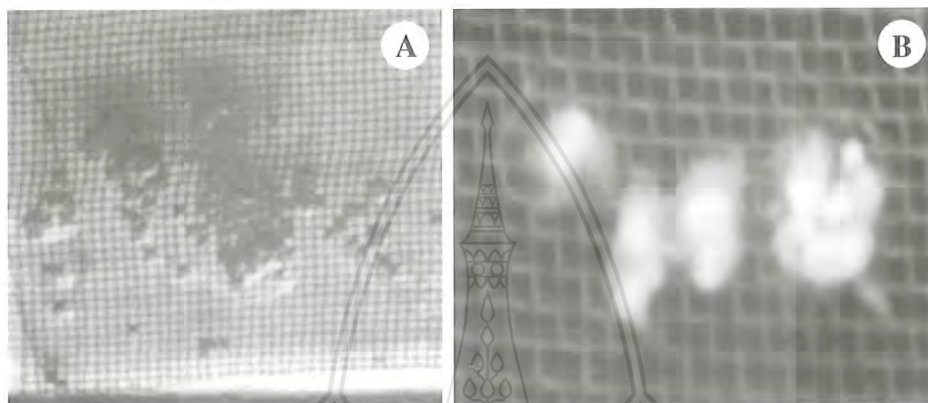


Figure 1. African catfish eggs. (A.) Healthy eggs. (B.) Infected eggs showing fungal hyphae.

6. Data analysis

Percentages of infection and hatching rates of fish eggs were calculated by formula of Barnes et al. (1998).

$$\text{Infection rate} = \frac{\text{number of eggs covered with fungal mycelia} \times 100}{\text{total number of fish eggs}}$$

$$\text{Hatching rate} = \frac{\text{number of hatching eggs} \times 100}{\text{total number of fish eggs}}$$

Percentage of corrected mortality rate of fish eggs was calculated by Abbott's formula

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100$$

Pt = percentage of corrected mortality

Po = mortality rate of test group

Pc = mortality rate of control group

Comparison data of percentages of infection, hatching and mortality rates of fish eggs between the control and test groups were analyzed with One-way ANOVA by SPSS 9.0 for Window (Norusis, 1993).

RESULTS

Effects of 4 fungal isolates on infection, hatching and mortality rates on African catfish eggs

The result showed that all isolates: *S. diclina* MHSK 01, *Achlya* sp. MHSK 06, *A. ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 could infect on both live and dead eggs. At the beginning, fungal mycelia developed on some dead eggs and then infested the adjacent live eggs. The comparison study of virulence effect of each isolate at various concentrations showed that *A. ambisexualis* MHSK

07 at concentration 1×10^4 zoospores/mL exhibited the highest infection rate (93.98 ± 1.44 %), the lowest number of hatching rate (8.80 ± 1.25 %) and the highest corrected mortality rate (89.92 ± 2.37 %) with significant difference from other concentrations ($P < 0.05$) but it was not significantly different from *S. diclina* MHSK 01 at 1×10^4 zoospores/mL (88.06 ± 11.33 % infection rate, 10.85 ± 1.30 % hatching rate and 85.90 ± 1.68 % mortality rate, respectively) ($P > 0.05$) (Figure 2). *Achlya* sp. MHSK 06 at 1×10^4 zoospores/mL could also cause higher infection rate, lower hatching rate and higher mortality rate significantly different from those of 1×10^1 , 1×10^2 and 1×10^3 zoospores/mL ($P < 0.05$) (Figure 2A, B, C). Although *Aphanomyces*

sp. MHSK 12 at 1×10^1 and 1×10^2 zoospores/mL were able to infect the healthy eggs (4.85 ± 0.40 and 8.09 ± 2.53 % infection rate) with no significant difference ($P > 0.05$) but it was significantly different from the control group ($P < 0.05$), whereas their effects on hatching and mortality rates were not significantly different from the control group ($P > 0.05$) (Figure 2B, C).

As shown in Figure 2A, B and C, the comparison study of virulence effect of different isolates showed that *S. diclina* MHSK 01 and *A. ambisexualis* MHSK 07 caused higher percentage of infection rate, lower percentage of hatching rate and higher percentage than those of *Achlya* sp. MHSK 06 and *Aphanomyces* sp. MHSK 12

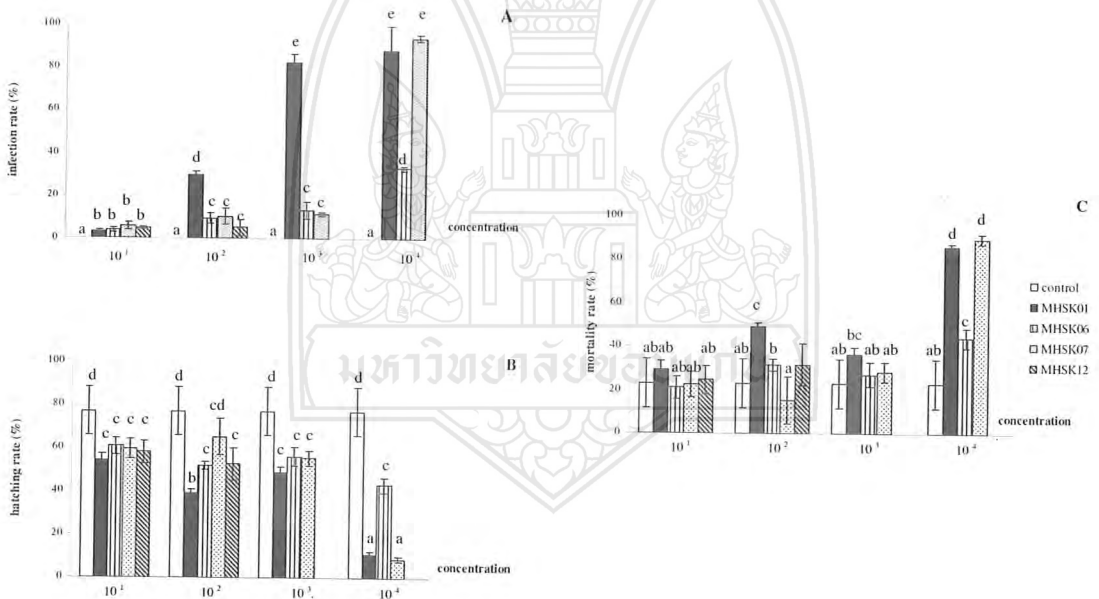


Figure 2. Effects of various concentrations (zoospores/mL) of fungi on African catfish eggs for (A.) Infection rate, (B.) Hatching rate, and (C.) Mortality rate. Error bars are + 1SD. Histograms with the same letter are not significantly different, where the different letters are significantly different among isolates and concentration ($p < 0.05$).

Histopathological examination

As presented in Figure 3A, the histological examination of normal healthy eggs of the control group showed 2 layers of egg envelopes. The outer envelope was dark pink and the inner envelope was pale pink (H&E, PAS and Giemsa stain) and was stained black with Grocott which could not distinguish the two layers. The cytoplasm was composed of yolk granules which were blue with H&E stain, positively with PAS reaction, pink with Giemsa and dark brown with Grocott. Histopathological examination of infected eggs showed numerous fungal hyphae attached on the outer surface of the whole eggs (Figure 3B). There were numerous pores on egg envelopes and fungal hyphae could penetrate through egg envelope (Figure 3C) and accumulated in yolk granules (Figure 3D). Some infected eggs showed germinated zoospores in the cytoplasm (Figure 3E). The width range of accumulated hyphae in yolk granules from each test group was also measured as follows: 1.25 - 7.50, 2.50 - 12.50, 1.25 - 7.50 and 1.25 - 2.50 μm ($n = 10$), respectively, which was the same width range of hyphae of *S. diclina* MHSK 01, *Achlya* sp. MHSK 06, *A. ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12, respectively, which were used in this experiment. Therefore, it could be confirmed that accumulated hyphae in yolk granules germinated from the same zoospores of each isolate that was used for this experimental infection.

DISCUSSION AND CONCLUSION

Outbreaks of waterborne fungal infections are a common problem in aquaculture facilities and fish hatcheries and are wide spread in all stages of

life cycle of fish from egg to adult stage (Hussein et al., 2000). According to Scott and O'Bier (1962), members of the family Saprolegniaceae, particularly genus *Saprolegnia* could be isolated from many species of fish eggs such as rainbow trout (*Salmo gairdneri*), brook trout (*Salvelinus fontinalis*), largemouth bass (*Micropterus salmoides*), Northern pike (*Esox lucius*), brown trout and genus *Aphanomyces* from rainbow trout. Kitancharoen and Hatai (1997) could isolate *Aphanomyces frigidophilus* from eggs of Japanese char (*Salvelinus leucomaenis*). Hussein and Hatai (1999) reported *S. salmonis* from eggs of sockeye salmon (*Onchrhynchus nerka*). According to Yuasa et al. (2000), *S. diclina* was isolated from infected eggs of African catfish (*Clarius gynepinus*), *Saprolegnia* sp. from snakeskin gourami (*Trichogaster pectoralis*), *Achlya flagellata* from eggs of common carp (*Cyprinus carpio*), *Achlya* sp. from eggs of snakeskin gourami, *Achlya bisexualis* from eggs of barb (*Labiobarbus burmanicus*) and *Aphanomyces* sp. from eggs of tilapia (*Oreochromis niloticus*) and eggs of rohu (*Labeo rohita*) from fish hatcheries, Northeast, Thailand.

In the present study, zoospores of *S. diclina* MHSK 01, *Achlya* sp. MHSK 06, *A. ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 could infect the healthy fish eggs of African catfish which was depending on the concentrations of zoospores. Especially 1×10^4 zoospores/mL of *S. diclina* MHSK 01, *Achlya* sp. MHSK 06, *A. ambisexualis* MHSK 07 were more effective than those of 1×10^1 , 1×10^2 and 1×10^3 zoospores/mL. Therefore, apparently increasing of zoospore numbers led to an increased degree of infection and mortality rates while the degree of hatching rate declined corresponding to the number of zoospores. Whereas

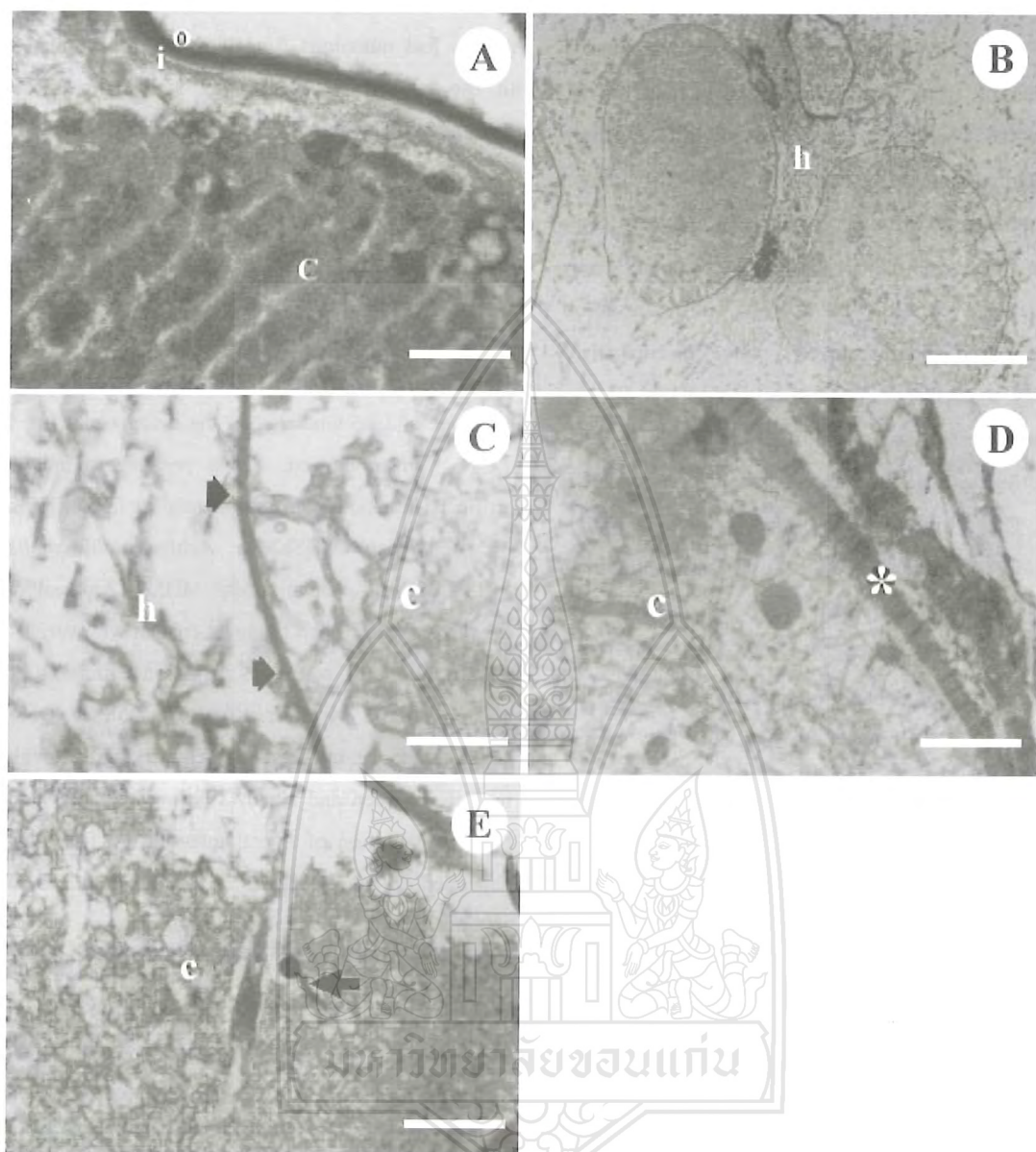


Figure 3. Cross-section of African catfish eggs. (A) Normal live egg of the control group showing two layers of egg envelope (o = outer envelope, i = inner envelope, c = cytoplasm). (H&E, bar = 25 μ m) (B) Eggs of the test group showing numerous fungal hyphae (h) attached on egg envelopes. (PAS, bar = 250 μ m) (C - D) Eggs of the test group showing fungal hyphae (h) penetrated (arrow) into cytoplasm (c) and numerous pores (*) on egg envelope. (PAS, H&E, bar = 25 μ m) (E) Some eggs of the test group showing germinated zoospore (arrow) in cytoplasm (c). (H&E, bar = 25 μ m).

effect on infection rate of *Aphanomyces* sp. MHSK 12 at 1×10^2 zoospores/mL was not significantly different from that of 1×10^1 zoospores/mL ($P > 0.05$) but they were significantly different from that of the control group ($P < 0.05$). Furthermore, the effect of *Aphanomyces* sp. MHSK 12 on hatching and higher mortality rates were not significantly different from those of 1×10^1 , 1×10^2 zoospores/mL and the control group ($P > 0.05$). The comparison study showed that *A. ambisexualis* MHSK 07 was more virulent than those of *S. diclina* MHSK 01, *Achlya* sp. MHSK 06 and *Aphanomyces* sp. MHSK 12. This is similar to those reports that of *S. diclina* NJM 005 saprophyte could cause less infection than *S. parasitica* NJM 8604 and *S. ferax* NJM 9580 which were pathogens (Kitancharoen and Hatai, 1997). The results of isolates on infection, hatching and mortality rates of African catfish eggs may suggest that *A. ambisexualis* MHSK 07 is a pathogen, whereas *S. diclina* MHSK 01, *Achlya* sp. MHSK 06 and *Aphanomyces* sp. MHSK 12 are saprophytes.

According to Post (1983); Kitancharoen and Hatai (1996); Paxton and Willoughby (2000), the occurrence of dead eggs seemed to be an enriched nutritional substrate and an important condition for fungal growth and also enhanced their growth distribution to the surrounding healthy eggs. Kitancharoen and Hatai (1997) stated that surrounding hyphal growth could reduce the water circulation around the live eggs and block oxygen transportation, thus contributing to the death of the eggs. In the observation in this study, the dead eggs were susceptible to fungal infection but not to the live eggs. From the present study, the artificial infection was successful and was revealed with fungal hyphae in infected eggs from histopathological

examination. It showed that dead eggs of the test groups had numerous fungal mycelia accumulated in the cytoplasm which was similar to those reported by Paxton and Willoughby (2000). Numerous pores were also found on the egg envelopes of the infected eggs. This is possibly due to the normal characteristics of dead eggs which were damaged or the fungi released enzymes to degrade the structural components of the egg envelope (Rand and Munden, 1992) in having hyphae penetrated across the egg envelope and accumulated in the cytoplasm.

In conclusion, it was possible to induce artificial infections of *Saprolegnia diclina* MHSK 01, *Achlya* sp. MHSK 06, *Achlya ambisexualis* MHSK 07 and *Aphanomyces* sp. MHSK 12 to healthy eggs of African catfish by direct contact with various concentrations of zoospores. The concentrations of zoospores and virulent effect of isolates were suggested to be important which was agreed with by Kitancharoen and Hatai (1996), who had stated that the causation of fungal infection on fish eggs depended on fungal species, their concentrations of zoospores and species of fish eggs.

In addition, the importance of a routine protocol in the African catfish hatchery should eliminate the dead eggs to reduce the concentration of fungal zoospores during the incubation period in order to decrease the opportunity of fungal infection on fish eggs.

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Effect of Atrazine on Spermatogenesis of Mice

(*Mus musculus* Linn.)

ผลของสารอะทราซีนต่อการสร้างสเปิร์มของหนูเมาส์

(*Mus musculus* Linn.)

Ampa Luangpirom^{1*} and Tussaneeporn Junsanjuk²

Abstract

Atrazine is widely used as a non-selective herbicide. Earlier studies had shown that atrazine cause the production of aromatase, an enzyme that converts androgens (male sex hormones) into estrogens (female sex hormones). These results led us to hypothesize that this herbicide could disrupt endocrine function of male reproduction. To evaluate this hypothesis, we examined the effect of atrazine on the spermatogenesis of mice (*Mus musculus* Linn.) by gaving this herbicide at doses of 0, 200 and 300 mg/kg BW for 28 days and determined the result at 1, 14 and 28 days after treatment. Both treated groups after 1 and 14 days of atrazine withdrawal reveal that number of sperm count, number of viable sperms and number of normal moving sperms were significantly decreased at $p < 0.05$, but number of abnormal sperms was high in both treated groups. Histological examination also showed corresponding result with the decreasing number of spermatid and spermatocyte layers, which were significantly different from the control group at $p < 0.05$. This effect was temporary and reversible after 28 days of atrazine withdrawal.

บทคัดย่อ

อะทราซีนเป็นสารปราบวัชพืชแบบไม่เลือก มีการใช้อย่างกว้างขวางและสารนี้ยังสามารถเพิ่มการผลิตเอนไซม์อะโรมาเตสที่ใช้ในการเปลี่ยนฮอร์โมนแอนโดรเจนเป็นฮอร์โมนอีสโตรเจนในเพศผู้ การศึกษาครั้งนี้เพื่อตรวจสอบผลของสารอะทราซีนต่อการสร้างสเปิร์มของหนูเมาส์ โดยการป้อนสารขนาด 0, 200 และ 300 มิลลิกรัม/กิโลกรัมน้ำหนักตัว เป็นเวลา 28 วัน ตรวจสอบผลจากคุณภาพน้ำเชื้อและจุลพยาธิสภาพของอวัยวะหลังหยุคให้สาร 1, 14 และ 28 วัน พบว่าหลังหยุคให้สาร 1 และ 14 วัน กลุ่มทดลองทั้งสองกลุ่มมีจำนวน

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สเปิร์มทั้งหมด จำนวนสเปิร์มที่มีชีวิต และจำนวนสเปิร์มที่เคลื่อนที่ปกติ มีจำนวนลดลงอย่างมีนัยสำคัญที่ $P > 0.05$ แต่จำนวนจำนวนสเปิร์มที่มีรูปร่างผิดปกติมีจำนวน สเปิร์มเพิ่มขึ้นทั้งสองกลุ่ม และคุณภาพน้ำเชื้อ ไม่แตกต่างจากกลุ่มควบคุมหลังหยดให้สาร 28 วัน ส่วนผลการตรวจจุลพยาธิของอวัยวะ พบว่ามีผลสอดคล้อง กับคุณภาพน้ำเชื้อ โดยกลุ่มทดลองทั้งสองกลุ่มหลังหยดได้รับสาร 1 และ 14 วัน มีจำนวนชิ้นสเปิร์มมาติกและ จำนวนชิ้นสเปิร์มมาโตไซต์ลดลงอย่างมีนัยสำคัญที่ $P > 0.05$ และมีจำนวนชิ้นสเปิร์มมาโตไซต์ไม่แตกต่างจาก กลุ่มควบคุมหลังหยดให้สาร 28 วัน การศึกษานี้สรุปได้ว่าสารอะทราซีนมีผลรบกวนการสร้างสเปิร์มแบบ ชั่วคราวและสามารถกลับมาเป็นปกติหลังหยดให้สาร 28 วัน

Keywords: atrazine, mice, spermatogenesis

คำสำคัญ: อะทราซีน, แม้าส์, การสร้างสเปิร์ม

INTRODUCTION

Atrazine is a member of a family of chloro-s-triazine that is widely used as a nonselective herbicide. This is one of the top selling products from a large chemical company (Stevens et al., 1999). It inhibited photosynthesis in plants and has some neuromuscular toxicity at high dose, including motor in co-ordination, limb paralysis, respiratory distress and hypothermia in laboratory animals (IARC,

1991). Atrazine that is persistent in soil and water, for over one year is the most common contaminant of ground surface and river water (IARC, 1991).

Earlier studies have found that atrazine cause the production of aromatase, an enzyme that convert androgen (male sex hormone) into estrogens (female sex hormone) which are shown in Figure 1. (Sanderson et al., 2000; 2001)

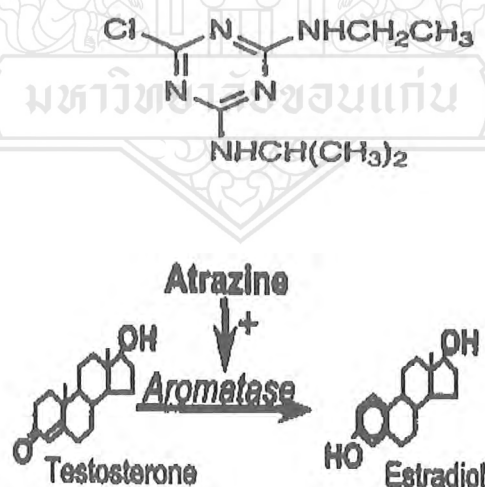


Figure 1. Conversion of androgen into estrogen by aromatase.

The adverse effect of atrazine on gonadal development was also found in african clawed frogs (*Xenopus laevis*) in the laboratory (Tavera-Mendoza et al., 2002). Carr (1992) reported that atrazine is an endocrine disruptor that demasculinized and feminized male amphibian. The adverse effect of this compound was also studied in rats (Kniewald et al., 2002), but its reverse effect have not been well characterized. These reasons led us to hypothesize that this herbicide may disrupt endocrine function of spermatogenesis of mice and may be reversible after withdrawal.

MATERIAL AND METHODS

Atrazine's solution

A commercial herbicide product containing atrazine 80 % (KEMER Co. Ltd.) was diluted with distilled water at dose of 200 and 300 mg/ml.

Animals

All experiments were conducted in the animal room of Biology department, at Khon Kaen University, Thailand. One week before the start of each experiment, eight week-old male mice were purchased and housed in room at temperature = 25 ± 2 °C, dark:light cycle = 10:14, food and water were provided *ad libitum* throughout the study.

Experiment

The total number of thirty mice were divided into 3 groups and each group was divided into 3 subgroups having 6 animals in each subgroup. Atrazine's solution was administered at

dose of 0, 200 and 300 mg/kg BW for 28 days into their respective subgroups. All groups were killed on day 1, 14 and day 28 after final feeding, respectively. The testes were dissected, fixed in Bouin's fluid, embedded in paraffin, sectioned at 7 μ m thickness and stained with hematoxylin and eosin. Section of testis were examined histology of spermatocyte and spermatid layers. Vas deferens and epididymis were dissected for seminal analysis.

Statistics

Statistical significance between the control and experimental data were subjected to analysis of variance (one way ANOVA) together with Duncan's multiple range test ($p < 0.05$)

RESULTS

Histological studies

Histological examination of the testes in the control group were found that spermatocyte and spermatid layers were 2.17 ± 0.18 and 3.76 ± 0.42 , respectively. Groups treated with atrazine at 200 and 300 mg/kg BW showed a significant decrease in the number of spermatocyte layers (1.70 ± 0.26 and 1.68 ± 0.24) and spermatid layers (2.35 ± 0.25 and 2.19 ± 0.28), respectively ($p < 0.05$) (Table 1, Figure 2: A1 - A3). After withdrawal on days 14, both treated groups found significant decrease in the number of spermatocyte and spermatid layers ($p < 0.05$). However, neither treated groups showed significant changes in the number of spermatid and spermatocyte layers from control group after 28 days of atrazine withdrawal (Figure 2: B1 - B3).

Table 1. Testis histological examination of mice after 1 days of atrazine withdrawal.

atrazine (mg/kg BW)	spermatogonium layer ($\bar{X} \pm SE$)	spermatocyte layer ($\bar{X} \pm SE$)	spermatid layer ($\bar{X} \pm SE$)
0	0.76 ± 0.25^a	2.17 ± 0.18^a	3.76 ± 0.42^a
200	0.47 ± 0.27^b	1.70 ± 0.26^b	2.35 ± 0.25^b
300	0.39 ± 0.26^c	1.68 ± 0.24^b	2.19 ± 0.28^c

$\bar{X}$ = mean, SE = standard error
Same alphabet = not significant different
Different alphabet = significant different at $p < 0.05$

Seminal analysis

Sperm count of control group $52.88 \pm 4.73 \times 10^6$ cell/ml. The mice given atrazine at dose of 200 and 300 mg/kg BW for 28 days showed a significant decrease in sperm count 43.50 ± 4.11 and $28.91 \pm 3.17 \times 10^6$ cell/ml (Table 2, Figure 3: 3A), number of moving sperms 48.63 ± 6.71 and $43.07 \pm 5.28 \%$ (Table 2, Figure 4: 4A) and number of viable sperms $45.23 \pm 4.59 \%$ and $40.78 \pm 4.83 \%$ ($p < 0.05$) (Table 2, Figure 5: 5A) with concomitant significant increase in the number of abnormal sperms $52.01 \pm 4.21 \%$ and $56.17 \pm 5.26 \%$ (Table 2, Figure 6: 6A). After atrazine withdrawal for 14 days, both treated groups still showed lower qualification of seminal fluid than control group (Figure 3: 3B, Figure 4: 4B, Figure 5: 5B and Figure 6: 6B). However, after 28 days of withdrawal, seminal analysis were found to be not significantly different from control group (Figure 3: 3C, Figure 4: 4C, Figure 5: 5C and Figure 6: 6C).

Table 2. Number of sperm count, moving sperms, viable sperms and abnormal sperms of mice after 1 day of 28 days.-long atrazine treatment.

atrazine mg/kg BW	sperm count (cells $\times 10^6$ /ml)	moving sperms (%)	viable sperms (%)	abnormal sperms (%)
0	52.88 ± 4.73^a	60.09 ± 4.76^a	68.41 ± 5.87^a	31.75 ± 6.11^a
200	43.50 ± 4.11^b	48.63 ± 6.71^b	45.23 ± 4.59^b	52.01 ± 4.21^b
300	28.91 ± 3.17^c	43.07 ± 5.28^b	40.78 ± 4.83^b	56.17 ± 5.26^b

Same alphabet = not significant different
Different alphabet = significant different at $p < 0.05$

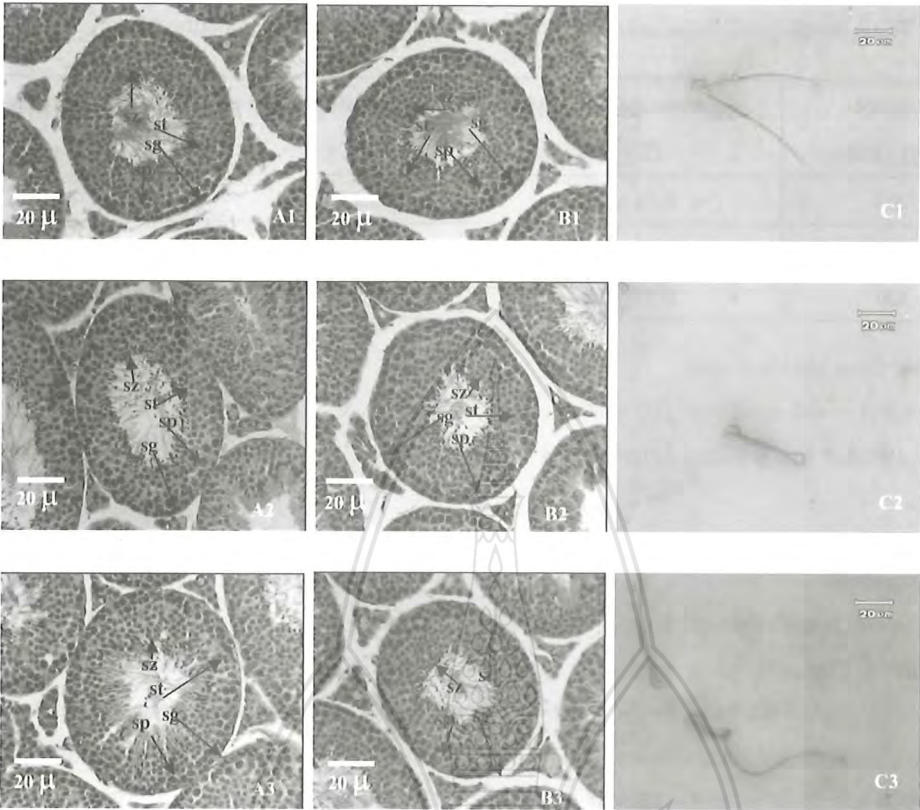


Figure 2. Cross section of testis: A = 1 day after final treatment; B = 28 days after 28 days long atrazine treatment, 1, 2, 3 = mice treated with atrazine 0, 200 and 300 mg/kg BW, sg = spermatogonium, sp = spermatocyte, st = spermatid, sz = spermatozoa; C = abnormal sperm: C1 = intermedial cytoplasmic droplet, C2 and C3 = bent tail sperm

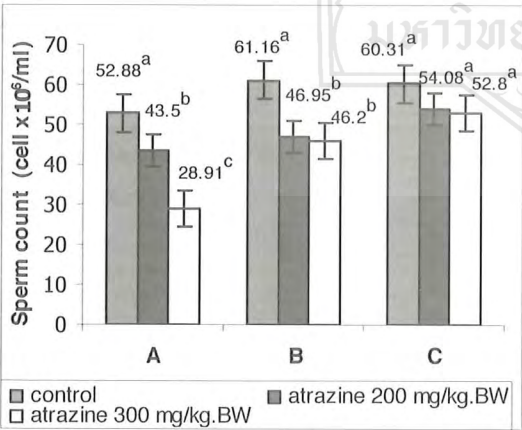


Figure 3. Sperm count of mice after atrazine treatment 1 (A), 14 (B) and 28 days (C).

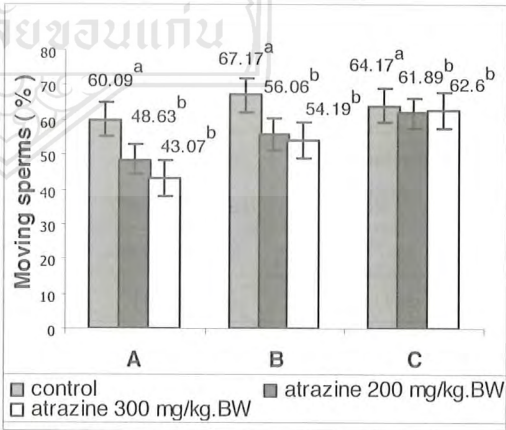


Figure 4. Moving sperms of mice after atrazine treatment 1 (A), 14 (B) and 28 days

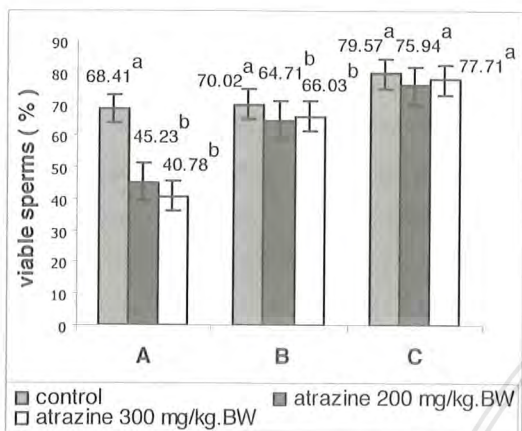


Figure 5. Viable sperms of mice after atrazine treatment 1 (A), 14 (B) and 28 days (C).

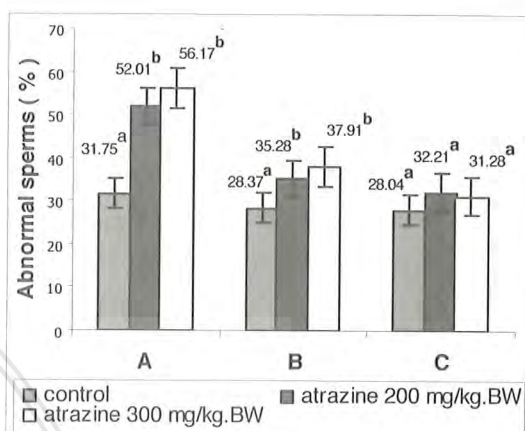


Figure 6. Abnormal sperms of mice after atrazine treatment 1 (A), 14 (B) and 28 days (C).

DISCUSSION

Our data demonstrated that atrazine at dose of 200 and 300 mg/kg BW. interrupted spermatogenesis of mice, evidence as Table 1, Figure 2 and Table 2. The result of seminal analysis was found that the sperm count, moving sperms and viable sperms were significantly decreased, but number of abnormal sperms showed high incidence in higher doses. Similar observation have also been studied by Kniewald et al. (2000) and Sanderson et al. (2001) which reported that atrazine cause the production of aromatase, an enzyme that converts androgen into estrogen. Hayes (2003) conducted laboratory studies and found that the blood testosterone level of male frogs decreased with the presence of atrazine 25 ppb in the water which they grew. However, our study also found that adverse effects of atrazine on spermatogenesis of mice reveal improvement by showing seminal qualification of both treated groups after atrazine withdrawal 28 days as control group. These are the results for interruption of testicular function.

This study also used histological examinations of the testis section. It was revealed that spermatocyte layers and spermatid layers significantly decreased which corresponded with the results of seminal analysis. When the treatment was ceased for 28 days, we found that histological result and seminal analysis were not different from the control group. Revised et al. (1996) reported that atrazine was metabolized and excreted by urine 20 %, feces 55 % and deposited in adipose tissue 25 % after 9 - 12 days of consumption. Our studies concluded that the effect of atrazine on the impairment of spermatogenesis was reversible after 28 days of atrazine withdrawal. This finding may have implication for human reproduction health.

ACKNOWLEDGMENTS

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Palynological Study of *Piper* L. (Piperaceae) in Thailand

การศึกษาเรณูของพืชสกุลพริกไทยในประเทศไทย

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Yupadee Paopun⁴

Abstract

A palynological study of 16 species of *Piper* L. in Thailand was undertaken using light and scanning electron microscopy. The pollen is monad, heteropolar, monocolpate, kidney shaped or spheroidal. The ornamentation of pollen can be classified into four types: psilate, scabrate, fossulate and rugulate with sparsely distributed spinules. Pollen data does not support macromorphological evidence for the identification of Thai *Piper* species.

บทคัดย่อ

ศึกษาสัณฐานวิทยาเรณูของพืชสกุลพริกไทยในประเทศไทยจำนวน 16 ชนิด ด้วยกล้องจุลทรรศน์และกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด พบว่าเป็นเรณูเดี่ยว มีขั้วชนิดขั้วต่าง มีช่องเปิดยาว 1 ช่อง รูปร่างเรณูคล้ายรูปไตหรือค่อนข้างกลม สามารถจำแนกสัณฐานเรณูออกเป็น 4 กลุ่ม คือ psilate scabrate fossulate และ rugulate ที่สันมี spinules ในการศึกษาครั้งนี้ลักษณะทางสัณฐานวิทยาเรณูที่ได้ ไม่สนับสนุนการนำมาจำแนกชนิดพืชสกุลพริกไทยในประเทศไทย

Keywords: pollen, *Piper*, Thailand

คำสำคัญ: เรณู, พืชสกุลพริกไทย, ประเทศไทย

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INTRODUCTION

The genus *Piper* L. is pantropical, with ca. 1,000 species and belongs to the family Piperaceae (Mabberley, 1987). The genus is a well-defined genus of woody climbers, herbs, small shrubs or scandents (Henderson, 1959; Huber, 1987). Thirty-four species are recognized in Thailand (Suwanphakdee, 2005). The leaves are often simple, entire and mostly alternate. The flowers are tiny, bisexual or unisexual, borne in compact spikes or rarely racemes which are usually leaf-opposed. The ovary is superior and surrounded by the bracts which are variable in shape. There is no perianth. The fruit is a small drupe. Lei and Xing (1998) investigated the pollen of some Chinese species. The results reveal that the pollen is 1-colpate and there is great uniformity in pollen morphology among the studied species except for differences of ornamentation which vary from verrucate to almost psilate. There is no detailed description and comprehensive study of the Thai *Piper* pollen, and therefore there is a need for this palynological research.

MATERIAL AND METHOD

Pollen grains of 16 species of the genus *Piper* in Thailand were examined by light microscopy (LM) and scanning electron microscopy (SEM). Pollen was obtained from material collected

in the wild by the first author. For LM studies, pollen was acetolysed according to Erdtman (1972), mounted in silicone oil and sealed with paraffin. Ten pollen grains of each species were measured. For SEM, pollen was directly collected from dried specimens and affixed to aluminum stubs with carbon tape and sputter-coated with a gold-palladium mixture. The samples were photographed and examined with a JEOL (JSM5600 LV) SEM at the Scientific Equipment Center, Research and Development Institute, Kasetsart University. The pollen data of the specimens examined is compiled in Table I. The descriptive terminology follows Erdtman (1972) and Faegri and Iversen (1975).

RESULTS

Table 1 summarizes the quantitative results obtained for the different parameters of the species studied. The pollen grains are more or less homogenous (Table 1, Figures 1 and 2). They are monads, heteropolar, monocolpate, spheroidal or kidney-shaped. The pollen size is minute to small ($P = 7 - 10 \mu\text{m}$, $E_1 = 8 - 14 \mu\text{m}$ and $E_2 = 6 - 9 \mu\text{m}$). The aperture is simple with a colpate form. The colpi are between 6 - 9 μm at distal ends. Exine is 0.7 - 1 μm thick. The exine sculpturing is psilate, scabrate, fossulate or rugulate with sparsely distributed spinules (Figure 3).

Table 1. Pollen morphology of studied species of Thai Piper.

No.	Species	Specimen/herbarium	P (μm)	E ₁ (μm)	E ₂ (μm)	Ex (μm)	C (μm)	Sc
1	<i>P. belle</i> L.	C. Suwanphakdee 103 (BKF)	8	8.3 ± 0.57 (7-9)	7.6 ± 0.36 (7-8)	0.8	7	rugulate+spinules
2	<i>P. boehmeriifolium</i> (Miq.) C.DC.	C. Suwanphakdee 42 (BKF)	8	9	7.3 ± 0.35 (7-8)	1	7	rugulate+spinules
3	<i>P. caninum</i> Blume	C. Suwanphakdee 114 (DMSC)	7	8.3 ± 0.31 (8-9)	7.8 ± 0.33 (7-8)	0.8	6	rugulate+spinules
4	<i>P. colubrinum</i> Link	C. Suwanphakdee 1 (DMSC)	8	10	9	0.7	7	scabrate
5	<i>P. kurzii</i> Ridl.	C. Suwanphakdee 120 (BKF)	9	10.7 ± 0.32 (10-11)	8.1 ± 0.78 (7-9)	0.8	7	fossulate
6	<i>P. longum</i> L.	C. Suwanphakdee 48 (KKU)	8	10	8.3 ± 0.35 (8-9)	1	7	scabrate
7	<i>P. nigrum</i> L.	C. Suwanphakdee 14 (DMSC)	8	10.6 ± 0.32 (10-11)	8.7 ± 0.36 (8-9)	-	-	rugulate+spinules
8	<i>P. polycarpa</i> Ridl.	C. Suwanphakdee 107 (KKU)	7.3 ± 0.34 (7-8)	8.9 ± 0.66 (8-10)	7.7 ± 0.36 (7-8)	1	6	rugulate+spinules
9	<i>P. ramipilum</i> C.DC.	C. Suwanphakdee 105 (BKF)	8.7 ± 0.38 (8-9)	9.6 ± 0.27 (9-10)	7.2 ± 0.34 (7-8)	0.8	7.2 ± 0.35 (7-8)	psilate
10	<i>P. sarmentosum</i> Roxb.	C. Suwanphakdee 147 (BK)	8	10.5 ± 0.29 (10-11)	9	0.8	-	scabrate
11	<i>P. umbellatum</i> L.	C. Suwanphakdee 76 (KKU)	9.8 ± 0.34 (9-10)	13.2 ± 0.95 (11-14)	10.2 ± 0.66 (9-11)	1	7.7 ± 0.34 (7-8)	fossulate
12	<i>P. sp. 1</i>	C. Suwanphakdee 100 (DMSC)	8.7 ± 0.34 (8-9)	11.9 ± 0.60 (11-13)	7.4 ± 0.37 (7-8)	1	6.5 ± 0.37 (6-7)	-
13	<i>P. sp. 2</i>	C. Suwanphakdee 101 (KKU)	7.8 ± 0.33 (7-8)	9.3 ± 0.70 (9-10)	7.2 ± 0.67 (6-8)	1	6.1 ± 0.69 (5-7)	rugulate+spinules
14	<i>P. sp. 3</i>	C. Suwanphakdee 145 (KKU)	9.6 ± 0.36 (9-10)	8.7 ± 0.32 (8-9)	8.6 ± 0.37 (8-9)	1	8.3 ± 0.73 (7-9)	rugulate+spinules
15	<i>P. sp. 4</i>	C. Suwanphakdee 124 (BKF)	8.8 ± 0.33 (8-9)	9.6 ± 0.31 (9-10)	8.7 ± 0.37 (8-9)	0.8	7.3 ± 0.67 (6-8)	fossulate
16	<i>P. sp. 5</i>	C. Suwanphakdee 119 (BKF)	7.6 ± 0.36 (7-8)	9.4 ± 0.31 (9-10)	7.6 ± 0.38 (7-8)	0.7	6.7 ± 0.34 (6-7)	rugulate+spinules

P = polar axis, E₁ = equatorial diameter 1, E₂ = equatorial diameter 2, Ex = exine thickness, C = colpus length, Sc = sculpturing at the SEM level.

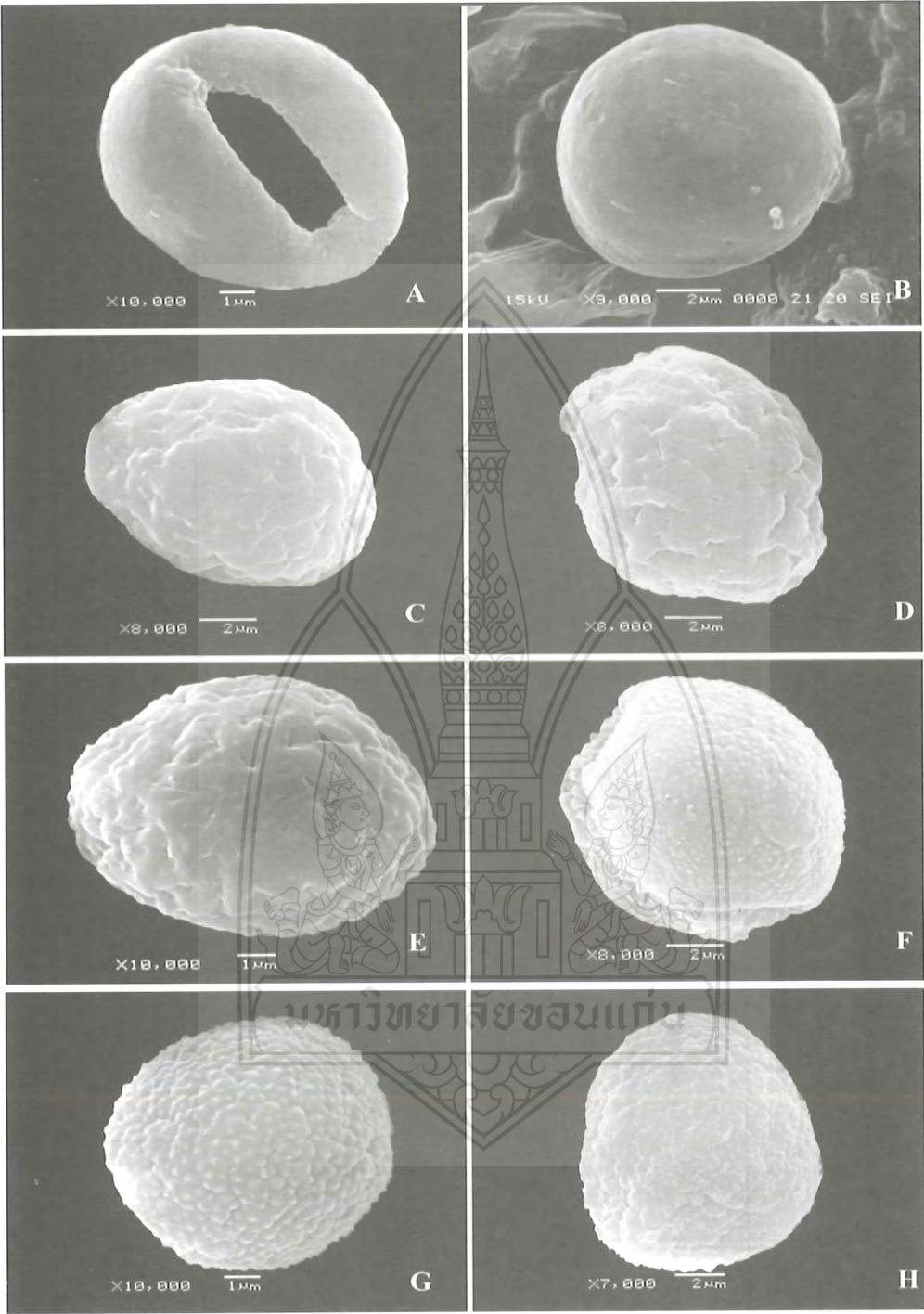


Figure 1. Scanning electron micrographs of pollen grain of genus *Piper* in Thailand: A. polar view of *P. ramipilum*, B. equatorial view of *P. ramipilum*, C. *P. kurzii*, D. *P. umbellatum*, E. *P. sp.4*, F. *P. colubrinum*, G. *P. longum* and H. *P. sarmentosum*.

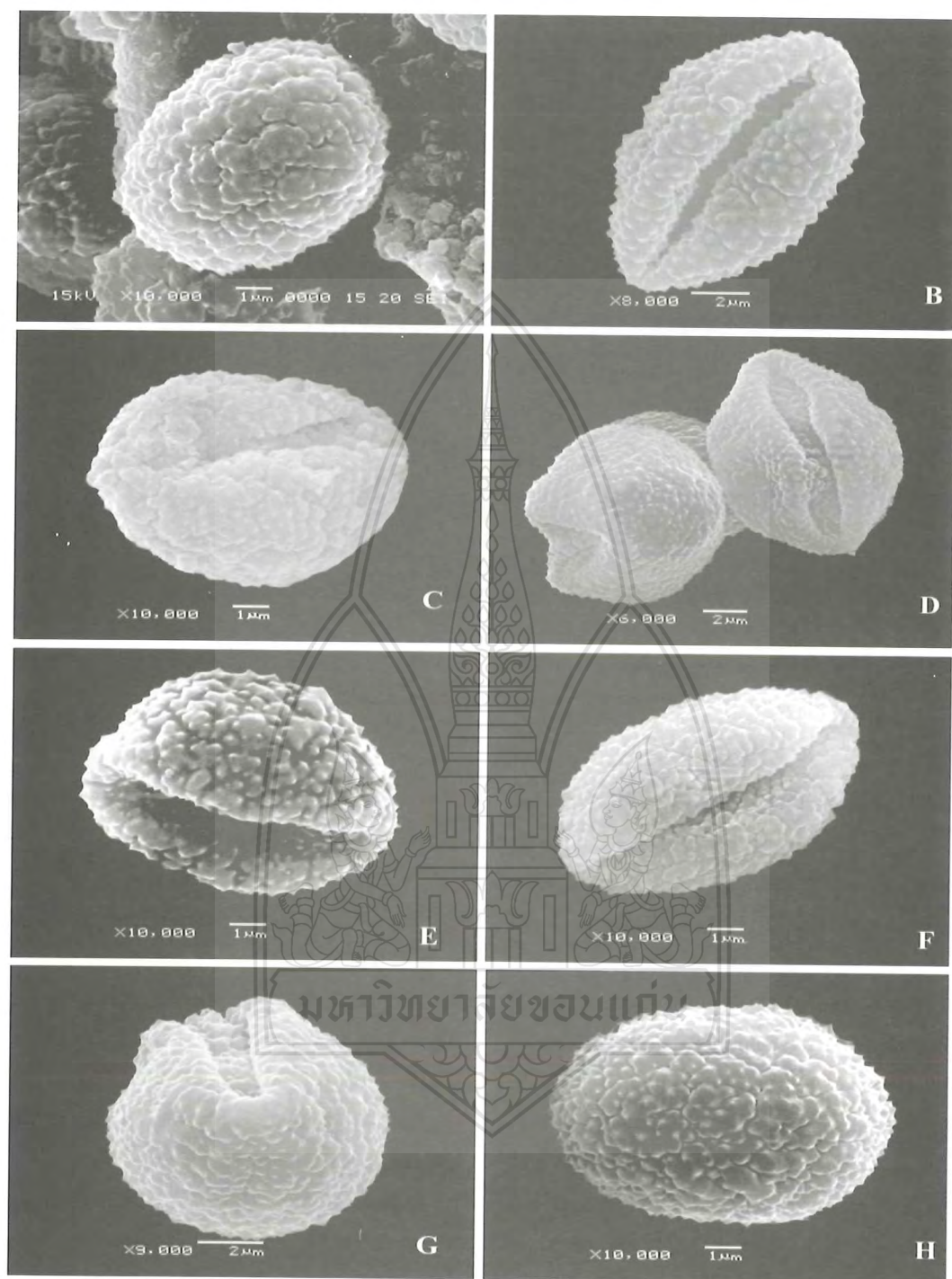


Figure 2. Scanning electron micrographs of pollen grain of genus *Piper* in Thailand: A. *P. betle*, B. *P. boehmeriifolium*, C. *P. caninum*, D. *P. nigrum*, E. *P. polycarpa*, F. *P. sp.2*, G. *P. sp.3*, H. *P. sp.5*.

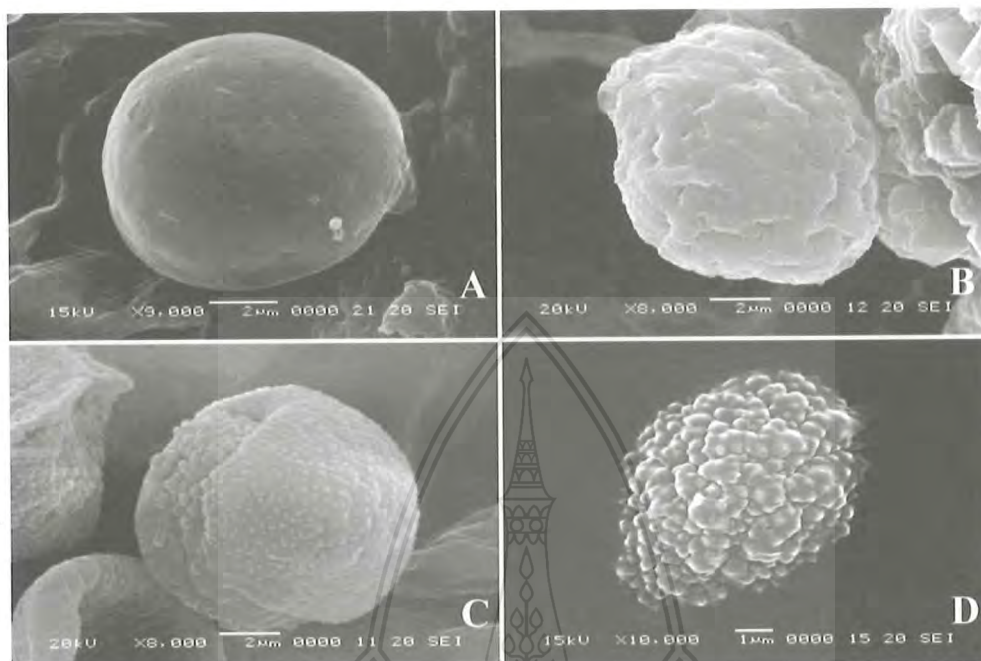


Figure 3. Four types of sculpture from scanning electron micrographs of genus *Piper* in Thailand: A. Psilate, B. Fossulate, C. Scabrate and D. Rugulate with spinules.

DISCUSSION AND CONCLUSIONS

In all the taxa studied pollen grains are generally homogenous and minute except small grains, 10 μm to 14 μm in *P. colubrinum*, *P. kurzii*, *P. longum*, *P. sarmentosum*, *P. umbellatum* and *P. sp.1*. The shape of the grains varies from spheroidal as in *P. betle*, *P. colubrinum*, *P. longum*, *P. nigrum*, *P. polycarpa*, *P. ramipilum* and *P. sp.3* to kidney-shaped in *P. boehmriifolium*, *P. caninum*, *P. kurzii*, *P. sarmentosum*, *P. umbellatum*, *P. sp.1*, *P. sp.2*, *P. sp.4* and *P. sp.5*. The ornamentation of all pollen grains is psilate in LM. The details shown in SEM are variable, e.g. psilate, scabrate, fossulate or rugulate with scabrate on the upper surface. The ornamentation can be used to divide grains into four distinct pollen types based on the details of ornamentation.

1. Psilate type: Occurs in *P. ramipilum* (Figure 1 A and B).
2. Fossulate type: Occurs in *P. kurzii* (Figure 1 C), *P. umbellatum* (Figure 1 D) and *P. sp.4*. (Figure 1 E)
3. Scabrate type: Occurs in: *P. colubrinum* (Figure 1 F), *P. longum* (Figure 1 G), *P. sarmentosum* (Figure 1 H)
4. Rugulate with sparsely distributed spinules type: Occurs in *P. betle* (Figure 2 A), *P. boehmriifolium* (Figure 2 B), *P. caninum* (Figure 2 C), *P. nigrum* (Figure 2 D), *P. polycarpa* (Figure 2 E), *P. sp.2* (Figure 2 F), *P. sp.3* (Figure 2 G) and *P. sp.5*. (Figure 2 H)

The exine sculpturing of the Thai *Piper* is different from Lei and Xing's (1998) work who reported only verrucate or almost psilate in the

Chinese *Piper* species. The size and pollen types of most species are similar to those of Erdtman (1972) and Walker and Doyle (1975). The pollen is heteropolar and minute to small in size. In addition, the pollen of studied species is heteropolar, kidney-shaped or spheroidal and corresponds to that of Walker and Doyle (1975) for subgenus *Artottonia*.

The data collected so far are not sufficient to offer hypotheses on the possible evolutionary trends in the pollen of *Piper* as a whole. No intensive studies on pollen of this genus have been made. However, the monocolpate form of the pollen is a primitive characteristic of the pollen of the Magnolian families such as the Magnoliaceae, Nymphaeaceae, Annonaceae etc. (Nair, 1970).

The pollen morphology does not appear to be particularly useful as a taxonomic technique in the identification of Thai *Piper* species.

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Leaf and Root Anatomy of *Acriopsis* Reinw. ex Blume (Orchidaceae) in Thailand

กายวิภาคศาสตร์ของใบและรากกล้วยไม้สกุลเอื้องนมหนู (*Acriopsis* Reinw. ex Blume) ในประเทศไทย

Narumol Piwpuan¹ and Achra Thammathaworn^{2*}

Abstract

There have been only two species of *Acriopsis* Reinw. ex Blume reported in Thailand, *A. indica* Wight and *A. liliifolia* (J. König) Ormerod. Their leaf and root anatomy were studied using paraffin embedded section, leaf epidermal scraping and light microscopy. The diameter of silica body bases, the thickness of cutin and the size of guard-cells in leaf and the number of arches in vascular cylinders in the root were found to be useful for identification.

บทคัดย่อ

ศึกษาลักษณะทางกายวิภาคศาสตร์ของใบและรากของกล้วยไม้สกุลเอื้องนมหนู (*Acriopsis* Reinw. ex Blume) ซึ่งมีรายงานพบเพียงสองชนิดในประเทศไทย ได้แก่ *A. indica* Wight และ *A. liliifolia* (J. König) Ormerod โดยใช้กรรมวิธีพาราฟิน การลอกผิวใบ และศึกษาลักษณะทางกายวิภาคศาสตร์ด้วยกล้องจุลทรรศน์แบบใช้แสง พบว่าลักษณะที่สามารถนำมาใช้ในการระบุชนิดพืชทั้งสองชนิดนี้ได้ ได้แก่ ความยาวเส้นผ่านศูนย์กลางของผลึกซิลิกา ความหนาของผิวเคลือบคิวทินที่แผ่นใบ ขนาดของปากใบ และจำนวนแขนงมัดท่อลำเลียงในราก

Keywords: *Acriopsis*, Anatomy, silica bodies

คำสำคัญ: กล้วยไม้สกุลเอื้องนมหนู, กายวิภาคศาสตร์, ผลึกซิลิกา

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INTRODUCTION

In Orchidaceae, some recognised vegetative anatomy is similar to other ordinary monocots. There are terrestrial roots with or without velamen. Vascular tissue in stems is in many scattered bundles, which are more numerous near the surface of the stems and embedded in the parenchyma. Leaves have many parallel veins. Four types of stomata have been reported in leaves; paracytic, diacytic, tetracytic and anomocytic stomata. Major orchid groups possess silica cells or stegmata, in which two types of silica bodies occur: conical-shaped and spherical-shaped silica bodies. Stegmata, if present, are associated with vascular bundle sclerenchyma and/or fiber bundles (Dressler, 1993).

Stern and Judd (2002) reported common features of root, leaf and stem anatomy in some representatives of each genera in the tribe Cymbidieae. Roots of Cymbidieae are velamentous except in *Govenia*. All velamen cells are thin walled except in *Grammatophyllum* and *Porphyroglottis*. Tilosomes appear in most taxa. Endodermal and pericyclic cells have O-thickened walls while pith is composed of thin-walled parenchymatous cells. Leaves of most taxa possess adaxial and abaxial fibre bundles, which are absent in *Ansellia* and *Pteroglossaspis* and in some species of *Cymbidium* and *Govenia*. Stomata predominantly appear in abaxial surfaces. Vascular bundles always contain both phloem and xylem sclerenchyma. Stegmata are associated with phloem and xylem sclerenchyma but are absent from xylem sclerenchyma of *Acriopsis*, *Bromheadia*, *Eulophia* and *Govenia* and not associated with either phloem and xylem sclerenchyma in *Diplodium*. Stegmata also occur along surfaces of the fibre bundles in most taxa except in *Grobya*.

Genus *Acriopsis* is one of 28 members in the tribe Cymbidieae, in which the most related genus to *Acriopsis* is *Thecostele* (Dressler, 1993). There are 12 genera of Cymbidieae distributed in Indochina including *Acriopsis*. The genus is distinguished from others by having lateral sepals that merge completely into a synsepal, four pollinia and minute vesidium. Two species of *Acriopsis* have been reported in Thailand, *A. indica* Wight and *A. liliifolia* (J. König) Ormerod, which are found in evergreen forests. The first species can be distinguished from the latter by the presence of broad ovate lip blades which continue from a narrow claw and have no distinct sidelobes (Seidenfaden, 1983; Sithisajatham and Krisanachandee, 2005). Note that Seidenfaden (1983) used the name *A. javanica* Reinw. which was changed to the accepted name, *A. liliifolia* (J. König) Ormerod, by Paul Ormerod in 1995 (Sithisajatham, 2006). Nowadays, there is no complete report for anatomical characters of both species; only information about *A. javanica* (Stern and Judd, 2002) has been reported.

The purpose of the present study is to reveal the significant taxonomic characters between the two studied species, which will be basic knowledge for future studies in orchid systematics.

MATERIAL AND METHODS

Leaves and roots of the plants were collected from living specimens (collection nos. are W. Tokaew 295 and W. Tokaew 62 for *A. indica* and *A. liliifolia*, respectively) in November 2005. Leaves and roots were cut from living plants and fixed in FAA 70 % for at least 24 hours before preparing by paraffin embedded section and

epidermal scraping of leaves. Sections were stained with Safranin and Fast green while epidermis was stained with Safranin. Anatomical characters were observed under a light microscope and photographed through a Normarski Olympus BX 51 microscope fitted with an Olympus DP11 digital camera.

RESULTS

Leaves of *Acriopsis* are isolateral. Hairs are absent from both species. The epidermis is composed of one-layer epidermal cells which are polygonal shaped with a thin cuticle. Tetracytic stomata are present in both adaxial and abaxial surfaces (Figure 1). The cuticle thickness and size of guard cell pairs are showed in Table 1. The hypodermis is present as fibre bundles in both adaxial and abaxial rows, with each bundle separated by chloroplast-containing cells (Figure 2). The mesophyll is composed of chloroplast-containing cells which are arranged in trilamellate form: a broad middle layer of larger cells between narrower rows of much smaller adaxial and abaxial cells. All mesophyll cells are closely packed with small intercellular spaces. Two types of crystaliferous idioblasts are present; raphide bundles occur in large and cleared cells while tiny druses are included in chloroplast-containing cells. Collateral vascular bundles are sited abaxially in one row, alternating between larger bundles and smaller ones. The smaller bundles occupy less than one-half of the mesophyll thickness and more than two-thirds for the larger ones. Sclerenchyma groups occur at both phloem and xylem poles; the phloem sclerenchyma is more prominent than the xylem sclerenchyma. Stegmata with conical, rough-surfaced silica bodies occur in fibre bundles (Figure 3) and both phloem and

xylem sclerenchyma. The difference in silica bodies' base diameters in both species are shown in Table 1. Bundle sheath cells are parenchymatous, and form a complete ring just around some smaller vascular bundles.

Acriopsis roots are velamentous. The velamen is 6 - 10 cell-layers wide in *A. indica*, and 4 - 8 cell-layers wide in *A. liliifolia*. Epivelamen cells are mostly rectangular, orientated in periclinal rows, while immediately subtending cells are polygonal or isodiametric, inner layer cells are polygonal and anticlinally orientated, and cell walls are thin with spiral thickening (Figure 4). Tilosomes and root stegmata are absent in both species. Exodermal cells are rectangular to polygonal, both periclinally and anticlinally orientated. Cell walls are thin in *A. indica* but quite thick in *A. liliifolia*, without thickening, while passage cells are intermittent and markedly nucleated (Figure 4). The cortex is 7 - 9 cell-layers wide, and the cells are mostly isodiametric. Inner layers have triangular intercellular spaces, and cell walls are thin with branched, banded thickenings (Figure 5). Raphide idioblasts occur and sclerenchyma are present in *A. liliifolia*. Endodermal cells are rectangular to polygonal and isodiametric. There are thick-walled cells adjacent to the phloem, and thin-walled cells adjacent to the xylem (Figure 6). Vascular cylinders are 6-arch in *A. indica* and 8-arch in *A. liliifolia*. The xylem occurs in radial rows alternating with clusters of the phloem elements. The vascular tissues are surrounded by thick-walled sclerenchyma cells. Pith cells are thick-walled parenchyma, isodiametric with intercellular spaces.

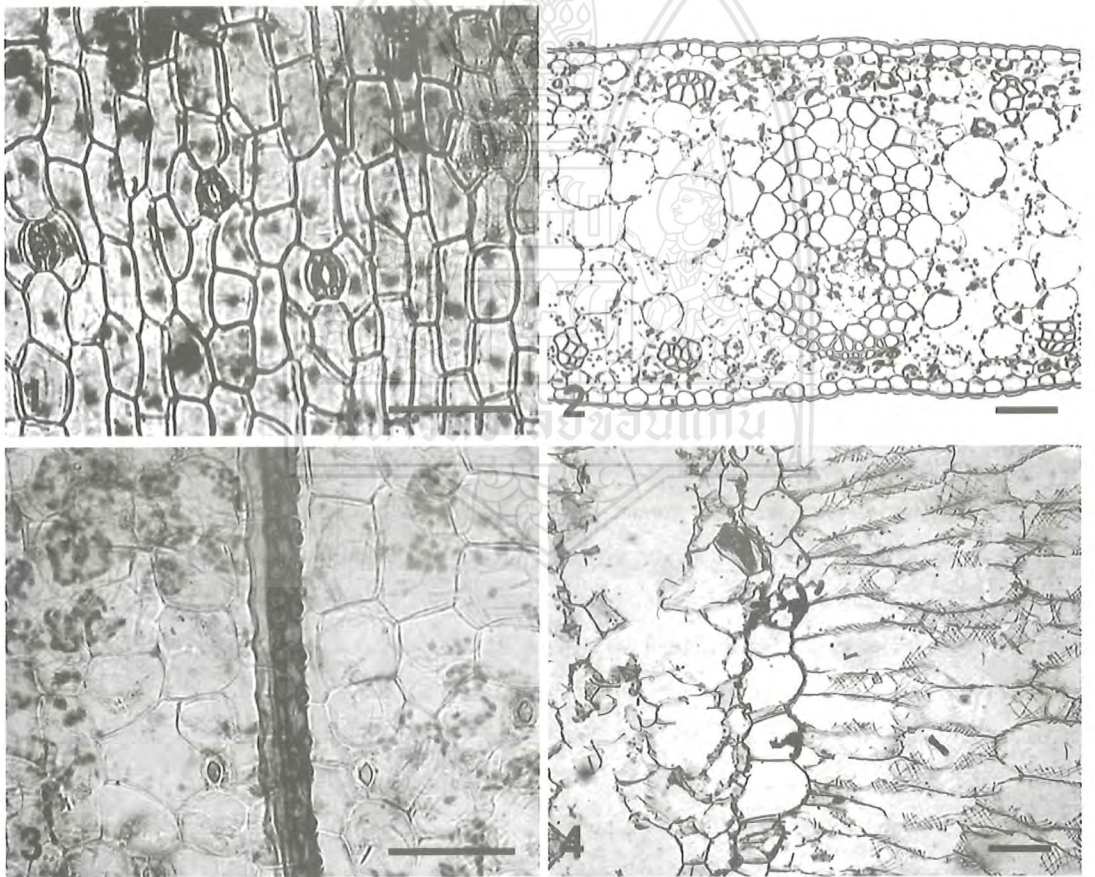
From leaf and root anatomical characters, the identification key can be provided as shown below.

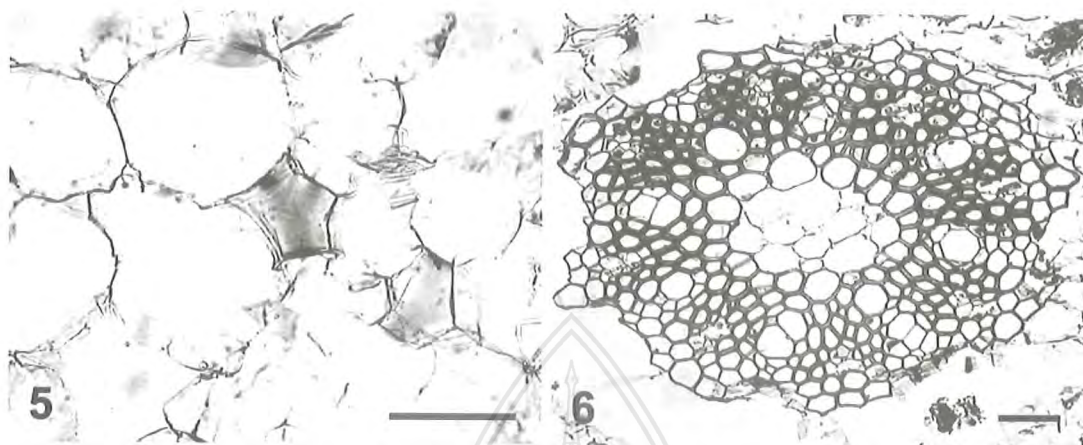
Key to species:

- a. Leaf stigmata with silica bodies base diameter 5 - 7.5 μm , cuticle thickness on leaf blade < 2.5 μm , 6-arch vascular cylinder in root *A. indica*
- b. Leaf stigmata with silica bodies base diameter < 5 μm , cuticle thickness on leaf blade $\geq$ 2.5 μm , 8-arch vascular cylinder in root *A. liliifolia*

Table 1. Leaf characters of *Acriopsis indica* and *A. liliifolia*

Species	Cuticle thickness (μm)			Size of guard cell pairs (μm)		Silica bodies' base diameter (μm)
	Leaf blade	Abaxial midrib	Leaf margin	Adaxial (l/w)	Abaxial (l/w)	
<i>A. indica</i>	< 2.5	2.5	2.5 - 4	20/16	20/18	5 - 7.5
<i>A. liliifolia</i>	2.5	5	7.5	19/13	18/14	< 5





Figures 1 - 6. Anatomical characters of root and leaf. Figures 1, 2, 6: *A. liliifolia*. Figures 3 - 5: *A. indica*;

Figure 1. Epidermis with tetracytic stomata.

Figure 2. Leaf in x-section showing trilamellate mesophyll, a vascular bundle with sclerenchyma and fibre bundles (arrows).

Figure 3. Stigmata on fibre bundle (arrow).

Figure 4. Velamen with spiral thickening (arrowhead) and exodermis with passage cells (arrow).

Figure 5. Branched, banded thickening in cortical cell (arrow).

Figure 6. Root vascular cylinder with xylems (arrows) alternating with phloems (white arrows) and parenchymatous pith cells. Scale bars = 50 μm .

DISCUSSION AND CONCLUSIONS

The results show that leaves and roots of *Acriopsis* species share most characters. Some leaf characters are: hairs absent, tetracytic stomata on adaxial and abaxial surfaces, trilamellate mesophyll, presence of fibre bundles and fibre bundle stigmata, conical-shaped silica bodies, phloem and xylem sclerenchyma which are more distinct in phloem poles and presence of stigmata on phloem and xylem sclerenchyma. Common characters of root anatomy are: thin and spiral thickening of velamen cell walls, epivelamen cells distinctive, tilosomes absent, vascular tissue surrounded by sclerenchyma,

root stigmata absent and parenchymatous pith. The combination of these characters, the diameter of silica bodies' base, the thickness of cutin, the size of guard-cells and the number of vascular cylinder arches in the root can be used for identifying the *Acriopsis* species.

Yukawa and Stern (2002) reported anatomical characters of *A. javanica* (the same species which was changed to *A. liliifolia* by Seidenfaden in 1995) that share many characters with the studied species. Some characters are however different. These are water storage cells which are absent in *A. indica* and *A. liliifolia* while

stigmata are present on xylem sclerenchyma but absent in *A. javanica*. The presence of tiny druses in mesophyll cells is also unreported in *A. javanica*. Because of the very small size of tiny druses, this might have caused them to be unnoticeable in the latest work, while the absence of stigmata on xylem sclerenchyma may be the same; they are unnoticed because of their small size and lower abundance than on the phloem. In order to store water in most epiphytes for adaptation to water shortage periods, epiphyte orchids often have an enlarged portion of the stem called pseudobulbs, thick and succulent leaves and additional water storage cells in their vegetative organs (Lüttge, 1989). However, we did not see adaptation like thick and succulent leaves and water storage cells in both species.

Stern and Judd (2002) combined anatomical characters in the cladistic analysis of Cymbidieae. The result showed that anatomical features provide support for the monophyly of a particular genus. *Acriopsis* is likewise distinguished by its trilamellate mesophyll and also because of having banded cells in the root cortex. Therefore, anatomical features found in this study still support the phylogeny among the Cymbidieae.

Compared with the most related genus, *Thecostele*, apart from differences in morphological details, the anatomical characters of these two genera are quite different. From the data reported in Yukawa and Stern (2002) the most important differences are having abaxial stomata only and homogenous mesophyll in *Thecostele*.

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Fatty Acids Content and Antioxidant Capacity of Peanut

ปริมาณกรดไขมันและความสามารถในการต้านการเกิดออกซิเดชันของถั่วลิสง

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Abstract

Peanut (*Arachis hypogaea* L.) is one of the important economic crops of Thailand. The aim of this study was to determine the antioxidant capacity and fatty acids content of 8 commercial varieties of peanut. Methanol extracts and oil extracts of peanut kernel from various seed lines (KKU72-1, KKU40, KKU1, Tainan9, Khonkaen5, Khonkaen6, Khonkaen60-1 and Khonkaen60-3) were tested for their antioxidant capacity by using spectrophotometric assays (DPPH assay and ABTS assay). Peanut oil was extracted from dried kernels by Soxtec system HT. Most peanut lines contained between 47 - 53 % oil. For fatty acids analysis, peanut oil contained oleic acid (C18:1) > linoleic acid (C18:2) > palmitic acid (C16:0) and the highest ratio of oleic acid : linoleic acid (O/L ratio) was obtained from Khonkaen6 and KKU 60-3 giving the O/L ratios of 3.83 ± 0.00 and 3.66 ± 0.02 , respectively. The oil extracted from Khonkaen5 and Khonkaen6 showed the highest antioxidant capacity giving IC_{50} values of 0.17 ± 12.37 % (w/v) and 0.24 ± 12.19 % (w/v), respectively, as determined by DPPH assay. The methanolic extracts from Khonkaen6, Khonkaen60-1 and Khonkaen60-3 showed the highest antioxidant capacity as determined by DPPH assay ($p < 0.05$) and KKU72-1, Khonkaen5, Khonkaen6, Khonkaen60-1 and Khonkaen60-3 showed the highest antioxidant capacity as determined by ABTS assay ($p < 0.05$). The total phenolics from methanolic extracts exhibited negative correlation with IC_{50} values obtained from both DPPH assay and ABTS assay.

บทคัดย่อ

ถั่วลิสง (*Arachis hypogaea* L.) จัดเป็นพืชที่มีความสำคัญทางเศรษฐกิจพืชหนึ่งของประเทศไทย การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาถึงความสามารถในการต้านการเกิดออกซิเดชัน ชนิดและปริมาณกรดไขมัน จากถั่วลิสงที่แนะนำให้เกษตรกรเพาะปลูกแปดสายพันธุ์ (มข 72-1 มข40 มข 1 Tainan 9 ขอนแก่น 5 ขอนแก่น

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6 ขอนแก่น 60-1 และขอนแก่น 60-3) ผลการวิเคราะห์ปริมาณน้ำมันโดยเครื่อง Soxtec พบว่ามีน้ำมันเป็นองค์ประกอบประมาณ 47 - 53 % และเมื่อวิเคราะห์ชนิดและปริมาณกรดไขมันด้วย Gas Liquid Chromatography พบว่ากรดไขมันที่มีมากที่สุดในทุกสายพันธุ์ คือ กรดโอเลอิก (C18:1) กรดลิโนเลอิก (C18:2) และกรดปาล์มิติก (C16:0) ตามลำดับ เมื่อพิจารณาถึงอัตราส่วนของกรดโอเลอิกต่อกรดลิโนเลอิก (O/L ratio) พบว่าสายพันธุ์ขอนแก่น 60-1 มีค่า O/L ratio สูงสุด คือ 0.00 ± 3.83 รองลงมาคือ ขอนแก่น 60-3 มีค่าเท่ากับ 0.02 ± 3.66 ($p < 0.05$) น้ำมันที่ได้จากสายพันธุ์ขอนแก่น 5 และขอนแก่น 6 มีความสามารถในการต้านการเกิดออกซิเดชันได้ดีที่สุดเมื่อทดสอบโดย DPPH assay โดยให้ค่าความเข้มข้นที่ยับยั้งการเกิดออกซิเดชันที่ 50% (IC50) ต่ำสุดคือ 0.17 ± 12.37 % (w/v) และ 0.24 ± 12.19 % (w/v) ตามลำดับ สารสกัดด้วยเมทานอลที่ได้จากสายพันธุ์ขอนแก่น 6 ขอนแก่น 60-1 และขอนแก่น 60-3 มีความสามารถในการต้านการเกิดออกซิเดชันได้ดีที่สุดเมื่อทดสอบโดย DPPH assay ($p < 0.05$) สารสกัดด้วยเมทานอลที่ได้จากสายพันธุ์ มข 72-1 ขอนแก่น 5 ขอนแก่น 6 ขอนแก่น 60-1 และขอนแก่น 60-3 มีความสามารถในการต้านการเกิดออกซิเดชันได้ดีที่สุดเมื่อทดสอบโดย ABTS assay ($p < 0.05$) นอกจากนี้ในการทดลองนี้ยังได้ศึกษาถึงความสัมพันธ์ของปริมาณฟีนอลทั้งหมดพบว่าความสัมพันธ์ของปริมาณฟีนอลทั้งหมดมีค่าผกผันกับค่า IC50 ทั้งจากวิธี DPPH assay และ ABTS assay

Keywords: peanut, antioxidant, fatty acid

คำสำคัญ: ถั่วลิสง, ความสามารถในการต้านการเกิดออกซิเดชัน, กรดไขมัน

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the important economic crops of Thailand. The kernels are used to make peanut butter, roasted snack peanuts, peanut confections, and peanut oil. The literature has reported many health benefits associated with consumption of peanuts including weight gain control (Alper and Mattes, 2002), prevention of cardiovascular diseases (Feldman, 1999), protection against Alzheimer disease (Peanut-Institute, 2002), and cancer inhibition (Awad et al., 2000). These benefits are mainly attributed to the fact that peanuts do not contain trans-fatty acids (Sanders, 2001), while being rich in mono- and polyunsaturated fatty acids (Kris-Etherton et al., 1999), micronutrients such as vitamin E, folate, minerals (potassium, magnesium, and zinc), fiber, and health promoting phytochemicals, particularly resveratrol

(Sanders et al., 2000; Sobolev and Cole, 1999) and other phenolic compounds. Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants (Randhir et al., 2004). These compounds, from one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), besides contributing towards the colour and sensory characteristics of fruits and vegetables (Alasalvar et al., 2001). Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Hertog et al., 1993; Parr and Bolwell, 2000). The beneficial effects derived from phenolic compounds have been

attributed to their antioxidant activity (Heim et al., 2002). Phenolic compounds could be a major determinant of antioxidant potentials of foods (Parr and Bolwell, 2000), and could therefore be a natural source of antioxidants. Therefore, this study was to determine antioxidant capacity and fatty acids content of 8 commercial varieties of peanut.

MATERIAL AND METHODS

1. Material

Eight commercial varieties of peanut were used in this study. There were KKKU72-1, KKKU40, KKKU1, Tainan9, Khonkaen5, Khonkaen6, Khonkaen60-1 and Khonkaen60-3. KKKU72-1 and Khonkaen60-3 are large-seeded peanut lines whereas the other peanut varieties are small-seeded peanut lines. KKKU1, KKKU40 and KKKU72-1 varieties were bred and developed by the Faculty of Agriculture, Khon Kaen University whereas Khonkaen5, Khonkaen6, Khonkaen60-1 and Khonkaen60-3 varieties were bred and developed by Khon Kaen Field Crops Research Center. Tainan9 was brought from Taiwan. This variety can adapt and grow well in Thailand. Standards including quercetin, vitamin E, vitamin C and trolox were purchased from Sigma Chemical Co. 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were also purchased from Sigma Chemical Co.

2. Methods of drying and oil extraction

Two grams of peanut sample was dried at 70 °C for several hours. Moisture content was measured by weight difference. Two grams of dried peanut was extracted for oil by the soxtec extractor (50 ml of petroleum ether was used as a solvent).

The extracted oil was determined for antioxidant capacity by DPPH assay.

3. Fatty acid analysis

The oil was prepared for fatty acid analysis by gas liquid chromatography (GLC). Fatty acid methyl esters (FAME) were prepared by adding 1 ml of 2.5 % $H_2SO_4/MeOH$ in 10 mg of oil sample and 100 μ l C17:0 (0.01 g/ml) (used as an internal standard). The mixture was incubated at 80°C for 2 hrs. After that, 200 μ l NaCl (0.9 % w/v) and 200 μ l heptane were added to the mixture. The FAME samples (2 μ l) were subjected to GLC (with Flame Ionization Detector: FID) for fatty acids analysis. Fatty acids analysis was conducted on a Shimadzu Gas Chromatograph GC-14B-CR7A and SGE fort GC capillary column (30 m X 0.25 mm ID BPX70 0.25 μ m) was used. Helium was the carrier gas at a flow rate of 30 ml/min. The oven temperature was maintained at 130 °C for 2 min. Then it was programmed at 5 °C/min to 220 °C and held at this temperature for 8 min. The injector temperature and detector temperatures were 250 °C and 300 °C, respectively.

The standard fatty acids that were used to identify fatty acid content in commercial varieties of peanut consisted of myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosenoic acid, behenic acid, erucic acid and lignoceric acid.

4. Methanolic extraction

Forty milliliters of methanol was added to a beaker containing 2 g of ground peanut. The mixture was stirred for 2 hrs at room temperature. Then the mixture was filtered through filter paper

(Whatman no.4). The crude extract was determined for total phenolic content by the Folin-Ciocalteu method and antioxidant capacity by DPPH assay and ABTS assay.

5. Determination of total phenolics

Total phenolics in methanolic extracts were determined by the Folin-Ciocalteu method (Torres et al., 1987). Gallic acid was used as the standard and the result was calculated as gallic acid equivalent (mg GAE/100 g). Three milliliters of water was added to a tube containing 0.05 ml of various concentrations of crude extract (100, 50 and 25 % v/v). Then 2.5 ml Folin-Ciocalteu's reagent was added followed by 0.75 ml of 20 % Sodium carbonate. The total volume was adjusted to 5 ml by adding water. The mixture was incubated at 50 °C for 2 hrs. The absorbance of total phenolics was recorded at 765 nm.

6. Antioxidant capacity of peanut

The methanolic extracts from peanut lines were determined for antioxidant capacity by DPPH assay (Leong and Shui, 2002) and ABTS assay (Miller et al., 1993). Vitamin C, vitamin E, Quercetin and trolox were used as the standards and the results were expressed as 50 % inhibitory concentration or IC_{50} .

The oil extracts from peanut lines were determined for antioxidant capacity by DPPH assay (Ramadan et al., 2003). Three milliliters of 10^{-4} M DPPH solution in toluene was added to tubes containing 0.2 ml of various concentrations of extracted oil (20, 10, 5 and 2.5 % w/v). After 2 min, the absorbance of DPPH was recorded at 515 nm. Vitamin E was used as a standard and the result was expressed as 50 % inhibitory concentration.

RESULTS AND DISCUSSION

1. Moisture and oil contents

The moisture of peanut had to be analyzed because it might affect the concentration of antioxidants in peanut. For the moisture content analysis, the moisture contents of peanut samples were about 4.8 to 6.4 %. The highest moisture contents were found in KKU72-1, KKU40 and Tainan9 compared with those of the other peanut lines (Table 1). The moisture contents of KKU72-1, KKU40 and Tainan9 were 6.39 ± 0.22 , 6.10 ± 0.11 and 6.41 ± 0.24 %, respectively. The moisture of peanut was low (4.8 - 6.4 %), therefore it should not affect the antioxidant capacity analysis. For the oil and fatty acid content analysis, we used dried samples of peanut.

For the oil content analysis, the oil content of peanut samples were about 47 to 53 % (of dry weight). The highest oil content was found in KKU72-1 compared with those of the other peanut lines (Table 1). The oil content of KKU72-1 was 53.64 ± 0.41 %. The extracted oil in this study was crude oil because it was composed of triglyceride, phospholipids, gum, fat soluble vitamins such as vitamin E and the other lipophilic compounds.

2. Fatty acid content

The standard fatty acid profile that was used to identify fatty acid content in peanut lines, consisted of myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosenoic acid, behenic acid, erusic acid and lignoseric acid (Figure 1).

Table 1. Moisture and oil contents in peanuts.

Peanut line	Moisture	Oil
	(%)	(%) / dry weight
KKU72-1	6.39 ± 0.22 ^a	53.64 ± 0.41 ^a
KKU40	6.10 ± 0.11 ^a	49.11 ± 0.74 ^{b,c}
KKU1	5.22 ± 0.31 ^b	50.38 ± 0.11 ^{b,c}
Tainan 9	6.41 ± 0.24 ^a	47.89 ± 0.88 ^b
KK5	5.32 ± 0.18 ^b	48.05 ± 1.36 ^b
KK6	5.69 ± 0.06 ^c	48.06 ± 1.88 ^b
KK60-1	5.14 ± 0.27 ^{b,d}	48.88 ± 1.66 ^{b,c}
KK60-3	4.82 ± 0.07 ^d	51.07 ± 1.03 ^{a,b}

Different superscripts are significantly different (p < 0.05).

For fatty acid analysis, peanut oil consisted of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosenoic acid, behedic acid and lignoceric acid (Figure 2).

Figure 2 shows the fatty acid profile of the KKU40 peanut line. For the other seven peanut lines, the fatty acid profiles were the same as that of KKU40 but the amount of each fatty acid was different. The most abundant fatty acid in eight peanut lines was oleic acid followed by linoleic acid and palmitic acid (Table 2).

From a previous study of Andersen et al. (1998), oleic acid was the major fatty acid in peanut oil. The oleic acid contents were 55 - 60 % whereas the values obtained in this study were 40.96 - 63.47 %.

The ratio of oleic acid to linoleic acid or O/L ratio was determined for each sample. KK6 and KK60-3 showed the highest O/L ratio over the rest (Table 2). The O/L ratio of KK6 was 3.83 ±

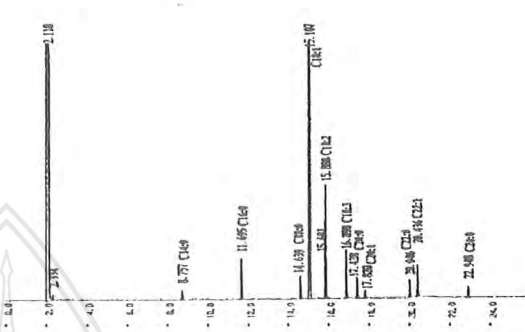


Figure 1. Standard fatty acid profile by Gas Liquid Chromatography.

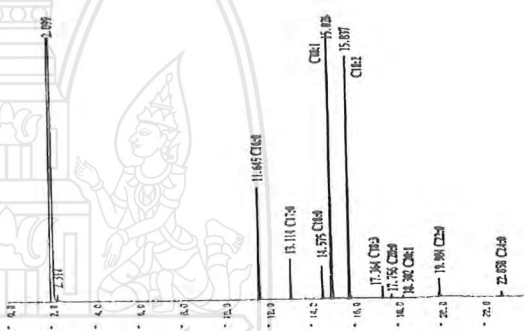


Figure 2. Fatty acid profile of KKU 40 by Gas Liquid Chromatography.

0.00 and the O/L ratio of KK60-3 was 3.66 ± 0.02. This ratio indicated the stability of peanut oil. A higher O/L ratio implies the higher stability of peanut oil.

3. Total phenolics of peanut

The methanolic extract was used for total phenolic analysis by Folin Ciocalteu's method and

Table 2. Fatty acids contents from peanut.

Line	Fatty acids contents of peanut (percent fatty acid)										O/L ratio
	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1 n-9	Linoleic acid C18:2 n-6	Linolenic acid C18:3 n-3	Arachidic acid C20:0	Eicosenoic acid C20:1 n-9	Behenic acid C22:0	Lignoceric acid C24:0		
KKU 72-1	11.46 ± 0.11	4.10 ± 0.25	49.74 ± 0.81	28.38 ± 0.22	1.42 ± 0.41	0.81 ± 0.23	0.64 ± 0.04	2.33 ± 0.18	1.11 ± 0.03	1.75 ± 0.01 ^a	
KKU 40	14.23 ± 1.33	4.02 ± 0.18	46.32 ± 0.56	30.89 ± 0.53	1.18 ± 0.14	0.54 ± 0.10	0.35 ± 0.04	1.82 ± 0.59	0.64 ± 0.24	1.50 ± 0.04 ^a	
KKU 1	13.06 ± 0.12	4.51 ± 0.00	42.64 ± 0.12	33.43 ± 0.11	1.70 ± 0.02	0.60 ± 0.01	0.37 ± 0.01	2.74 ± 0.09	0.95 ± 0.02	1.28 ± 0.01 ^b	
Tainan 9	14.65 ± 0.72	4.07 ± 0.08	42.12 ± 1.00	34.78 ± 0.30	1.18 ± 0.01	0.54 ± 0.01	0.29 ± 0.03	1.83 ± 0.08	0.55 ± 0.03	1.21 ± 0.04 ^b	
KK 5	15.63 ± 1.08	4.00 ± 0.24	40.96 ± 0.83	34.66 ± 0.60	1.30 ± 0.14	0.50 ± 0.05	0.28 ± 0.02	2.03 ± 0.28	0.63 ± 0.11	1.18 ± 0.04 ^b	
KK 6	8.39 ± 0.06	4.76 ± 0.04	63.09 ± 0.01	16.47 ± 0.01	1.85 ± 0.07	0.89 ± 0.02	0.50 ± 0.00	2.90 ± 0.04	1.15 ± 0.01	3.83 ± 0.00 ^c	
KK 60-1	15.45 ± 0.30	3.56 ± 0.03	41.65 ± 0.08	35.09 ± 0.08	1.15 ± 0.04	0.52 ± 0.03	0.29 ± 0.01	1.75 ± 0.12	0.55 ± 0.06	1.19 ± 0.01 ^b	
KK 60-3	10.19 ± 0.35	3.56 ± 0.08	63.47 ± 0.22	17.35 ± 0.11	1.34 ± 0.11	0.81 ± 0.06	0.43 ± 0.03	2.05 ± 0.28	0.80 ± 0.13	3.66 ± 0.02 ^d	

Different superscripts are significantly different (p < 0.05).

Table 3. Total phenolics in peanut.

Peanut Line	Gallic acid equivalent
	(mg/100 g)
KKU72-1	1.31 ± 0.04 ^a
KKU40	1.14 ± 0.01 ^b
KKU1	1.00 ± 0.01 ^c
Tainan 9	1.13 ± 0.02 ^b
KK5	1.44 ± 0.03 ^d
KK6	1.24 ± 0.02 ^c
KK60-1	1.41 ± 0.02 ^d
KK60-3	1.43 ± 0.01 ^d

Different superscripts are significantly different (p<0.05).

Table 4. Antioxidant capacity of peanut.

Peanut Line	50 % Inhibitory Concentration (IC ₅₀)		
	Oil extracted ¹ % (w/v)	Methanolic extracted ²	Methanolic extracted ³
		% (v/v)	% (v/v)
KKU 72-1	14.09 ± 0.15 ^a	1.15 ± 0.01 ^a	0.04 ± 0.00 ^a
KKU 40	13.99 ± 0.17 ^a	1.92 ± 0.04 ^b	0.08 ± 0.02 ^{c,d}
KKU 1	14.69 ± 0.59 ^a	1.49 ± 0.05 ^c	0.09 ± 0.01 ^d
Tainan 9	14.80 ± 0.00 ^a	1.18 ± 0.17 ^a	0.08 ± 0.01 ^{c,d}
KK 5	12.37 ± 0.17 ^b	1.15 ± 0.01 ^a	0.05 ± 0.00 ^{a,b}
KK 6	12.19 ± 0.24 ^b	0.98 ± 0.03 ^d	0.07 ± 0.01 ^{b,c,d}
KK 60-1	17.47 ± 0.57 ^c	0.94 ± 0.00 ^d	0.06 ± 0.00 ^{a,b,c}
KK 60-3	17.77 ± 1.12 ^c	0.82 ± 0.01 ^d	0.06 ± 0.01 ^{a,b,c}

Different superscripts are significantly different (p < 0.05) within the same column.

¹ The antioxidant capacity determined by DPPH assay using toluene as a solvent.

² The antioxidant capacity determined by DPPH assay using methanol as a solvent.

³ The antioxidant capacity determined by ABTS assay.

was reported as gallic acid equivalent (mg/100 g). The methanolic extract from KK5, KK6 and KK60-3 showed the highest total phenolics by giving gallic acid equivalents of 1.44 ± 0.03 mg/100 g, 1.41 ± 0.02 mg/100 g and 1.43 ± 0.01 mg/100 g, respectively (Table 3). The total phenolics appeared to have a positive correlation with antioxidant capacity determined by DPPH assay and ABTS assay. Moreover, the results show that low moisture peanut contains high total phenolics, especially in KK60-3 and KK5.

4. Antioxidant capacity of peanut

In this study, the antioxidant capacity was expressed as IC_{50} or 50 % inhibitory concentration. The IC_{50} is the concentration that inhibits 50 % of oxidation. If the IC_{50} is low, the antioxidant capacity is high.

The extracted oils from peanut lines were tested for antioxidant capacity by DPPH assay. The oil extracts from KK5 and KK6 showed the highest antioxidant capacity by giving IC_{50} values of 12.37 ± 0.17 % (w/v) and 12.19 ± 0.24 % (w/v), respectively (Table 4). This might be due to the antioxidative property obtained from tocopherols in peanut (Kornsteiner et al., 2006). In reference to nutrition, low levels of vitamin E could be associated with a potentially higher risk of atherosclerosis or other degenerative diseases and increased intake appears to be protective against these diseases. One of the most developed theories for the cardioprotective effect appears to be concerning tocopherol induced inhibition of LDL oxidation, which is proposed to be a key role in the atherogenic process (Elmadfa and Wagner, 2003). On the other hand, Miller et al. (2005) showed that high dosage (> 400 IU/d)

vitamin E supplements may increase all-cause mortality. However, low quantities which can be obtained from average nut consumption have been shown to be beneficial to coronary heart disease (Bramley et al., 2000). In addition, γ -tocopherol is assumed to be the most active form in humans, however α -tocopherol is the most prevalent form of vitamin E in plant seeds, especially in nuts (Jiang et al., 2001; Wagner et al., 2004).

The methanolic extracts from peanut lines were determined for antioxidant capacity by DPPH assay and ABTS assay. The methanolic extracts from KK6, KK60-1 and KK60-3 showed the highest antioxidant capacity by giving IC_{50} values of 0.98 ± 0.03 % (v/v), 0.94 ± 0.00 % (v/v) and 0.82 ± 0.01 % (v/v) determined by DPPH assay, respectively (Table 4). The methanolic extracts from KKKU72-1 showed the highest antioxidant capacity by giving IC_{50} value of 0.04 ± 0.00 % (v/v) determined by ABTS assay (Table 4).

This might be due to the antioxidative property obtained from phenolic compounds. Henn and Stehle (1998) have shown that the antioxidant activities of fruit juices, wines and teas are highly correlated with total phenolic content.

In our previous study, it was reported that the methanolic extracts from the (China 97-2 X KK60-3) F6-8-1 peanut line showed the strongest antioxidant capacity determined by DPPH assay and ABTS assay (Swatsitang et al., 2003). In this study, the antioxidant capacity of methanolic extracts from KK6, KK60-1 and KK60-3 were lower than that of (China 97-2 X KK60-3) F6-8-1 determined by DPPH assay. However the antioxidant capacity of methanolic extracts from KKKU72-1 was higher than that of (China 97-2 X KK60-3) F6-8-1 determined by ABTS

assay. The antioxidant capacity in peanut lines of this study was due to the total biological matters in peanut seed such as vitamin E in oil or chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, flavonoids and stilbene (resveratrol) (Yu et al., 2005).

Since it was reported that the antioxidant activity of a plant extract often originates from phenolic compounds (Lee et al., 2004), the antioxidant activity is closely related to the content of phenolic compounds. The results of this study showed a similar trend of correlation of total phenolics and antioxidant capacity. The negative correlations between total phenolics and IC₅₀ values were obtained from both DPPH assay and ABTS assay.

CONCLUSIONS

The results of this study indicate that the moisture contents and the oil contents in eight commercial varieties of peanut were about 4.8 to 6 % and 47 to 53 %, respectively. The highest fatty acid in eight commercial varieties of peanut was oleic acid followed by linoleic acid, palmitic acid, stearic acid, behenic acid, linolenic acid, lignoceric acid, arachidic acid and eicosenoic acid, while the highest O/L ratios were found in KK6 and KK60-3.

Eight commercial varieties of peanut contained small amount of total phenolics (1.00 - 1.44 mg gallic acid equivalent/100 g). The antioxidant capacity of the methanolic extracts appeared to be directly proportional to the concentration of total phenolics. The antioxidant capacity of methanolic extracts giving IC₅₀ values ranging from 0.82 to 1.92 % (v/v), KK6, KK60-1 and KK 60-3 showing the highest antioxidant capacity determined by DPPH assay. The antioxidant capacity of the methanolic extracts determined by ABTS assay gave IC₅₀

values ranging from 0.04 to 0.09 % (v/v), with KKU72-1 showing the highest antioxidant capacity. The antioxidant capacity of the extracted oils gave IC₅₀ values ranging from 12.19 to 17.77 % (w/v), with KK5 and KK6 showing the highest antioxidant capacity determined by DPPH assay.

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Antioxidant Capacity and Nutritional Values of Pak-Wanpa (*Melientha suavis* Pierre.)

ความสามารถในการต้านออกซิเดชันและคุณค่าทางโภชนาการ ของผักหวานป่า (*Melientha suavis* Pierre.)

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Abstract

Pak-Wanpa (*Melientha suavis* Pierre.) is a traditional vegetable in Thailand and Southeast Asia. Pak-Wanpa has nutritional value and contains antioxidants which can delay or inhibit cell destruction by oxidation. The aims of this study were to 1) determine antioxidant capacity by using two different spectrophotometric methods (DPPH assay and 2-deoxyribose assay) 2) determine vitamin C and total phenolic contents, which are related to antioxidant capacity by using Titrimetric method and Folin-Ciocalteu assay, respectively and 3) determine its nutritional values (moisture, protein, lipid, carbohydrate, fiber and ash). Pak-Wanpa exhibited antioxidant capacity giving IC_{50} values of 0.23 ± 0.00 % (v/v) and 0.05 ± 0.00 % (v/v) determined by DPPH assay and 2-deoxyribose assay, respectively. Vitamin C and total phenolic contents were 96.20 ± 0.55 mg/100 g and 370.69 ± 8.74 mg gallic acid equivalent/100 ml, respectively. The water, protein, lipid, carbohydrate, fiber and ash contents of Pak-Wanpa were 78.16 ± 0.71 %, 7.43 ± 0.10 %, 0.52 ± 0.04 %, 8.45 ± 0.05 %, 3.90 ± 0.12 % and 1.54 ± 0.16 %, respectively.

บทคัดย่อ

ผักหวานป่า (*Melientha suavis* Pierre.) เป็นไม้พื้นเมืองที่มีถิ่นกำเนิดอยู่ในประเทศไทยและประเทศในแถบเอเชียตะวันออกเฉียงใต้ เป็นพืชที่มีคุณค่าทางโภชนาการและมีสารแอนติออกซิเดนต์ (antioxidant) ซึ่งเป็นสารที่สามารถยับยั้งหรือต้านการทำลายเซลล์ที่เกิดจากปฏิกิริยาออกซิเดชันได้จากการศึกษาพบว่าความสามารถในการต้านออกซิเดชันของผักหวานป่าที่วิเคราะห์โดยวิธี DPPH assay ให้ค่าความเข้มข้นที่สามารถยับยั้งการเกิดออกซิเดชันที่ 50 % หรือ IC_{50} คือ 0.23 ± 0.00 % (v/v) และวิธี 2-deoxyribose assay พบว่าค่า IC_{50} คือ 0.05

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$\pm 0.00\%$ (v/v) ปริมาณวิตามินซีและปริมาณฟีนอลิกทั้งหมดพบว่ามีค่าเท่ากับ 96.20 ± 0.55 มก./100 ก. และ 370.69 ± 8.74 มก. gallic acid equivalent/100 มล. ตามลำดับ ส่วนคุณค่าทางโภชนาการ ได้แก่ ปริมาณน้ำ โปรตีน ไขมัน คาร์โบไฮเดรต เส้นใยและเถ้า มีค่าดังนี้ คือ $78.16 \pm 0.71\%$, $7.43 \pm 0.10\%$, $0.52 \pm 0.04\%$, $8.45 \pm 0.05\%$, $3.90 \pm 0.12\%$ และ $1.54 \pm 0.16\%$ ตามลำดับ

Keywords: *Melientha suavis* Pierre., Antioxidant capacity, Total phenolics

คำสำคัญ: ผักหวานป่า, ความสามารถในการต้านออกซิเดชัน, ปริมาณฟีนอลิกทั้งหมด

1. INTRODUCTION

Pak-Wanpa (*Melientha suavia* Pierre.) is a deciduous tree, commonly found in mixed deciduous forest and dry dipterocarp forest in Thailand. Historically, Pak-Wanpa is an important plant used as a vegetable by people in Thailand, Lao People, Democratic Republic (LPDR) and Southeast Asia. During the early dry season (February to April), young leaves and flowers of Pak-Wanpa are sought out and extensively collected from wild populations by local people. Young leaves, young and/or blooming flowers are used as edible parts. They are always found in local markets around Thailand, even in Bangkok. The price of Pak-Wanpa is relatively high (about 80 - 100 baht per kilogram) (Prathepha, 2000). The ripe fruits are also edible (juicy mesocarp) and in Vietnam the seeds are eaten in the same way as groundnut after boiling or frying. The wood is often used for making charcoal in Thailand. Fresh shoots and leaves of Pak-Wanpa contain per 100 g edible portion: water 76.6 g, protein 8.2 g, carbohydrates 10.0 g, fiber 3.4 g, ash 1.8 g, carotene 1.6 mg, vitamin C 115 mg and the energy value is about 300 kJ/100 g (Frits Stoepman, 1994).

Epidemiological studies have shown that increased consumption of fruits and vegetables has been associated with protection against various forms

of cancer (Abdille et al., 2005), a number of chronic diseases, such as neoplasm, cardiovascular diseases, inflammation, neurodegenerative pathologies, cataracts, diabetes as well as the ageing process (Papetti et al., 2006; Toor, et al., 2006).

Fruits and vegetables are major sources of dietary antioxidant vitamins such as vitamin C (ascorbic acid), vitamin E (tocopherol), precursors of vitamin A i.e., β -carotene (Block et al., 1992) and phenolic compounds which are also antioxidant and are numerous and widely distributed in the plant Kingdom (Namiki, 1999). Phenolic constituents, such as flavonoids and phenolic acids are especially worthy of notice due to their high antioxidative activity (Pilarski et al., 2006). The antioxidants are known to play an important role in protection against disorders caused by oxidant damage (Shyamala et al., 2005). Antioxidants refers to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. They act in one or more of the following ways: as reducing agents, by free radical scavenging, and as quenchers of singlet oxygen (Chanwitheesuk et al., 2005).

The aims of this study were to determine antioxidant capacity by using two different spectrophotometric methods (DPPH assay and 2-deoxyri-

bose assay), and to determine vitamin C and total phenolic contents, which were related to antioxidant capacity by using the Titrimetric method and the Folin-Ciocalteu assay, respectively, as well as to determine nutritional values (moisture, protein, lipid, carbohydrate, fiber and ash) of Pak-Wanpa.

2. MATERIAL AND METHODS

2.1 Chemicals

2,6-dichloroindophenol (DCIP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Metaphosphoric acid (HPO_3), L-ascorbic acid (vitamin C), 2-deoxyribose, Thiobarbituric acid (TBA), and Gallic acid were purchased from Sigma, USA. Methanol (MeOH), Acetic acid, Hydrogen peroxide (H_2O_2), Ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), Potassium permanganate (K_2MnO_4), Sodium hydroxide (NaOH), and Folin-Ciocalteu reagent were purchased from BDH, UK. Ethylene diamine tetra acetic acid (EDTA) and Potassium dihydrogen phosphate (KH_2PO_4) were purchased from Fluka, Switzerland. Hydrochloric acid (HCl) and Sodium carbonate (Na_2CO_3) were purchased from Scharlau, Spain. Petroleum ether was purchased from Lab-Scan, Thailand. Sulfuric acid (H_2SO_4) was purchased from J.T Baker, USA. Trichloroacetic acid (TCA) was purchased from Merck, Germany.

2.2 Vegetable samples

The young leaves of Pak-Wanpa were purchased from Saraburi, Thailand.

2.3 Sample preparation

The young leaves of vegetables were cleaned and cut into small pieces, then homogenized and filtered. The juice was prepared by making

two-fold serial dilution, used for antioxidant capacity and total phenolics determination. For nutritional values, the young leaves were cleaned and cut into small pieces before being dried in an oven at 100°C to determine water content. After that, the residues were ground to fine powder in a blender and kept at room temperature prior to being used for the analyses of protein, lipid, fiber and ash.

2.4 Antioxidant capacity

DPPH assay (Leong and Shui, 2002)

The antioxidant capacity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical. The 200 μl aliquot of sample or 200 μl of aliquot of distilled water (control) was added to 3 ml of 1×10^{-4} M methanolic solution of DPPH and mixed well. After that, it was centrifuged at 3000 rpm for 5 min to separate the particles. The absorbance of supernatant was read at 515 nm using a spectrophotometer (Shimadzu UV-160A, Japan). Ascorbic acid was used as a standard and the assessment of antioxidant capacity was expressed as the 50 percent inhibitory concentration (IC_{50}).

2-deoxyribose assay (Chung et al., 1997)

The antioxidant capacity of the vegetable juice was determined by 2-deoxyribose assay based on the inhibition of the deoxyribose degradation caused by the attack of hydroxyl radicals. The hydroxyl radicals were induced in the system by the Fenton reaction. In the final volume of 2 ml, the reaction mixtures contained the following reagents: 100 μl of 10 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 μl of 10 mM EDTA, 200 μl of 10 mM 2-deoxyribose, 20 μl of sample, 1.38 ml of 0.1 mM Phosphate buffer pH 7.4 and 200 μl of 10 mM H_2O_2 . The reaction mixture was mixed well and incubated at 37°C for

1 h. Then, oxidized products of 2-deoxyribose were determined by boiling with 1 ml of 2.8 % trichloroacetic acid solution and 1 ml of 1 % thiobarbituric acid solution for 10 min. Then, tubes were cooled on ice. The absorbance of thiobarbituric acid reactive substances was recorded at 532 nm using a spectrophotometer (Shimadzu UV-160A, Japan). The antioxidant capacity of the sample was expressed as the IC_{50} .

2.5 Vitamin C content by the Titrimetric method

The content of vitamin C in the sample was determined by the Titrimetric method (AOAC, 1990). In brief, the sample was cleaned and cut into small pieces and then 18 g of sample was extracted by 250 ml of metaphosphoric-acetic acid ($HPO_3/HOAc$) solution at room temperature and filtered through a filter paper. Two ml of extracted sample was transferred into a 150 ml flask that contained 5 ml of $HPO_3/HOAc$ solution. After that, titration with 2,6-dichloroindophenol was performed. The amount of vitamin C in a sample was determined by redox titration using the reaction between ascorbic acid and 2,6-dichloroindophenol acting as a self-indicator in the titration. In acidic solutions 2,6-dichloroindophenol is red, but if ascorbic acid is present, it will be reduced to a colorless substance. The solution will remain colorless as more 2,6-dichloroindophenol is added until all of the ascorbic acid has reacted. As soon as the next drop of 2,6-dichloroindophenol solution is added, the solution will be light red, due to the excess 2,6-dichloroindophenol and the end point of the titration has been reached. The content of vitamin C was then determined by referring to the calibration graph, using ascorbic acid solution as a standard.

2.6 Determination of total phenolics content

Total phenolics content of young leaves was determined by Folin-Ciocalteu assay (Torres et al., 1987). In brief, 50 μ l of sample was added to the mixtures of distilled water (3 ml), 250 μ l of Folin-Ciocalteu reagent, 750 μ l of 20 % Na_2CO_3 and the volume was made up to 5 ml by adding distilled water. Then, it was mixed well and incubated at 50 °C for 2 h. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu UV-160A, Japan). The concentration was calculated using gallic acid as a standard, and the results were expressed as gallic acid equivalents.

2.7 Nutritional values

The nutritional value of the sample derived from water, protein, lipid, carbohydrate, fiber and ash.

Water content

Water content was determined by the drying method. The sample was cleaned and cut into small pieces. After that, 10 g of sample was transferred into a plate before drying at 100 °C for 4 h. The sample was weighed and dried again until the difference of two successively dried weights should not exceed 0.003 g. Water content was calculated by the following formula.

$$\text{Water content} = (a - b) \times 100/a$$

where a is the fresh weight and b is the dried weight.

Protein content

Protein content was determined by the Kjeldahl method. 0.5 g of dried sample was digested with 2 tablets of Kjeltabs and 10 ml of conc. H_2SO_4 in a digestion tube at 420 °C for 30 - 45 min. After that, 50 ml of distilled water was

added and distilled with 40 % NaOH in order to obtain ammonia which was collected in 25 ml of 4 % Boric acid. The amount of nitrogen was calculated from the volume of 0.1 M HCl used for titration.

$$N (\%) = 1.401 \times (V_1 - V_2) \times M_{HCl} / W_s$$

where V_1 and V_2 are the volumes of HCl used for titration of the sample and blank, respectively. M_{HCl} is the concentration of HCl and W_s is the weight of sample.

The amount of nitrogen was converted to protein content by using a constant factor.

$$\text{Protein } (\%) = N (\%) \times 6.25$$

Lipid content

Lipid content was determined by Soxtec (Soxtec system HT, 1043 Extraction unit, Tecator).

Three g of dried sample was packed in the filter paper and transferred to the thimble. The sample was extracted by 50 ml of petroleum ether in the extraction cup for 1.30 h. Then, the extraction cup was dried in an oven at 100 °C for 30 min. The lipid content was obtained by means of weighing and calculated by the following formula.

$$\text{Lipid } (\%) = (W_2 - W_1) \times 100 / W_s$$

where W_1 and W_2 are the weights of extraction cup before and after extracting lipid and W_s is the weight of sample.

Fiber and ash content

Fiber content was determined by Fibertec. The crucible was cleaned and dried in an oven before weighing (W_1). About 0.5 g of dried sample was weighed in a crucible (W_2) and transferred to the Fibertec system (1020 Hot extractor, Tecator). The sample was hydrolyzed with 150 ml of hot 0.128 M H_2SO_4 and boiled for 30 min. Then, it was rinsed with 30 ml of hot distilled water 3 times,

followed by hydrolyzing with 150 ml of hot 0.223 M KOH and boiled for 30 min and then rinsed with 30 ml of hot distilled water 3 times. Then, it was washed with 20 ml of acetone 3 times in the cold extraction unit. After that, the crucible was dried at 130 °C for 2 h and weighed (W_3). The residue was composed of crude fiber and ash. The residue was burned at 500 °C for 3 h in a muffle furnace (NEY model 6-1350A). After the crucible was cooled in a desiccator, it was weighed (W_4). Fiber and ash contents were calculated by the following formulae.

$$\text{Fiber } (\%) = W_3 - W_4 \times 100 / W_s$$

$$\text{Ash } (\%) = W_4 - W_1 \times 100 / W_s$$

where W_s is the weight of sample.

Carbohydrate content

Carbohydrate content was calculated by subtracting water content, protein content, lipid content, fiber content and ash content from 100.

3. RESULTS AND DISCUSSION

The antioxidant capacity was determined using two different chemical assays. For the first assay, the free radical scavenging activity of the vegetable was tested with DPPH assay. The role of antioxidant is its interaction with oxidative free radicals. The essence of DPPH assay is that the antioxidant reacts with the stable free radical i.e. 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1,1-diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample antioxidant. In the present study, Pak-Wanpa was able to decolorize DPPH and the free radical scavenging potential of the vegetable was expressed as the 50 percent inhibitory concentration (IC_{50}). For DPPH assay, antioxidant capacity (IC_{50} value) of standard

antioxidant (ascorbic acid) was $20.64 \pm 0.07 \mu\text{M}$ and antioxidant of Pak-Wanpa was $21.94 \pm 0.73 \mu\text{M}$ vitamin C equivalent. In the present study, antioxidant capacity of Pak-Wanpa was comparable

to that of vitamin C in this assay as shown in Figure 1. For 2-deoxyribose assay, antioxidant capacity (IC_{50} value) of Pak-Wanpa was $0.05 \pm 0.00 \%$ (v/v).

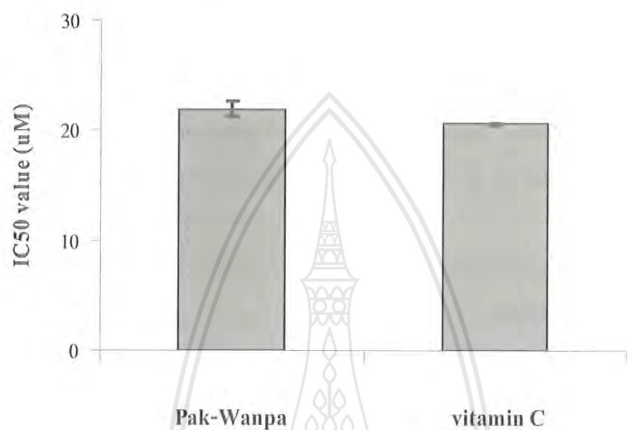


Figure 1. Antioxidant capacity of Pak-Wanpa and vitamin C.

Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (i.e. L-ascorbate anion). As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical and singlet oxygen. Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules. The ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate.

The content of vitamin C of Pak-Wanpa was $96.20 \pm 0.55 \text{ mg}/100 \text{ g}$ and the total phenolics was $370.69 \pm 8.74 \text{ mg gallic acid equivalent}/100 \text{ ml}$. Total phenolics determined in this assay are not absolute measurements of the amounts of phenolic compounds, but are in fact based on their chemical

reducing capacity relative to gallic acid (Abdille et al., 2005). The nutritional values of Pak-Wanpa are shown in Table 1.

Table 1. The nutritional values of Pak-Wanpa.

Nutritional values (%)	
water	78.16 ± 0.71
protein	7.43 ± 0.10
lipid	0.52 ± 0.04
carbohydrate	8.45 ± 0.05
fiber	3.90 ± 0.12
ash	1.54 ± 0.16

Table 2 shows the nutritional values of Pak-Wanpa in the present study and nutritional values of Pak-Wanpa and other vegetables (obtained from different trees) analyzed by the Nutrition

Division (2001). In this study, water is the major component of Pak-Wanpa followed by carbohydrate, protein, fiber, ash and lipid. The data obtained from the Nutrition Division also shows that water is the most abundant component of Pak-Wanpa followed by protein, carbohydrate, fiber, ash and lipid. The differences of the same components of Pak-Wanpa obtained from this study and the Nutrition Division might be due to the variation of the origins of the

plant and the harvest seasons. All vegetables (Table 2) show that water is the highest component whereas the other components are much smaller. Vegetable is well recognized as a good source of minor nutrients such as vitamins and minerals, and the other important components including dietary fibers and phytochemicals, which can play important roles in the human body.

Table 2. The nutritional values of Pak-Wanpa and some vegetables in Thailand.

Nutritional values (%)	water	protein	lipid	carbohydrat	fiber	ash
Pak-Wanpa*	78.16	7.43	0.52	8.45	3.90	1.54
Pak-Wanpa [†]	87.1	8.2	0.6	2.3	2.1	1.8
Pak-meg [†]	79.1	3.0	1.0	15.8	3.2	1.1
Pak-kadone [†]	76.8	2.6	0.5	18.8	1.9	1.3
Sesbania, tender tips [†]	74.4	8.3	2.6	12.8	5.1	1.9
Cassia, tips and leaves [†]	78.4	7.7	1.4	10.9	5.6	1.6

*present study

[†]Nutrition Division

4. CONCLUSION

Pak-Wanpa exhibited antioxidant capacity giving IC₅₀ values for two different spectrophotometric assays (DPPH assay and 2-deoxyribose assay). Pak-Wanpa is a good source of vitamin C and total phenolics. The major content of Pak-Wanpa is water followed by carbohydrate, protein, fiber, ash and lipid. This is the first report that has revealed the antioxidant capacity and total phenolics content of Pak-Wanpa. This vegetable could be a good source of antioxidants. Further studies are needed for the isolation of active compounds and also in

vivo studies are needed for better understanding of their mechanisms of action as antioxidant.

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Antioxidant Capacity of Vegetable Juices

ความสามารถในการต้านออกซิเดชันของน้ำผัก

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Abstract

Vegetables are a good source of crucial nutrients such as vitamins and minerals and the other important components including dietary fiber and phytochemicals, which can delay the progress of degenerative diseases. Twenty vegetable juices were evaluated for their antioxidant capacity using 1, 1-diphenyl-2-picrylhydrazyl assay (DPPH assay) and 2-Deoxyribose assay. These juices were also examined for vitamin C and total phenolics contents. Neem juice exhibited the strongest activity against DPPH radicals, followed by red pepper juice with their IC_{50} values of 0.07 ± 0.00 % (v/v) and 0.25 ± 0.00 % (v/v), respectively. In good correlation with the antioxidant activity, neem contained a very high content of total phenolics (643.65 ± 86.74 mg gallic acid equivalent/100 mL) compared to other vegetable juices whereas red pepper contained the greatest amount of vitamin C (69.16 ± 5.32 mg/100 mL). Spinach juice showed the highest inhibition of damage to 2-deoxyribose from hydroxyl radical ($\cdot OH$), giving an IC_{50} value of 0.64 ± 0.02 % (v/v), although its total phenolics was 202.51 ± 13.3 mg gallic acid equivalent/100 mL and vitamin C was 7.76 ± 0.67 mg/100 mL. The amount of total phenolics and vitamin C appears to correlate with the antioxidant capacity of some vegetable juices.

บทคัดย่อ

ผักเป็นแหล่งที่ดีของสารอาหารที่ร่างกายต้องการในปริมาณน้อยซึ่งได้แก่วิตามินและแร่ธาตุ อีกทั้งยังมีสารที่มีความสำคัญอื่นๆ รวมอยู่ด้วย ได้แก่ ใยอาหารและสารพฤกษเคมี ซึ่งสามารถช่วยชะลอการเกิดโรคเนื่องจากการเสื่อมสภาพของร่างกาย การศึกษานี้ได้ทำการวิเคราะห์ความสามารถในการต้านออกซิเดชันของน้ำผัก 20 ชนิด ด้วยวิธีที่ใช้สาร 1,1-diphenyl-2-picrylhydrazyl (DPPH) และวิธีที่ใช้สาร 2-Deoxyribose นอกจากนี้ยังวิเคราะห์หาปริมาณวิตามินซีและปริมาณฟีนอลิกทั้งหมดด้วยผลการวิเคราะห์แสดงให้เห็นว่าน้ำจากใบสะเดามีความสามารถในการต่อต้านอนุมูล DPPH ได้สูงที่สุด ตามมาด้วยน้ำพริกชี้ฟ้าแดง โดยมีค่า IC_{50} เท่ากับ 0.07 ± 0.00 % (v/v)

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และ 0.25 ± 0.00 % (v/v) ตามลำดับ องค์ประกอบที่คาดว่าทำให้น้ำผักดังกล่าวมีความสามารถในการต้านอนุมูลอิสระได้ดี ได้แก่ สารฟีนอลิกทั้งหมดซึ่งมีค่าสูงมากในน้ำสะเดา (643.65 ± 86.74 มก. เทียบเท่ากับกรดแกลลิก/100 มล.) และปริมาณวิตามินซีในน้ำพริกขี้หนูแดง (69.16 ± 5.32 มก./100 มล.) น้ำผักโขมสามารถป้องกันการทำลาย 2-Deoxyribose โดยอนุมูลไฮดรอกซิลได้ดีที่สุด โดยให้ค่า IC_{50} เท่ากับ 0.64 ± 0.02 % (v/v) แม้ว่าจะมีปริมาณฟีนอลิกทั้งหมดเท่ากับ 202.51 ± 13.3 มก. เทียบเท่ากับกรดแกลลิก/100 มล. และมีวิตามินซีเท่ากับ 7.76 ± 0.67 มก./100 มล. อย่างไรก็ตามปริมาณฟีนอลิกทั้งหมดและวิตามินซีดูเหมือนว่าจะมีผลต่อความสามารถในการต้านออกซิเดชันของน้ำผักจำนวนหนึ่ง

Keywords: antioxidant capacity, vegetable juice, vitamin C, total phenolics

คำสำคัญ: ความสามารถในการต้านออกซิเดชัน, น้ำผัก วิตามินซี, ฟีนอลิกทั้งหมด

1. INTRODUCTION

Vegetable can be any part of a plant that can be used for cooking or consumption. It can be a root, stem, shoot, leaf, flower, fruit, and even a seed. Vegetables are a good source of minor but crucial nutrients such as vitamins, minerals and the other important components such as dietary fibers and phytochemicals. Several studies showed that high consumption of vegetable and fruit had an inverse relationship with the incidence of degenerative diseases including heart diseases (Hertog et al., 1993; Lee et al., 2004), cancer (Block et al., 1992; Byers and Perry, 1992; Sugimura, 2002), inflammation (Giugliano et al., 2006; Rahman et al., 2006) and brain dysfunction (Youdim and Joseph, 2001; Trewavas and Stewart, 2003).

Free radicals can cause oxidative damage to all biomolecules and initiate a chain reaction which results in physiological damage. This physiological damage can be repaired but may also accumulate over a period of time and cause many degenerative diseases (Ames et al., 1993). The role of antioxidants is to protect all biomolecules from the oxidative damage of free radicals, therefore

antioxidants can delay the progress of degenerative diseases. Human body can produce some antioxidants especially many enzymatic systems (superoxide dismutase, catalase and glutathione peroxidase) and can obtain some low molecular weight antioxidants from foods, in particular vegetables and fruits (vitamin C, vitamin E, carotenoids and phenolic compounds), which can scavenge some reactive species (superoxide anion and hydrogen peroxide) and inhibit a chain reaction. Most information available for vegetables is about their nutritional values. There is insufficient data about antioxidant capacity of vegetables. Therefore, the objective of this study was to determine the antioxidant capacity, vitamin C content, and total phenolics content of some vegetable juices. The relationships between antioxidant capacity and vitamin C content or phenolics content, were also determined.

2. MATERIAL AND METHODS

2.1 Vegetable samples

Twenty vegetables were purchased from 4 markets in Khon Kaen, Thailand. The common and scientific names of these vegetables are given in Table 1.

Table 1. List of twenty vegetable samples.

No.	Common name	Scientific name
1.	Neem	<i>Azadirachta indica</i> A. Juss.
2.	Red, long pepper	<i>Capsicum annuum</i> Linn. Var. <i>acuminatum</i> Fingerth
3.	Sweet basil	<i>Ocimum basilicum</i> Linn.
4.	Sacred basil	<i>Ocimum sanctum</i> Linn.
5.	Dill	<i>Anethum graveolens</i> L.
6.	Purple egg plant	<i>Solanum melongena</i>
7.	Spinach	<i>Spinacia oleracia</i> Linn.
8.	Woolly morning glory	<i>Ipomoea aquatica</i>
9.	Lime	<i>Citrus aurantifolia</i> Swig.
10.	Chinese kale	<i>Brassica oleracea</i> cv. group Chinese kale
11.	Tomato	<i>Lycopersicum esculentum</i> Mill.
12.	Chinese radish	<i>Raphanus sativus</i> Linn.
13.	Pakchoi	<i>Brassica napus</i> L. var <i>Chinensis</i>
14.	Lettuce	<i>Lactuca sativa</i> Linn.
15.	Spring onion, flower	<i>Allium ascalonicum</i> Linn.
16.	Chinese celery	<i>Apium graveolens</i> Linn.
17.	Carrot	<i>Daucus carota</i> Linn.
18.	Sweet pepper	<i>Capsicum annuum</i> Linn.
19.	Broccoli	<i>Brassica oleracea</i> Linn.
20.	Cabbage	<i>Brassica oleracea</i> Linn. var. <i>capitata</i>

Edible parts of vegetable were cut into small pieces and ground with pestle and mortar. The juice was obtained by squeezing the vegetable pulp through a filter cloth. The vegetable juice is then diluted with distilled water in order to make the 2-fold serial dilutions for antioxidant capacity analysis.

2.2 Chemicals

The major chemicals were purchased from Sigma (U.S.A) including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,6-dichloroindophenol (DCIP), 2-deoxyribose, 2-thiobarbituric acid (TBA), gallic acid and L-ascorbic acid. The other chemicals and reagents used in this study were of analytical grade.

2.3 Antioxidant capacity by DPPH assay (Leong and Shui, 2002)

Two hundred microliters of vegetable juice was mixed with 3 mL of 100 μ M DPPH (in methanol) and centrifuged at 3,000 rpm for 5 min. The absorbance of the supernatant was read at 515 nm. The antioxidant capacity was expressed as 50 % inhibitory concentration (IC₅₀) which could be obtained from a graph plotted with the concentrations of vegetable juice versus the absorbance of DPPH. The lower IC₅₀ indicates the higher antioxidant capacity of vegetable juice.

2.4 Antioxidant capacity by 2-deoxyribose assay (Chung et al., 1997)

Twenty microliters of vegetable juice was added to a mixture of 0.2 mL of 10 mM 2-deoxyribose, 0.1 mL of 10 mM FeSO₄·7H₂O, 0.1 mL of 10 mM EDTA and 1.38 mL of 0.1 M phosphate buffer pH 7.4. Two hundred microliters of 10 mM H₂O₂ was then added to the mixture, mixed, and incubated at 37 °C for 1 hr. After incubation, 1 mL of 2.8 % trichloroacetic acid and 1 mL of 1 % thiobarbituric acid was added the reaction tube which was then mixed, and then boiled for 10 min. The tube was cooled and the absorbance of thiobarbituric acid reactive substances (TBARS) was recorded at 530 nm. The antioxidant capacity was expressed at 50 % inhibitory concentration (IC₅₀) as mentioned above.

2.5 Vitamin C content of vegetable juice

(The association of official analytical chemists, 1990)

Vegetable juice was mixed with metaphosphoric/acetic acid solution (HPO₃/HOAc) at a ratio of 1:1. Then 7 mL of the mixture was titrated with 2,6-dichloroindophenol solution to the end point which was pale pink. L-ascorbic acid was used as a standard vitamin C.

2.6 Total phenolic content of vegetable juice

(Torres et al., 1987)

Fifty microliters of vegetable juice was added to a tube containing 3 mL distilled water, 0.25 mL Folin-ciocalteu's reagent, 0.75 mL of 20 % Na₂CO₃. The total volume was adjusted to 5 mL by distilled water then mixed, and incubated at 50 °C for 2 hrs. The absorbance of each tube was recorded at 765 nm. The total phenolics in juice was expressed as mg gallic acid equivalent per 100 mL (mg GAE/100 mL)

3. RESULTS

3.1 Antioxidant capacity of vegetable juice by DPPH assay

The antioxidant capacity (IC₅₀ values) of 20 vegetable juices is shown in Table 2 and Figure 1. Neem juice exhibited the highest antioxidant capacity by giving an IC₅₀ value of 0.07 ± 0.00 % (v/v) followed by red & long pepper, sweet basil, sacred basil, dill, purple egg plant, spinach, wooly morning glory, lime, Chinese kale, Chinese radish, pakchoi, lettuce, flower of spring onion, Chinese celery, carrot, sweet pepper, broccoli and cabbage.

Table 2. Antioxidant capacity of 20 vegetable juices determined by DPPH assay.

No. of Vegetable	IC ₅₀ (% v/v)	No. of Vegetable	IC ₅₀ (% v/v)
(1) Neem	0.07 ± 0.00	(11) Tomato	1.31 ± 0.16
(2) Red, long pepper	0.25 ± 0.00	(12) Chinese radish	1.78 ± 0.03
(3) Sweet basil	0.34 ± 0.18	(13) Pakchoi	1.78 ± 0.32
(4) Sacred basil	0.66 ± 0.01	(14) Lettuce	2.06 ± 0.08
(5) Dill	0.72 ± 0.14	(15) Flower of spring onion	2.12 ± 0.03
(6) Purple egg plant	0.78 ± 0.01	(16) Chinese celery	2.22 ± 0.54
(7) Spinach	0.87 ± 0.00	(17) Carrot	2.39 ± 0.09
(8) Woolly morning glory	0.90 ± 0.02	(18) Sweet pepper	3.51 ± 0.06
(9) Lime	0.91 ± 0.00	(19) Broccoli	3.61 ± 0.45
(10) Chinese kale	1.28 ± 0.12	(20) Cabbage	5.42 ± 1.96

Figure 1 shows the histogram of IC₅₀ values of all juices starting from the lowest, neem juice, to the highest, cabbage juice. The letters “a, b, c, d, e and f” indicate significant difference of IC₅₀ values, with different letters being significantly different (p < 0.05).

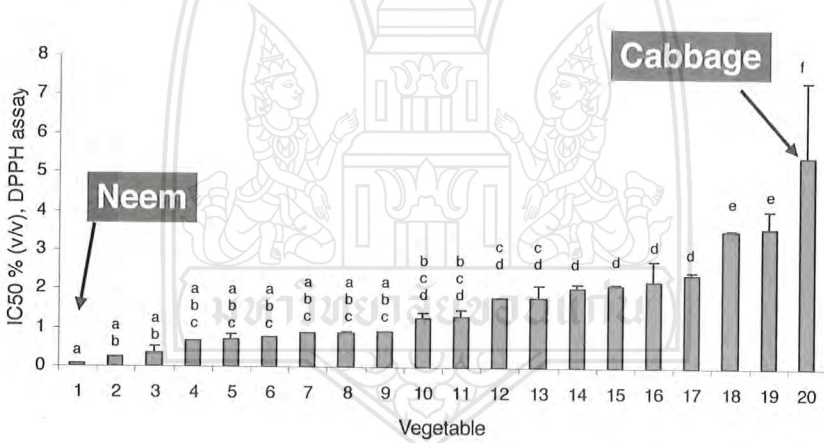


Figure 1. IC₅₀ values of 20 vegetable juices determined by DPPH assay.

3.2 Antioxidant capacity of vegetable juice by 2-deoxyribose assay

The antioxidant capacity (IC₅₀ values) of 20 vegetable juices is shown in Table 3 and Figure 2. Spinach juice exhibited the highest antioxidant capacity with an IC₅₀ value of 0.63 ± 0.02 % (v/v)

followed by dill, pakchoi, woolly morning glory, Chinese kale, Chinese celery, sweet basil, broccoli, purple egg plant, red & long pepper, carrot, flower of spring onion, sacred basil, lettuce, sweet pepper, Chinese radish, neem, cabbage, tomato and lime.

Table 3. Antioxidant capacity of 20 vegetable juices determined by 2-deoxyribose assay.

No. of Vegetable	IC ₅₀ (% v/v)	No. of Vegetable	IC ₅₀ (% v/v)
(1) Spinach	0.63 ± 0.02	(11) Carrot	1.21 ± 0.00
(2) Dill	0.64 ± 0.00	(12) Flower of spring onion	1.24 ± 0.08
(3) Pakchoi	0.64 ± 0.01	(13) Sacred basil	1.28 ± 0.56
(4) Woolly morning glory	0.74 ± 0.05	(14) Lettuce	1.30 ± 0.20
(5) Chinese kale	0.75 ± 0.04	(15) Sweet pepper	1.31 ± 0.14
(6) Chinese celery	0.80 ± 0.11	(16) Chinese radish	1.62 ± 0.33
(7) Sweet basil	1.00 ± 0.01	(17) Neem	1.94 ± 0.53
(8) Broccoli	1.12 ± 0.05	(18) Cabbage	2.45 ± 0.56
(9)Purple egg plant	1.18 ± 0.01	(19) Tomato	2.48 ± 0.23
(10) Red & long pepper	1.20 ± 0.12	(20) Lime	2.98 ± 0.07

Figure 2 shows the histogram of IC₅₀ values of all juices starting from the lowest, pakchoi juice, to the highest, lime juice. The letters “a, b, c, d, e, f, g and h” indicate significant difference of IC₅₀ values, with different letters being significantly different (p < 0.05).

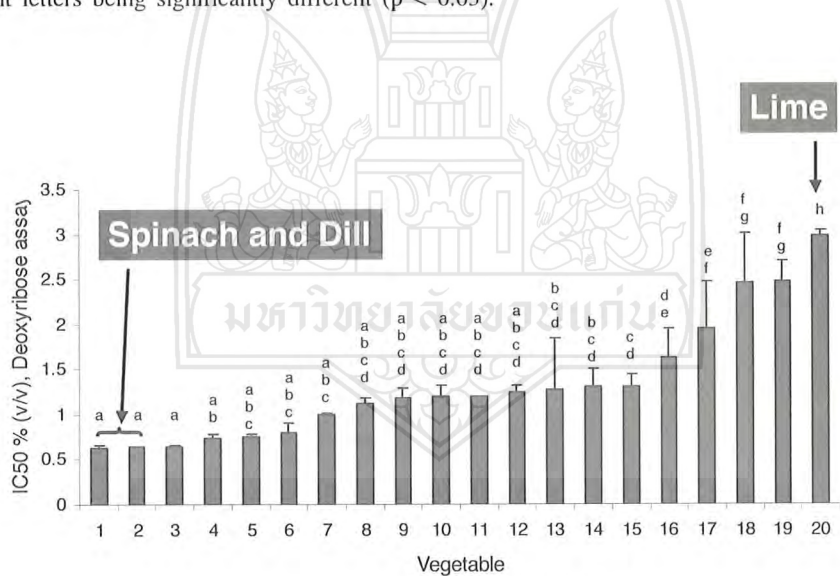


Figure 2. IC₅₀ values of 20 vegetable juices determined by 2-deoxyribose assay.

3.3 Vitamin C content of 20 vegetable juices

The vitamin C content of 20 vegetable juices is shown in Table 4 and Figure 3. The juice of purple egg plant gave the lowest vitamin C

content, 1.41 ± 0.17 mg/100 mL, whereas the juice of red & long pepper had the highest amount of vitamin C (69.16 ± 5.32 mg/100mL).

Table 4. Vitamin C content of 20 vegetable juices.

No. of Vegetable	Vitamin C (mg/100mL)	No. of Vegetable	Vitamin C (mg/100mL)
(1) Purple egg plant	1.41 ± 0.17	(11) Woolly morning glory	7.29 ± 2.00
(2) Cabbage	2.12 ± 0.29	(12) Spinach	7.76 ± 0.67
(3) Broccoli	3.76 ± 1.00	(13) Tomato	8.24 ± 0.87
(4) Flower of spring onion	4.12 ± 0.67	(14) Pakchoi	8.24 ± 2.66
(5) Sweet pepper	4.71 ± 1.66	(15) Chinese radish	8.88 ± 0.29
(6) Dill	5.41 ± 0.00	(16) Sacred basil	9.41 ± 0.67
(7) Chinese celery	5.41 ± 0.67	(17) Chinese kale	11.29 ± 2.00
(8) Lettuce	5.65 ± 0.00	(18) Sweet basil	11.29 ± 0.67
(9) Neem	6.12 ± 2.33	(19) Lime	20.41 ± 0.29
(10) Carrot	6.59 ± 0.67	(20) Red & long pepper	69.16 ± 5.32

Figure 3 shows the histogram of the vitamin C content in 20 vegetable juices. The highest vitamin C content was found in the juice of red & long pepper followed by lime, sweet basil, Chinese kale, sacred basil, Chinese radish, pakchoi, tomato, spinach, woolly morning glory, carrot, neem, lettuce, Chinese celery, dill, sweet pepper, flower of spring onion, broccoli, cabbage and purple egg plant. The letters “a, b, c, d, e, f, g and h” indicate significant difference in vitamin C content, with different letters being significantly different ($p < 0.05$).

3.4 Total phenolic content of 20 vegetable juices

The total phenolic content of 20 vegetable juices is shown in Table 5 and Figure 4. Neem juice contains the highest amount of total phenolics, 643.64 ± 86.74 mg GAE/100mL, followed by sweet basil (327.32 ± 74.8 mg GAE/100 mL), whereas carrot juice showed the lowest amount of total phenolics (10.78 ± 0.72 mg GAE/100 mL).

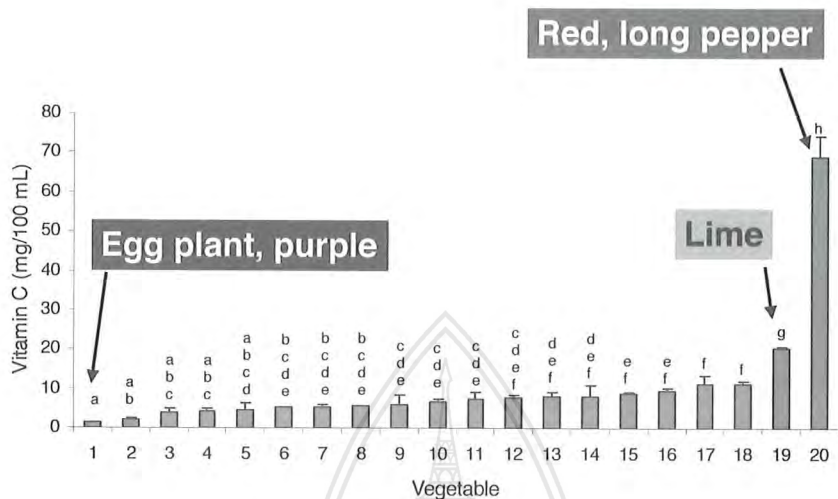


Figure 3. Vitamin C content of 20 vegetable juices.

Table 5. Total phenolic content of 20 vegetable juices.

No. of Vegetable	Total phenolics (mgGAE/100 mL)	No. of Vegetable	Total phenolics (mgGAE/100 mL)
(1) Carrot	10.78 ± 0.72	(11) Purple egg plant	116.00 ± 10.21
(2) Cabbage	15.06 ± 4.7	(12) Sweet pepper	116.84 ± 5.10
(3) Chinese radish	20.92 ± 1.04	(13) Chinese kale	121.24 ± 3.82
(4) Lime	29.60 ± 1.83	(14) Dill	169.17 ± 40.86
(5) Flower of spring onion	32.69 ± 1.43	(15) Woolly moring glory	171.24 ± 3.35
(6) Tomato	51.95 ± 3.03	(16) Spinach	202.51 ± 13.30
(7) Lettuce	52.12 ± 6.93	(17) Red & long pepper	219.52 ± 9.80
(8) Chinese celery	77.52 ± 13.38	(18) Sacred basil	280.46 ± 69.54
(9) Pakchoi	78.48 ± 11.23	(19) Sweet basil	327.32 ± 74.80
(10) Broccoli	91.72 ± 21.67	(20) Neem	643.64 ± 86.74

Figure 4 shows the histogram of the total phenolic content in 20 vegetable juices. The highest total phenolic content was found in the juice of neem followed by sweet basil, sacred basil, red & long pepper, spinach, woolly morning glory, dill, Chinese kale, sweet pepper, purple egg plant,

broccoli, pakchoi, Chinese celery, lettuce, tomato, flower of spring onion, lime, Chinese radish, cabbage and carrot. The letter “a, b, c, d, e, f, g and h” indicate significant difference of total phenolic content, different letters were significantly different (p < 0.05).

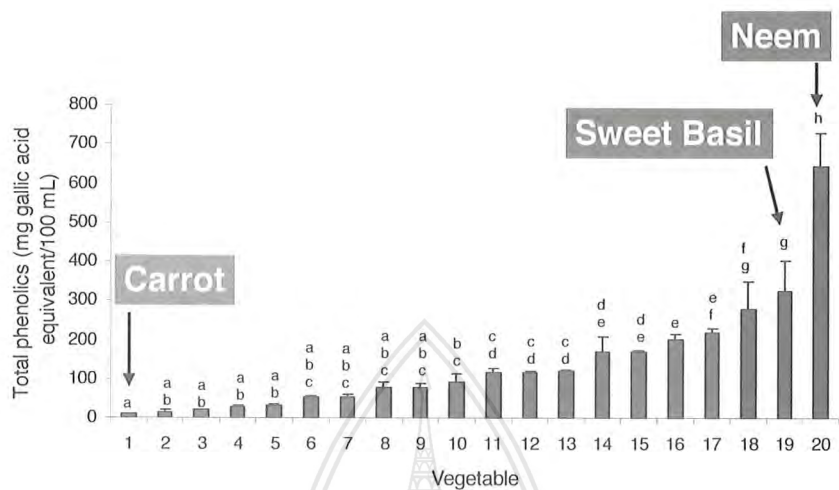


Figure 4. Total phenolic content of 20 vegetable juices.

3.5 Correlation coefficients of antioxidant capacity and vitamin C or total phenolics in juices

Table 6 shows the correlation coefficient values of antioxidant capacity of vegetable juices determined by 2 assays and vitamin C content, or total phenolics of those juices. The values indicated the trend of the relationship between antioxidant capacity and vitamin C content, or total phenolics of vegetable juices. The inverse relationship of IC₅₀ value and vitamin C, or total phenolics, implied that vitamin C and total phenolics contributed to antioxidant capacity of these vegetables. The vegetable

juice that contained high amount of vitamin C or total phenolics would give low IC₅₀ value. The lower the IC₅₀ value indicates higher antioxidant capacity of the juice. The correlation coefficients of antioxidant capacity and vitamin C content, or total phenolic content, were determined from 20 values obtained from 20 vegetables, all of which were very low. Therefore, the correlation coefficient values obtained in Table 6, are the values after some vegetables were discarded from the calculation until the values were as high as possible. The superscript of each r value represents the vegetables in each group.

Table 6. Correlation coefficients of IC50 values and vitamin C or total phenolics.

	Correlation coefficient (r)
IC ₅₀ (DPPH) & vitamin C	-0.51 ^w
IC ₅₀ (Deoxyribose) & vitamin C	-0.26 ^x
IC ₅₀ (DPPH) & total phenolics	-0.62 ^y
IC ₅₀ (Deoxyribose) & total phenolics	-0.65 ^z

w Red & long pepper, purple egg plant, Chinese kale, tomato, lettuce, carrot, flower of spring onion, Chinese celery, sweet pepper, broccoli and cabbage

x Purple egg plant, lettuce, carrot, neem, flower of spring onion, Chinese celery, sweet pepper, broccoli and cabbage

y Chinese radish, lettuce, carrot, neem, flower of spring onion, sweet basil, lime, sacred basil, broccoli and cabbage

z Chinese radish, lettuce, carrot, broccoli, flower of spring onion, lime, spinach, dill, cabbage and Chinese celery

4. DISCUSSION

The antioxidant capacity of vegetable juices were determined by two assays. The first one was DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and the second one was 2-deoxyribose assay. DPPH is a stable free radical which has maximum absorption at 515 nm. In the presence of antioxidants, DPPH is reduced to a non radical form and the maximum absorbance at 515 nm is decreased. The antioxidant capacity of vegetable juice was determined by the IC_{50} value which was obtained from a graph plotted between the concentrations of vegetable juice and the corresponding absorbances. DPPH assay is a simple and effective assay to evaluate antioxidant capacity of plant extracts or juices of vegetable and fruit. The result of this study showed that neem juice exhibited the highest antioxidant capacity determined by the DPPH assay. Sithisarn et al. (2005) showed that neem leaf (aqueous extract) exhibited antioxidant capacity about 50 % of that of butylated hydroxytoluene (BHT), which had the highest antioxidant capacity among the other extracts of this plant (flower, fruit, seed, and stem bark). The antioxidant activity of five vegetables (kale, spinach, cabbage, shallot and swamp cabbage) was

determined by carotene bleaching method (Amin and Tan, 2002) done in Malaysia. Among the five vegetables, shallot showed the highest antioxidant activity, followed by spinach, swamp cabbage, cabbage, and kale. Sourti et al. (2004) reported that the methanolic extracts of savory, radish leaf, garden cress, spearmint, leek, lettuce and dill showed antioxidant activity comparable to those of dl- α -tocopherol and quercetin, determined by the inhibition of linoleic acid peroxidation. The result from 2-deoxyribose assay showed that the juices of spinach, dill, and pakchoi exhibited the highest antioxidant capacity in this study. The free radical in 2-deoxyribose assay is hydroxyl radical (OH^*) which is generated from the reaction of ferrous ion with hydrogen peroxide. The hydroxyl radical is the most reactive oxygen radical and can induce severe damage to adjacent biomolecules (Gutteridge, 1984). The top ten most potent antioxidant capacity obtained from the 2 assays showed that 7 of them were the same vegetables including red & long pepper, sweet basil, dill, purple egg plant, spinach, woolly morning glory and Chinese kale. Dasgupta and De (2006) used 4 assays to analyze antioxidant activity of 11 leafy vegetables of India. One of the

assays used was similar to 2-deoxyribose assay done in this study. It was shown that *Ipomoea reptans* (woolly morning glory) exhibited the highest antioxidant activity. They also found that there was no correlation between antioxidant activity and total phenol/flavonoid content of these vegetables whereas Zhou and Yu (2006) found that total phenolic content and the measured antioxidant properties were correlated with each other. In this present study, antioxidant capacity of only some vegetables that correlated with total phenolics whereas vitamin C content revealed less correlation with antioxidant capacity of some vegetable juices. Actually pure vitamin C and many phenolic compounds can act as good antioxidants in DPPH assay but not in 2-deoxyribose assay. Vegetable juice is a very complex mixture of various substances which can exhibit antioxidant activity or can be prooxidant in different tested systems. However, total phenolics and vitamin C content appeared to be the compounds of interest in determining the antioxidant capacity of plant materials.

5. CONCLUSION

The present study found that neem juice exhibited the highest antioxidant capacity, determined by DPPH assay, whereas spinach juice, dill juice, pakchoi juice, woolly morning glory juice, Chinese kale juice and Chinese celery juice showed the most potent antioxidant capacity determined by 2-deoxyribose assay. Red & long pepper juice contained the greatest amount of vitamin C whereas neem juice contained the highest amount of total phenolics. The vitamin C and total phenolic contents seemed to play an important role in antioxidant capacity of some vegetable juices.

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A Preliminary Study of Aquatic Insect Diversity and Water Quality of Mekong River, Thailand

การศึกษาเบื้องต้นความหลากหลายของแมลงน้ำและคุณภาพน้ำในแม่น้ำโขง ประเทศไทย

Isara Thani¹ and Chitchol Phalaraksh^{2*}

Abstract

This study was aimed to study the diversity of aquatic insects and physicochemical factors of water in Mekong River. The aquatic insects were collected using pond net (0.5 mm) from 14 study sites during December 2005 and January 2006. Seven orders and 23 families were found, with the highest number of aquatic insects from the family Ephemeroptera. The most abundant family were Baetidae and Caenidae respectively, which were very commonly found in almost all sampling sites, Plecoptera as found in the upper sampling sites, which showed less polluted. The classification method of cluster analysis (UPGMA) and ordination by PCA were used to classify the sampling site. Physicochemical values, water and air temperature, pH, DO, BOD₅ and nutrient were measured, only BOD₅ value in some study sites was greater than class 4 standard value of the surface water quality standard of Thailand.

บทคัดย่อ

การศึกษานี้มีจุดประสงค์เพื่อหาความหลากหลายของแมลงน้ำและศึกษาคุณภาพน้ำทางกายภาพและเคมีของแม่น้ำโขงที่ไหลผ่านประเทศไทย โดยใช้สวิงขนาดตาข่าย 0.5 มิลลิเมตร เก็บตัวอย่างแมลงน้ำในเดือนธันวาคม 2548 และเดือนมกราคม 2549 ผลการศึกษาพบแมลงน้ำทั้งหมด 7 อันดับ 23 วงศ์ โดยอันดับที่พบจำนวนมากที่สุดคืออันดับแมลงจีบะขาว (Ephemeroptera) โดยวงศ์ที่เด่น ได้แก่ วงศ์ Baetidae และ Caenidae ซึ่งเป็นธรรมดาทั่วไปในเกือบทุกจุดเก็บตัวอย่าง แมลงเกาะหิน (Plecoptera) พบในจุดเก็บตัวอย่างส่วนต้นน้ำซึ่งพบมลพิษต่ำ การจำแนกกลุ่มของจุดเก็บตัวอย่างใช้วิธี UPGMA และ PCA ส่วนคุณสมบัติทางกายภาพและเคมีของน้ำ อุณหภูมิของน้ำและอากาศ ค่าความเป็นกรด-ด่าง (pH) ค่าออกซิเจนที่ละลายในน้ำ (DO) ค่าออกซิเจนที่

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จุลินทรีย์ใช้ย่อยสลายสารอินทรีย์ (BOD_5) และปริมาณสารอาหาร จากคุณสมบัติต่างๆ ของน้ำที่วัดได้มีเพียงค่า BOD_5 ในบางจุดที่มีปริมาณเกินค่ามาตรฐานคุณภาพน้ำในแหล่งน้ำผิวดินประเภทที่ 4

Keywords: Diversity, Aquatic insect, Mekong river

คำสำคัญ: ความหลากหลาย, แมลงน้ำ, แม่น้ำโขง

INTRODUCTION

The Mekong river is one of the main rivers in the southeast Asia. It's source is in the south-eastern Himalaya mountains and flows 4,200 kilometres to its mouth, in Vietnam into the South China Sea. This makes it the twelfth longest river. It ranks tenth in terms of discharge and top three rivers in the world (after the Amazon and Zaire) in term of fish species richness. The Upper Mekong River flows through Yunnan province, China, and western Burma. The lower basin covering parts of Thailand, Vietnam, Cambodia and the Lao People's Democratic Republic (Lao P.D.R.) Biodiversity of invertebrates in Asian rivers has not been studied thoroughly. The general composition of the benthos of large Asian rivers appears similar to that of such habitats found worldwide and includes Tubificidae, Chironomidae (Chironominae), Gastropoda (Prosobranchia) and Bivalvia (Hynes, 1970). Among aquatic insects, the diversity of plecopteran families is low in tropical Asian streams. The order is typically found in cooler, more northern latitudes, and is represented mainly by Perlidae (especially Neoperlinae) plus Nemouridae, Leuctridae, and Peltoperlidae. Odonata and Naucoridae (Hemiptera: Heteroptera) are conspicuous elements of the zoobenthos in many tropical Asian streams, and Lepidoptera (Pyralidae) may be diverse and abundant (Dudgeon, 2000).

Although most invertebrate species are still undescribed, a high diversity is displayed by certain taxa. For example, Schmid (1984) estimated that India alone may support 4,000 species of caddisfly (Trichoptera), with perhaps 50,000 species in the Oriental Region as a whole and the single genus *Chimarra* (Phillopotamidae) may contain 500 Southeast Asian species (Malicky, 1989).

The two most controversial current issues are the building of dams and the blasting of rapids. This has been criticised on grounds of cost as well as damage to the environment and to the livelihoods of affected villagers. Many researches have suggested that the changes to the Mekong river caused by upper Mekong development have affected the local livelihoods in many ways. During the last few years, changed waterways, bank erosion, and water fluctuation have led to the destruction of the riverine ecosystems, reduction of fish population and Kai (macroalgae; Cladophora). Bank erosion have caused villagers to lose their homes and farmlands. River bank vegetable gardens have also been affected by the unusual water fluctuation.

The objective of this study was a preliminary survey of the effect of anthropogenic activity on aquatic insect diversity in the Mekong river and its tributaries. Furthermore, the study aimed to investigate the relationship between physicochemical parameters and species richness from 14 sampling sites along the Mekong river.

MATERIAL AND METHODS

Sampling sites

Fourteen sampling sites were selected along the Mekong river. The first sites is the Golden Triangle, (20° 20' 8'' N, 100° 04' 48'' E) Chiang Saen district, Chiang Rai province. The end of the

sampling sites is at the Kaeng Sa-phue, (15° 14' 12'' N, 105° 14' 63'' E) Phibun Mangsahan district, Ubon Rachathani province (Figure 1, Table 1).



Figure 1. Location of the 14 sampling sites (MK1-MK14) along the Mekong river.

Table 1. Description of the 14 sampling sites along the Mekong river.

Code	Location of sampling sites	Substrate types
MK1	Golden triangle, Chiang Saen, Chiang Rai	Mainly sand, gravel, and some aquatic plants
MK2	Mae Kok river, Chiang Rai	Mainly cobbles, gravel and sand
MK3	Mekong river, Chiang Khong, Chiang Rai	Mainly Gravel, sand and macro algae (Cladophora)
MK4	Mekong river, Kaen Kud Ku, Chiang Khan, Loei	Mainly bed rock, some silt and clay
MK5	Mae Nam Heung, Tha Li, Loei	Mainly sand, silt and clay with some aquatic plants
MK6	Mekong river, Phon Phi Sai, Nongkhai	Mainly silt, clay and some aquatic plants
MK7	Mae Nam Loung, Phon Phi Sai, Nongkhai	Mainly big boulders, solid rock
MK8	Songkram river, Kham Ta Kla, Nakhonphanom	Mainly silt and clay and boulders
MK9	Mekong river, Muang, Nakhonphanom	Mainly sand, silt and clay
MK10	Mekong river, Kaeng Kabou, Wan Yai, Mukdahan	Mainly big boulders, bed rock
MK11	Mekong river, Don Tan, Mukdahan	Mainly big boulders, bed rock and some aquatic plants
MK12	Kaen Hin Khan, Cha Nu Man, Amnat Charoen	Mainly big boulders, bed rock and some aquatic plants
MK13	Mekong river, Khong Chiam, Ubon Ratchatani	Mainly big boulders, bed rock, gravel and some aquatic plants
MK14	Mae Mun river, Phibunmangsaan, Ubon Ratchatani	Mainly big boulders, bed rock and some aquatic plants

Aquatic insect sampling

Aquatic insect samples were collected once from 14 sites. The pond net, kicking then picking sample method was used for collecting aquatic insects from several habitats. There were two

replications in each sampling site. The pond net was put on the bottom against the stream current then the area in front of the net was disturbed by the foot. The insects which live in the gravel and sand drifted into the sieve bag. All samples were

preserved in 80 % ethanol for sorting and identification in the laboratory. The aquatic insects were sieved by using a 0.5-mm stainless steel sieve, sorted into a petri-dish, and identified by using a taxonomical key to the family level or to the lowest possible level. The numbers of individuals in each family were counted. Identification was done using, McCafferty (1983), Merritt and Cummins (1984), and Dudgeon (1992; 1999).

Physicochemical parameters of the study sites were collected on sites and include water temperature using hand mercury thermometer, pH using a pH meter, conductivity and total dissolved solid using a conductivity/TDS meter, turbidity using a spectrophotometer (HACH model DR/2000) by the absorptometric method, water alkalinity by phenolphthalein methyl orange indicator method, dissolved oxygen and biochemical oxygen demand (BOD_5) by the azide modification method, nitrate-nitrogen, ammonia-nitrogen and orthophosphate using a spectrophotometer (HACH model DR/2000) by cadmium reduction method, Nessler method and ascorbic acid method, respectively. These were determined in accordance with the standard method procedures (APHA, AWWA, WPCF, 1998)

Statistical analysis

The results of the physicochemical parameters of the study sites and the number of aquatic insects were analyzed by Multivariate Statistical Package (MVSP). Principal Component Analysis (PCA) was used to study the relative proportion of insect family compared to water quality. Cluster analysis was used to group the sampling sites by the similarity of physicochemical parameters and aquatic insects.

RESULTS AND DISCUSSION

Aquatic insect diversity

The analysis was based on 10 sampling sites because 4 sites showed an absence of aquatic insect species. The most abundant family was Baetidae (Ephemeroptera), followed by Chironomidae (Diptera), Corixidae (Hemiptera) and Hydropsychidae (Trichoptera) at 68, 14, 9 and 4 %, respectively (Figure 2, Table 2). Similarly, Chiangthong (2005) found that the most abundant family found during the year was Baetidae in Mae Kham watershed. Only one family of Plecoptera was found in this study. In the same way, the diversity of Plecopteran families is low in tropical Asian streams (Dudgeon, 2000). The most abundant of the individuals was Ephemeroptera (408 individuals) and the least was Coleoptera (4 individuals) (Table 2). The highest number of aquatic insects was found at the first sampling site (MK1-Golden Triangle) because there were several types of micro-habitats with aquatic plants, gravel and sand. On the other hand, the lowest number was found from MK10 (Mekong river, Kaeng Kabou, Wan Yai, Mukdahan) which might be the result of human impact such as agriculture and livestock. Moreover, the substrate of this site was big boulders and bed rock, which is not suitable for aquatic insect survival (Table 2).

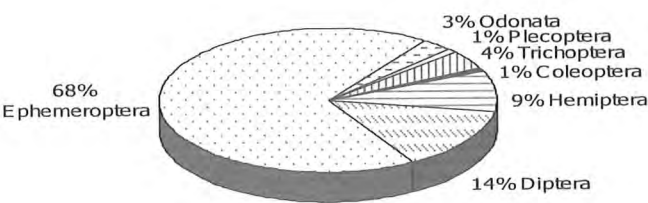


Figure 2. Percentage composition of aquatic insect orders in Mekong River.

Table 2. Orders and Families of aquatic insects found at 10 sampling sites.

Order	Family	Study sites									
		MK1	MK2	MK3	MK4	MK5	MK10	MK11	MK12	MK13	MK14
Ephemeroptera	Baetidae	110	83	37	117	10		2	23	7	19
	Caenidae	1	7	3		1		4	14	2	
	Heptageniidae	1	2	3				1			
	Ephemeridae	3	1		1						
	Ephemerellidae		1	2		1					
	Leptophlebiidae		2	1							
Odonata	Gomphidae			3	4			2	1		
	Libellulidae				5						
	Protoneuridae										2
Plecoptera	Perlidae	1	1	3	1						
Trichoptera	Hydropsychidae	13	7	1		1			1		
	Leptoceridae	3		1							
Coleoptera	Dytiscidae		1	1							
	Naucoridae			2							
Hemiptera	Corixidae		2	1				50			
	Gerridae		2		4						
Diptera	Chironomidae	26	39	4	1	1	1		1		
	Simuliidae				9						
	Culicidae	1									
	Tipulidae	1				3				1	
	Ceratogonoponidae	1		1							
Taxa richness		11	12	14	8	6	1	5	5	3	2
Total		172	148	63	142	17	1	59	40	10	21

Physicochemical parameters of water

For water temperature, the results showed that the lowest temperature (19.3 °C) was detected at MK 1 (Figure 3). The difference in water temperature may depend on the climate and the environment nearby the stream as well as sampling time, wind, water mixing, and the amount of sun light. Water temperature affects the number of aquatic insects since each species requires in a specific range of water temperature to live in because of their different respiratory rate and metabolism.

For Conductivity and turbidity, the highest value of conductivity was 484 μ S/cm at MK8 and the lowest value (135 μ S/cm) was measured at MK2. The high conductivity was caused by the high rate of soil erosion and that many ions were dissolved in the water. Conductivity due to all the ions dissolved in the water is related to alkalinity. The water volume changed in the different seasons. The dry season had less water volume thus the ion-concentration might be higher. Moreover, the conductivity was affected by the impacts of human activities such as agriculture, for example the raising of livestock and the discharge of urban wastewaters (Figures 4, 5). MK8 had a higher turbidity than other sites, with the highest value of 288 NTU. The underlying reason for the high turbidity in this watershed was soil erosion, as well as landscape morphology and seasonal changes.

For the pH and alkalinity, pH value of water samples from each sampling sites were not much different. Interestingly low values of pH were found, with the lowest at 6.6 in MK8. The pH range of surface water quality standard of Thailand is 5 to 9. The results from MK5 were higher than other sites because of high values of carbonates and

bicarbonates discharged into the water (Figure 6). The alkalinity values varied from 23 to 91 mg/L. In MK5 had the highest alkalinity value, while the lowest values were found at MK7 and MK8 (not showed). Alkalinity indicated that water was contaminated with ions, carbonate, bicarbonate, and hydroxide. Two main sources of carbonate and bicarbonate are rain and soil, including phosphate, borate and silicate (Traichaiyaporn, 2000). The alkalinity depended on the geology of the watershed area and the presence of limestone which is a natural source of alkalinity (Figure 7).

For Dissolved Oxygen (DO) and Biochemical Oxygen Demand ((BOD₅); Normal DO level is in running water are 4.6 - 8.6 mg/L. The lowest DO was measured at MK7 which might be caused by contamination from community wastewater. High oxygen levels were found at all upper sampling sites. The main source of dissolved oxygen for running water is from the atmosphere from the mixing process. It is different for standing water due to photosynthesis of micro-organisms (Figure 8). The highest BOD₅ (5.2 mg/L) was measured at MK14. BOD₅ value was high when the water body contained high levels of organic matters. At MK1, MK5, MK6 and MK9-MK14, BOD₅ values exceeded the water quality standard of Thailand (> 0.5 mg/L) Because of organic matters being discharged by the community into the rivers.

For Orthophosphate (PO₄³⁻), nitrate nitrogen (NO₃⁻-N), and ammonia nitrogen (NH₃-N). Low concentration of PO₄³⁻ values were found in MK2, MK8 and MK14. The highest concentration (0.33 mg/L) was measured at MK9 and the lowest concentration (0.04 mg/L) was measured at MK2 (Figure 9). The PO₄³⁻ might have been contaminated

by fertilizer used in agriculture and also from detergents that are widely used. The nitrate nitrogen values varied from 0.3 to 1.8 mg/L. The highest value was detected at MK8 while, the lowest was found at MK10 (Figure 10). The NO_3^- did not exceed the water quality standard of Thailand (less than 5.0 mg/L). There were two sources of nitrate, agricultural lands and domestic waste. The Ammonia nitrogen was highest at MK14 (0.4 mg/L.) (Figure 11). At all of the sampling sites, NH_3

level did not exceed the water quality standard of Thailand (0.5 mg/L). The high concentration of NH_3 was caused by community wastewater from livestock, agricultural wastes and fertilizers being discharged into the environment. The higher level was found at MK8 and MK14. High ammonia level indicated that the water had just been contaminated. Ammonia could be transformed to nitrate by the nitrification process and also transformed back to ammonia by denitrification.

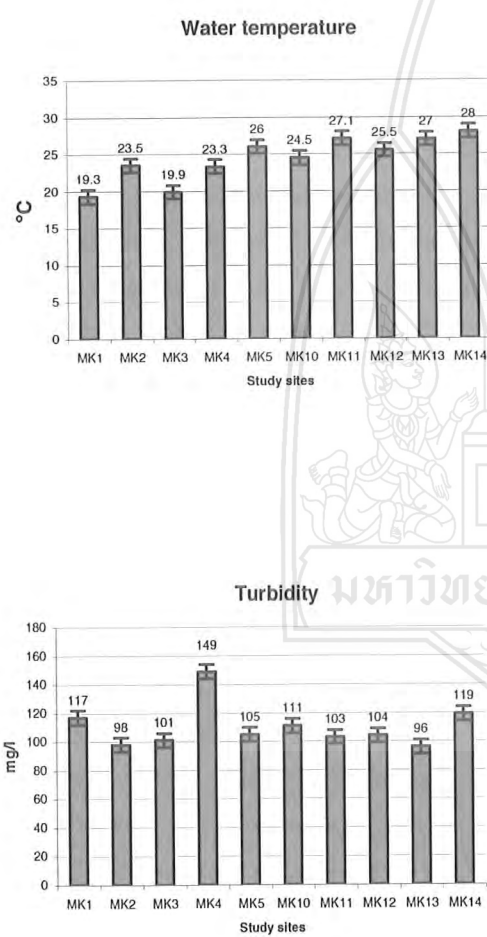


Figure 5. Water turbidity levels.

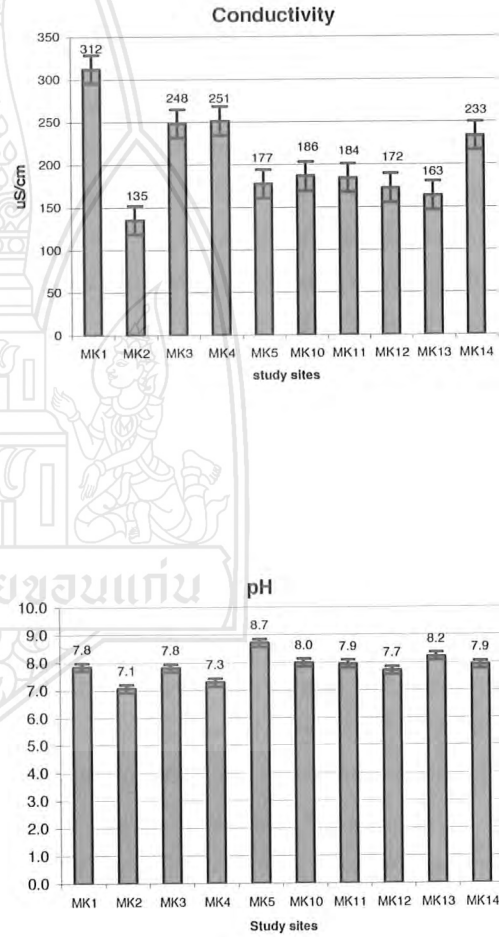


Figure 6. The pH levels.

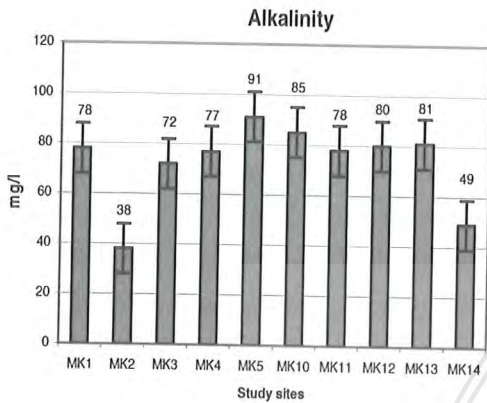


Figure 7. Alkalinity levels.

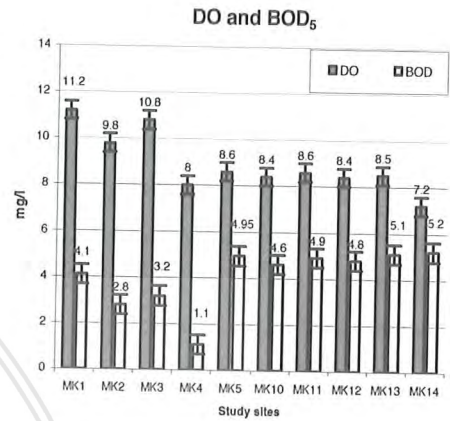


Figure 8. DO and BOD₅ levels.

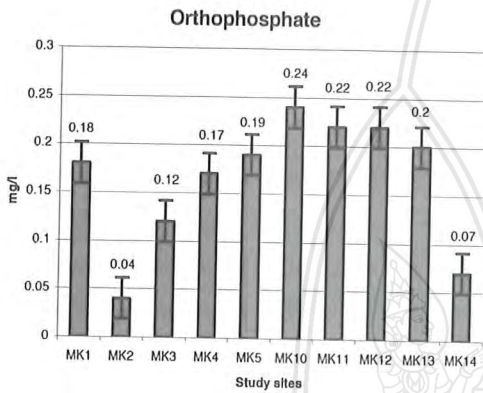


Figure 9. Orthophosphate levels.

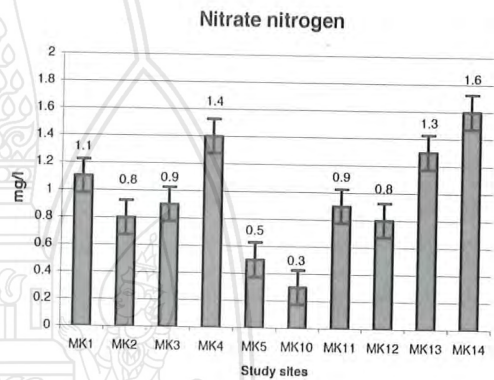


Figure 10. Nitrate nitrogen levels.

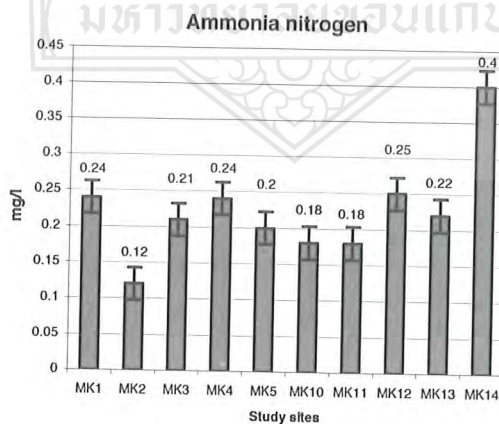


Figure 11. Ammonia nitrogen levels.

Cluster analysis and ordination by PCA

The Multivariate Statistical Package (MVSP) program was used to analyze the data of this research. The classification method cluster analysis (UPGMA) was used to classify the sampling site. By comparing the physicochemical data with the diversity of aquatic insects, the sampling sites could be classified into 3 groups (Figure 12).

The first group includes MK1, MK2 and MK4, was classified as slightly polluted water and high taxa richness. The most abundant family was Baetidae, which was very common and found at almost all the sampling sites in this group. Plecoptera associated with the less polluted sites was also found in these sites. In addition, these families might be linked to the substrate type which mainly consists of cobbles, gravel, sand and some aquatic plants. The second group includes at MK3, MK10, MK12, MK13, MK14, and MK15. These sites were classified together because they had similar substrate types. In which case, the diversity and distribution of aquatic insects was low in these groups. The third group included MK11. The most abundant aquatic insect at MK11 was Hemiptera,

family Corixidae while at other sites, it was very rare or absent. Therefore, this site was typically separated from the other sites.

In addition, they could also be classified by physicochemical properties. However, the UPGMA showed 4 groups (Figure 13). The first group included MK1, the substrates were mainly sand, gravel and cobbles, including macro algae and aquatic plants. For this, several microhabitats were suitable for aquatic insect life. The second group included MK3, MK4 and MK14, the water quality was affected by community activities surrounding rivers and tributaries. Also, these sites contained the same type of substrates. The third group included MK2, this site was influenced by tourist activities and sand transportation by boat. For these reasons, DO was disturbed and turbidity increased. Finally, the remaining sites, included rapid sites, with the exception for MK5 (Mac Nam Hueng) which was not a rapid site. The Principal Component Analysis (PCA) was used to analyze the correlation of all sampling sites (Figures 12, 13). The results of cluster analysis were also supported by PCA.

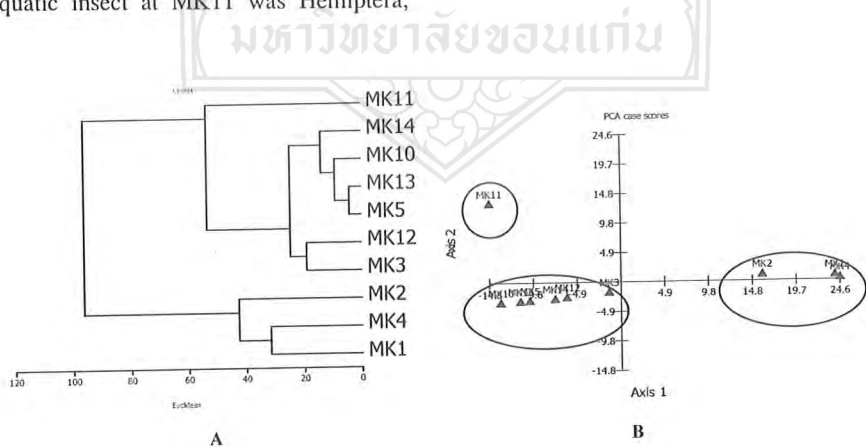


Figure 12. The cluster analysis (A) and ordination (by PCA) (B) of the study sites (based on aquatic insect richness).

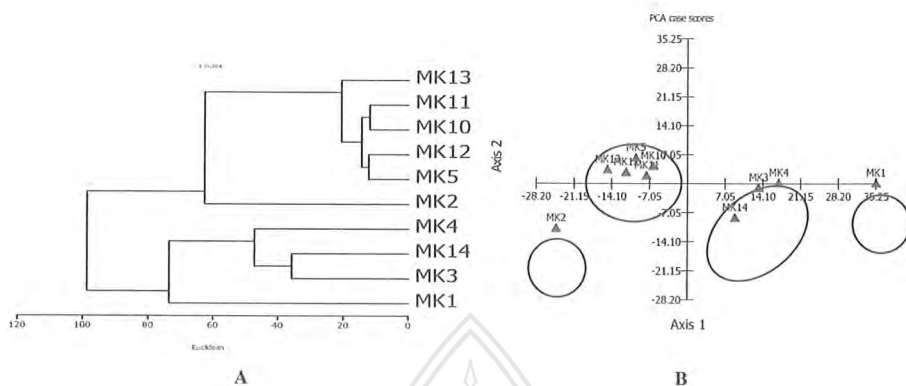


Figure 13. The cluster analysis (A) and ordination (by PCA) (B) of the study sites (based on physicochemical factors).

CONCLUSION

Seven orders and 23 families of aquatic insects were found, with the highest number of aquatic insects found from the order Ephemeroptera.

The most abundant mayfly family was Baetidae and Caenidae, respectively. For physicochemical values, only BOD₅ value in MK5-MK6 (Mae Nam Heung, Tha Li, Loei; Mekong river, Phon Phi Sai, Nongkhai) and MK9-MK14 (Mekong river, Muang, Nakhonphanom; Mekong river, Kaeng Kabou, Wan Yai, Mukdahan; Mekong river, Don Tan, Mukdahan; Kaen Hin Khan, Cha Nu Man, Amnat Charoen; Mekong river, Khong Chiam, Ubon Ratchatani; Mae Mun river, Phibunmangsan, Ubon Ratchatani) was greater than the class 4 standard value of the surface water quality standard of Thailand. Using cluster analysis, the sampling sites could be classified into 3 groups by using the diversity of aquatic insects. At the same time, by using the physicochemical properties, they could be classified into 4 groups.

However, we have shown that the diversity of aquatic insects and water quality of

Mekong River analyzed with ordination methods that could lead to interesting results and could serve as a basis for further studies.

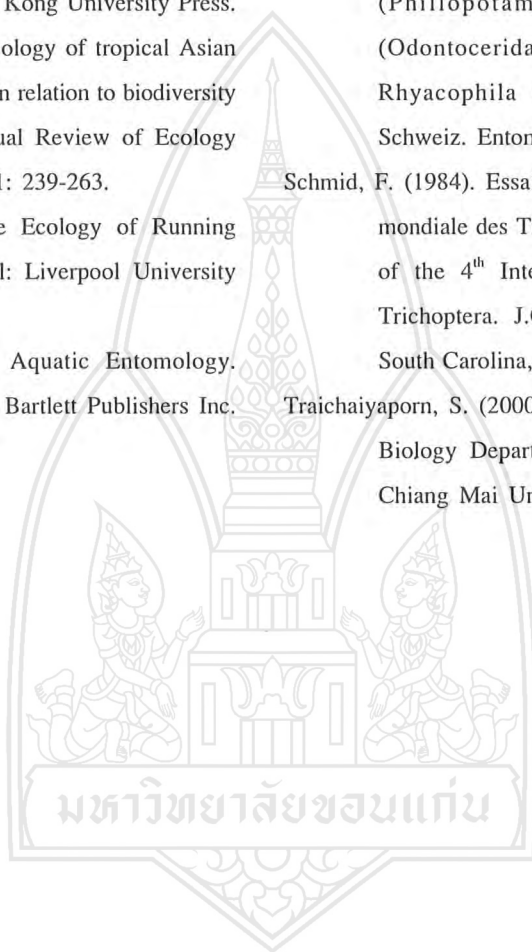
ACKNOWLEDGMENTS

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Diversity of Stream Benthic Macroinvertebrates at the Loei River and Adjacent Catchments, Northeastern Thailand

ความหลากหลายของสัตว์ไม่มีกระดูกสันหลังหน้าดินในลำธาร
ต้นน้ำเลยและต้นน้ำใกล้เคียง ภาคตะวันออกเฉียงเหนือ ประเทศไทย

Boonsatien Boonsoong¹ and Narumon Sangpradub^{2*}

Abstract

In the series of studied on rapid bioassessment of Thai streams, 20 sampling sites were collected from the headwater streams of the Loei River catchment, the San River catchment, and the Pa Sak River catchment on 4 collection dates (November 2004, March 2005, November 2005 and March 2006). All sampling sites were surrounded by the different land use (forested, agricultural and residential sites). Benthic macroinvertebrates were sampled using a D-frame net and followed by the U.S. EPA procedure. Benthic samples were subsampled in gridded trays at the maximum target sample size of 500 individuals. Macroinvertebrates were identified to the lowest practical taxonomic level. Of 62,176 invertebrate individuals identified, 161 insect and 21 non-insect taxa representing 19 orders 113 families and 180 genera of benthic macroinvertebrates were found from all sampling sites. The Trichoptera is the most diverse group followed by Ephemeroptera and Diptera, respectively. The most abundant taxa consisted of Chironomidae (14.45 %), *Cheumatopsyche* (Hydropsychidae) (12.85 %), and Elmidae (7.05 %) respectively. Four described species (*Kangella brocha* (Ephemerellidae), *Polyplocia orientalis* (Euthyplocidae), and *Prosopistoma annamense* (Prosopistomatidae)) are the first record of mayflies from Thailand.

บทคัดย่อ

ศึกษาการประเมินคุณภาพน้ำทางชีวภาพแบบเร็วในลำธารของประเทศไทย ทำการเก็บตัวอย่างทั้งหมด 20 สถานี จำนวน 4 ครั้ง ในลำธารต้นน้ำเลย ต้นน้ำสาน และต้นน้ำป่าสัก ในเดือนพฤศจิกายน 2547 มีนาคม 2548 พฤศจิกายน 2548 และ มีนาคม 2549 สถานีเก็บตัวอย่าง ครอบคลุมบริเวณที่มีการใช้พื้นที่แตกต่างกัน

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3 บริเวณ คือ พื้นที่ป่า พื้นที่เกษตรกรรม และเขตชุมชน เก็บสัตว์ไม่มีกระดูกสันหลังน้ำจืดโดยใช้สวิงรูปตัว D ตามคู่มือ และวิธีการมาตรฐานขององค์การพิทักษ์สิ่งแวดล้อมสหรัฐอเมริกา จากนั้นนำตัวอย่างที่ได้มาสุ่มแยกตัวอย่างสัตว์ในภาชนะที่แบ่งเป็นช่องเท่าๆ กัน จำนวนสูงสุดถึง 500 ตัว และจัดจำแนกตัวอย่างในระดับต่ำสุดเท่าที่ทำได้ จากการจำแนกตัวอย่างสัตว์จากสถานีทั้งหมด จำนวน 62,176 ตัว พบสัตว์ไม่มีกระดูกสันหลังน้ำจืดทั้งหมด 19 อันดับ 113 วงศ์ 180 สกุล ได้แก่ กลุ่มแมลงน้ำ 161 ชนิด และกลุ่มไม่ใช่แมลงน้ำ 21 ชนิด แมลงน้ำในอันดับ Trichoptera เป็นกลุ่มที่มีความหลากหลายมากที่สุด รองลงมา คือ อันดับ Ephemeroptera และอันดับ Diptera ตามลำดับ หนอนแดง (วงศ์ Chironomidae) เป็นกลุ่มสัตว์ที่มีความชุกชุมมากที่สุด (14.45 %) รองลงมา คือ แมลงหนอนปลอกน้ำสกุล *Cheumatopsyche* (12.85 %) และคั้งน้ำวงศ์ Elmidae (7.05 %) ตามลำดับ พบแมลงชีปะขาวที่ยังไม่มีรายงานมาก่อนในประเทศไทย 3 ชนิด คือ *Kangella brocha* (วงศ์ Ephemereillidae) *Polyplocia orientalis* (วงศ์ Euthyplocidae) และ *Prosopistoma annamense* (วงศ์ Prosopistomatidae)

Keywords: benthic macroinvertebrates, the Loei River catchment

คำสำคัญ: สัตว์ไม่มีกระดูกสันหลังน้ำจืด, ดินน้ำเลย

INTRODUCTION

Benthic macroinvertebrates perform a variety of functions in freshwater ecosystem. They have an important influence on nutrient cycles, primary productivity, decomposition and translocation of material (Wallace and Webster, 1996; Covich et al., 1999). They are the most commonly used for biomonitoring in lotic habitat worldwide (Rosenberg and Resh, 1993; Bonada et al., 2006).

In tropical Asian countries, riverine biodiversity is threatened by catchment deforestation, habitat degradation and agricultural expansion (Dudgeon, 1999). Biomonitoring tools are being developed to assess the Ecological health of the Lower Mekong River (Campbell et al., 2005). Invertebrate biodiversity and ecological studies in tropical Asian streams were reviewed by Dudgeon (1999). It revealed that the knowledge of aquatic invertebrate fauna in the region is very scarce.

In Thailand, the potential for using invertebrate fauna as indicators of freshwater

ecosystem health was documented (Sangpradub et al., 1998; Thorne and Williams, 1997; Mustow, 2002). Baseline data for aquatic invertebrate fauna of Thailand is poorly reported. Previous research on diversity of stream macroinvertebrates in Thailand is scattered and localized. The EPT are the most interesting group for Thai researchers (Sangpradub et al., 1998; Malicky and Chantaramongkol, 1998; Sites et al., 2001; Parnrong et al., 2002; Sangpradub et al., 2002). Only trichopteran adult was well studied, particularly in the North. At least 500 species were reported (Malicky and Chantaramongkol, 1998), however, knowledge of larval stage is limited. Although many macroinvertebrate biodiversity studies have been carried out in Thailand, overall knowledge is fragmented. Data on the distribution and abundance of the freshwater invertebrates group in Thailand is limited. Sangpradub and Boonsoong (2006) developed an illustrated key to family of freshwater invertebrates of the Mekong Basin and adjacent area. These taxonomic keys provided more

understanding of the taxonomic status of macroinvertebrates in Thailand.

The objectives of this paper are 1) to present a preliminary assessment of the diversity and distribution of stream macroinvertebrates in the headwater streams of the Loei River and adjacent catchments, and 2) provide basic taxonomic status of macroinvertebrates in Thailand.

MATERIAL AND METHODS

Study sites

Twenty sampling sites were selected from the headwater streams of the Loei River catchment,

the San River catchment, and the Pa Sak River catchment (Figure 1). All sampling sites were surrounded by the different land use (forested, agricultural and residential sites). Reference sites where (mostly located in the Phu Luang Wildlife Sanctuary) represented as unimpaired or least impaired a condition as possible; whereas, test sites were known to be influenced by varying levels of anthropogenic stressors. Typical riparian vegetation consists of *Elaeocarpus hainanensis* Oliv. and *Homonoia riparia* Lour. The streams of all reference sites flow through mixture of deciduous forest.

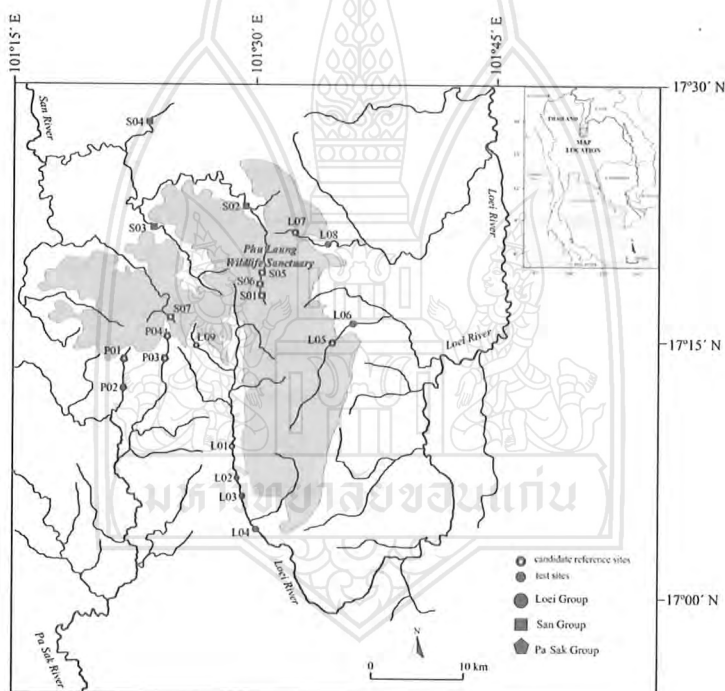


Figure 1. Map of study area showing the distribution of sampling sites.

Sampling and laboratory procedures

Macroinvertebrates were collected from each site using a D-frame dipnet (0.3-m wide, 500- μ m mesh) following the U.S. EPA procedure (Barbour

et al., 1999, multihabitat approach). In the laboratory, benthic samples were subsampled in gridded trays at the maximum target sample size of 500 individuals. Macroinvertebrates were identified to the lowest

practical taxonomic level using Sangpradub and Boonsoong (2006), usually genus or species, except for Collembola, Coleoptera, Diptera, Odonata (family); Acarina (order) and Oligochaeta (class). Those individuals were assigned to operational taxonomic units (OTUs). The level of the OTUs varied from species to class, but 74 % of OTUs were genera and species.

RESULTS AND DISCUSSION

All 62,176 invertebrates collected from all sampling sites during November 2004 - March 2006 were identified. Nineteen orders of benthic macroinvertebrates were found, consisting of 161 insect and 21 non-insect taxa (species, genera, families). Trichoptera is the most diverse group (20 families, 10 genera) followed by Ephemeroptera (15 families, 39 genera), and Diptera (14 families, 23 genera), respectively. The most common insect families consisted of Hydropsychidae (Trichoptera), Chironomidae (Diptera), Baetidae (Ephemeroptera), Perlidae (Plecoptera), Elmidae (Coleoptera), and Gomphidae (Odonata).

The most abundant taxa consisted of Chironomidae (14.45 %), *Cheumatopsyche* (12.85 %), and Elmidae (7.05 %) respectively. 13 OTUs taxa occurred at only one site and low abundance (rare taxa) (Table 1).

The genera such as *Sphaeronemura* (Plecoptera: Nemouridae), *Cryptoperla* (Plecoptera: Peltoperlidae), *Rhopalopsale* (Plecoptera: Leuctridae), *Epeorus* (Ephemeroptera: Heptageniidae), *Gratia* (Ephemeroptera: Baetidae), *Glossosoma* (Trichoptera: Glossosomatidae) and *Lepidostoma* (Trichoptera: Lepidostomatidae), *Molanna* (Trichoptera: Molannidae), and *Rhyacophila* (Trichoptera:

Rhyacophilidae) characterize the streams within riparian forests. The genera such as *Choroterpides* (Ephemeroptera: Leptophlebiidae), *Cheumatopsyche* (Trichoptera: Hydropsychidae), and *Baetis* (Ephemeroptera: Baetidae) were characteristic streams with agricultural and residential areas.

All together, 113 families and 180 genera of macroinvertebrates have been identified so far in the Loei River and adjacent catchments. The fauna was dominated by immature aquatic insects, and also included members of Oligochaeta, Acarina, Isopoda, Decapoda, and Mollusca. Chironomid and net-spinning caddisfly were the most abundance groups. Taxa list data showed that taxa richness vary with season. This may be related to difference in stream temperature and diel flight pattern. Declines in zoobenthos abundance might be expected during the wet season (Sangpradub and Naknan, 1998; Dudgeon, 1999).

The Trichoptera is a diverse order of aquatic insects that occurs in tropical Asian streams (Dudgeon, 1999). The Hydropsychidae is the most diverse family of the identified Trichoptera. These results represent the same diversity pattern found previously in Thailand (Watanasit, 1996; Sangpradub et al., 1998). Malicky and Chantaramonkol (1998) estimated that Thailand may contain 494 species of adult caddisfly. While Ephemeroptera is one of the most abundant and common components of running water. They are often found in mid-stream, where substratum, food and oxygen are abundant. Sites et al. (2001) reported 13 families and 28 genera of mayflies from southern Thailand. From this study, 3 genera (*Heterocloen*, *Chromarcys* and *Uracanthella*) and 4 described species (*Kangella brocha* (Ephemereillidae), *Polyplocia orientalis*

(Euthyplocidae), and *Prosopistoma annamense* (Prosopistomatidae)) are the first record of mayflies from Thailand.

Among the EPT insect orders, the diversity of plecopteran families is low in tropical Asian streams (Covich, 1988). The order is typical of cooler, more northern latitudes, and is represented mainly by Perlidae, Nemouridae, Leuctridae, and Peltoperlidae (Dudgeon, 1999). These families were found in many Thai streams and in this study (Sangpradub et al., 1998).

Taxonomic keys for Thailand have been constructed which can be used to identify macroinvertebrate families and genera, however, generic-level keys are lacking for some insect orders (e.g. Coleoptera, Odonata). The lack of identification keys limits the ability to assign-genus/species-level taxonomic names to specimen collected; however, at least some of these specimens can be identified to genus-level. Clearly, this is easier for some taxa (e.g. genera of Ephemeroptera, Plecoptera, and Trichoptera) than others (e.g. Odonata). Nevertheless, there is still an urgent need to produce a “*Guide to Freshwater Invertebrates of Thailand*”.

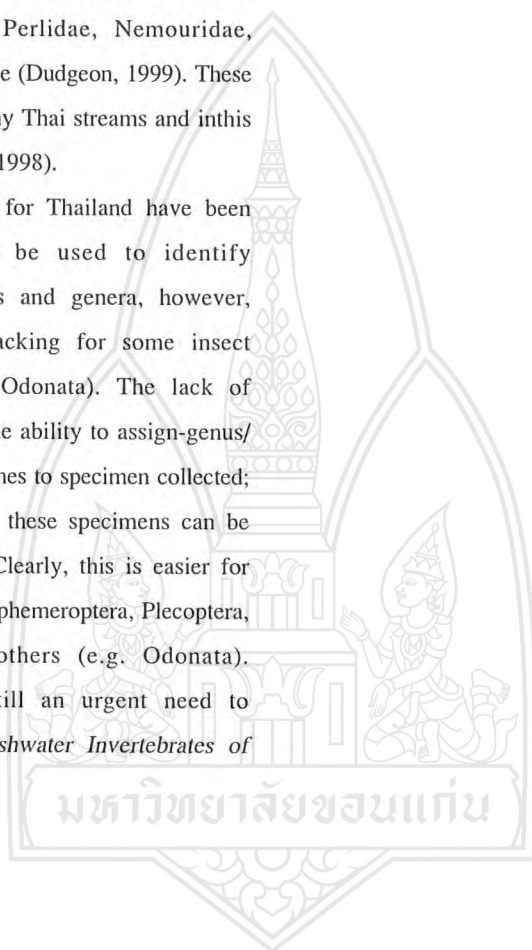


Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments.

Taxon		Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Annelida								
Oligochaeta	Oligochaeta		X		259	0.417	11	55
Arthropoda								
Arachnida								
Acarina	Acarina		X		59	0.095	13	65
Crustacea								
Decapoda								
Atylidae								
	Caridina		X		6	0.01	1	5
Palaeomonidae								
	Macrobrachium		X		87	0.14	10	50
	Macrobrachium lancesteri			X	1	0.002	1	5
Parathelphusidae			X		55	0.088	8	40
Potamidae			X		66	0.106	16	80
Insecta								
Coleoptera								
Chrysomellidae			X		5	0.008	2	10
Curculionidae				X	1	0.002	1	5
Dytiscidae			X		156	0.251	17	85
Elmidae		X			4383	7.049	20	100
Gyrinidae			X		233	0.375	17	85
Hydrophilidae								
	Berosus		X		158	0.254	15	75
	Hydrophilidae	X			655	1.053	19	95
Lampyridae			X		291	0.468	13	65
Psephenidae								
	Eubrianax		X		303	0.487	15	75
	Psephenoides		X		89	0.143	15	75
Ptilodactylidae			X		3	0.005	2	10
Scirtidae			X		338	0.544	18	90
Staphylinidae			X		100	0.161	13	65
Collembola								
	Isomotidae		X		48	0.077	9	45

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Diptera							
Athericidae							
<i>Atrichop</i>		X		79	0.127	9	45
<i>Suragina</i>		X		327	0.526	17	85
Blephariceridae							
<i>Blepharicera</i>		X		3	0.005	1	5
Ceratopogonidae							
<i>Bezzia</i>		X		205	0.33	18	90
<i>Forcipomyia</i>		X		18	0.029	5	25
Chaoboridae		X		16	0.026	1	5
Chironomidae							
Chironomidae	X			8986	14.453	20	100
<i>Stenochironomus</i>		X		69	0.111	14	70
Culicidae							
<i>Anopheles</i>		X		7	0.011	3	15
Culicidae		X		97	0.156	7	35
Dixidae		X		24	0.039	6	30
Empididae							
<i>Hemerodromia</i>		X		42	0.068	7	35
<i>Hilara</i>			X	1	0.002	1	5
Ephydriidae		X		11	0.018	3	15
Psychodidae		X		20	0.032	5	25
<i>Neotelmatoscopus</i>		X		2	0.003	2	10
Simuliidae							
	X			2574	4.14	19	95
Stratiomyidae							
<i>Odontomyia</i>		X		27	0.043	6	30
Tabanidae		X		11	0.018	5	25
Tipulidae							
<i>Antocha</i>		X		66	0.106	14	70
<i>Hexatoma</i>	X			698	1.123	18	90
<i>Tipula</i>		X		473	0.761	11	55
Tipulidae		X		96	0.154	13	65

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Ephemeroptera							
Baetidae							
<i>Acentrella</i>		X		16	0.026	2	10
Baetidae	X			1279	2.057	17	85
<i>Baetiella</i>			X	1	0.002	1	5
<i>Baetis</i>	X			1851	2.977	19	95
<i>Cloeon</i>		X		363	0.584	6	30
<i>Gratia narumonae</i>		X		36	0.058	3	15
<i>Heterocloeon</i>		X		35	0.056	7	35
<i>Platybaetis</i>	X			846	1.361	16	80
Caenidae							
Caenidae	X			1820	2.927	19	95
<i>Caenoculis</i>		X		34	0.055	4	20
<i>Clypeocaenis</i>		X		19	0.031	5	25
Ephemerellidae							
<i>Cincticostella insolta</i>			X	2	0.003	1	5
<i>Crinitella</i>		X		162	0.261	7	35
<i>Ephacerella commodema</i>			X	1	0.002	1	5
<i>Kangella brocha</i>		X		41	0.066	3	15
<i>Torleya</i>			X	4	0.006	3	15
<i>Uracanthella</i>	X			811	1.304	16	80
Ephemeridae							
<i>Ephemeru</i>		X		239	0.384	14	70
Euthyplocidae							
<i>Polyplocia orientalis</i>		X		29	0.047	9	45
Heptageniidae							
<i>Asionurus</i>		X		439	0.706	8	40
<i>Cinygmina</i>	X			2880	4.632	20	100
<i>Epeorus</i>			X	4	0.006	1	5
<i>Rhithrogena</i>		X		17	0.027	3	15
<i>Thalerosphyrus</i>		X		319	0.513	14	70
<i>Trichogenia maxillaris</i>			X	4	0.006	1	5
Isonymchiidae							
<i>Isonymchia</i>		X		24	0.039	3	15

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Ephemeroptera (contd.)							
Leptophlebiidae							
<i>Choroterpes</i>	X			1132	1.821	19	95
<i>Choroterpides</i>		X		3176	5.108	13	65
<i>Habrophlebiodes</i>		X		58	0.093	6	30
<i>Isca</i>		X		406	0.653	15	75
Neophemeridae							
<i>Potamanthellus edmundsi</i>		X		9	0.014	3	15
Oligoncuriidae							
<i>Chromarcys</i>			X	4	0.006	1	5
Potamanthidae							
<i>Rhoenanthus (P.) obscurus</i>		X		16	16	0.03	10
<i>Rhoenanthus (R.) distafurcus</i>		X		9	0.014	2	10
Prosopistomatidae							
<i>Prosopistoma annamense</i>		X		308	0.495	5	25
Teloganodidae							
<i>Teloganodes</i>		X		234	0.376	17	85
Tricorythidae							
<i>Tricorythus</i>		X		13	0.021	2	10
Hemiptera							
Aphelocheiridae							
<i>Aphelocheirus femoratus</i>		X		177	0.285	12	60
Corixidae			X	2	0.003	1	5
Gerridae		X		224	0.36	14	70
Hebridae							
<i>Timasius</i>		X		13	0.021	4	20
Helotrephidae							
<i>Fischerotrepes</i>		X		70	0.113	8	40
Leptopodidae							
<i>Leotichius</i>			X	1	0.002	1	5
<i>Valleriola</i>			X	4	0.006	1	5
Mesoveliidae							
<i>Mesovelia</i>		X		6	0.01	3	15
Micronectidae		X		271	0.436	8	40

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Hemiptera (contd.)							
Naucoridae							
<i>Laccocoris</i>		X		146	0.235	13	65
<i>Naucoris cheirochela</i>		X		164	0.264	9	45
Nepidae							
<i>Cercotmetus</i>			X	3	0.005	1	5
Veliidae							
<i>Rhagovelia sumatrensis</i>		X		72	0.116	15	75
<i>Strongylovelia</i>		X		7	0.011	5	25
Veliidae		X		32	0.051	11	55
Lepidoptera							
Crambidae							
<i>Elophila</i>		X		11	0.018	3	15
<i>Eoophyla</i>		X		15	0.024	9	45
<i>Paracymoriza</i>			X	4	0.006	2	10
Megaloptera							
Corydalidae							
		X		6	0.01	3	15
<i>Protohermes</i>		X		219	0.352	16	80
Odonata							
Aeshnidae		X		20	0.032	7	35
Amphipterygidae		X		12	0.019	6	30
Calopterygidae		X		18	0.029	5	25
Chlorocyphidae		X		16	0.026	9	45
Coenagrionidae			X	2	0.003	1	5
Cordulegastridae			X	4	0.006	3	15
Corduliidae		X		258	0.415	16	80
Euphaeidae	X			642	1.033	19	95
Gomphidae		X		588	0.946	18	90
Libellulidae		X		23	0.037	3	15
Platynemidae		X		21	0.034	4	20
Protoneuridae		X		7	0.011	2	10
Orthoptera							
Blaberidae		X		99	0.159	12	60
Tetrigidae		X		19	0.031	7	35

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Plecoptera							
Nemouridae							
<i>Amphinemura</i>		X		200	0.322	10	50
<i>Indonemoura</i>		X		536	0.862	14	70
<i>Sphaeronemoura</i>		X		81	0.13	6	30
Peltoperlidae							
<i>Cryptoperla</i>		X		469	0.754	9	45
Perlidae							
<i>Etrocorema</i>		X		393	0.632	13	65
<i>Neoperla</i>		X		366	0.589	19	95
<i>Phanoperla</i>		X		284	0.457	12	60
<i>Togoperla</i>		X		87	0.14	11	55
Leuctridae							
<i>Rhopalopsole</i>		X		8	0.013	2	10
Trichoptera							
Brachycentridae							
<i>Micrasema</i>			X	1	0.002	1	5
Calamoceratidae							
<i>Anisocentropus</i>		X		118	0.19	12	60
<i>Ganonema</i>		X		22	0.035	6	30
Dipseudopsidae							
<i>Dipseudopsis</i>		X		24	0.039	6	30
<i>Pseudoneureclipsis</i>		X		9	0.014	6	30
Ecnomidae							
<i>Ecnomus</i>		X		64	0.103	7	35
Glossosomatidae							
<i>Glossosoma</i>		X		132	0.212	8	40
Goeridae							
<i>Goera</i>		X		24	0.039	7	35
Helicopsychidae							
<i>Helicopsyche</i>		X		213	0.343	6	30

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Trichoptera (contd.)							
Hydropsychidae							
<i>Ceratopsyche</i>	X			2555	4.109	17	85
<i>Cheumatopsyche</i>	X			7991	12.852	19	95
<i>Hydatomanicus</i>		X		108	0.174	5	25
<i>Hydromanicus</i>		X		5	0.008	1	5
<i>Hydropsyche</i>		X		38	0.061	5	25
<i>Macrostemum</i>		X		1482	2.384	11	55
<i>Potamyia</i>		X		132	0.212	7	35
<i>Polymorphanisus</i>			X	1	0.002	1	5
<i>Pseudoleptonema</i>		X		88	0.142	5	25
Hydroptilidae							
<i>Hydroptila</i>			X	1	0.002	1	5
<i>Orthotrichia</i>		X		78	0.125	13	65
<i>Oxyethira</i>		X		51	0.082	13	65
<i>Ugandatrichia</i>			X	4	0.006	2	10
Lepidostomatidae							
<i>Lepidostoma</i>		X		560	0.901	13	65
Leptoceridae							
<i>Ceraclea</i>		X		6	0.01	2	10
<i>Leptoceridae</i>		X		322	0.518	18	90
<i>Leptocerus</i>		X		314	0.505	7	35
<i>Oecetis</i>		X		78	0.125	13	65
<i>Setodes</i>		X		16	0.026	6	30
Molannidae							
<i>Molanna</i>		X		7	0.011	1	5
Odontoceridae							
<i>Marilla</i>		X		235	0.378	13	65
Philopotamidae							
<i>Chimarra</i>	X			1444	2.322	17	85
<i>Wormaldia</i>		X		12	0.019	3	15
Polycentropodidae		X		20	0.032	11	55
Psychomyiidae							
<i>Tinodes</i>		X		10	0.016	7	35

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Trichoptera (contd.)							
Rhyacophilidae							
<i>Rhyacophila</i>		X		42	0.068	6	30
Stenopsychidae							
<i>Stenopsyche siamensis</i>		X		191	0.307	5	25
Xiphocentronidae			X	3	0.005	3	15
Mollusca							
Gastropoda							
Mesogastropoda							
Ancylidae							
<i>Ferrissia baconi</i>		X		26	0.042	4	20
Bithynidae							
<i>Bithynia</i>		X		73	0.117	1	5
Hydrobiidae							
Hydrobiidae			X	1	0.002	1	5
<i>Lacunopsis</i>		X		196	0.315	2	10
Tharidae							
<i>Brotia</i>		X		346	0.556	10	50
<i>Melanoides</i>		X		898	1.444	5	25
Lymnaeidae							
<i>Lymnaea (Radix) auricularia</i>		X		10	0.016	4	20
Pilidae							
<i>Pomacea</i>		X		67	0.108	1	5
Planorbidae							
<i>Indoplanorbis exustus</i>		X		25	0.04	4	20
Tharidae							
<i>Brotia</i>		X		346	0.556	10	50
<i>Melanoides</i>		X		898	1.444	5	25
Viviparidae							
<i>Indiopoma</i>		X		6	0.01	1	5
Viviparidae		X		78	0.125	3	15
Pelcypoda							
Arcoida							
Arcidae							
<i>Scaphula</i>		X		14	0.023	3	15

Table 1. Relative total abundance and occurrence of taxa collected from 20 macroinvertebrates sampling sites in the Loei River and adjacent catchments. (contd.)

Taxon	Abundant	Common	Rare	Total abundance	Percent abundance	No. of sites where taxon was collected	Percent occurrence
Pelycypoda (contd.)							
Unionoida							
Amblemidae							
<i>Scabies crispata</i>			X	1	0.002	1	5
Veneroida							
Corbiculidae							
<i>Corbicula</i>		X		214	0.344	11	55

[Taxa in alphabetical order and grouped by major taxonomic categories: **Abundant**, greater than 1 percent of total abundance and greater than or equal to 75 percent occurrence; **Common**, less than 1 percent but greater than 0.006 percent of total abundance, and greater than 5 percent but less than 75 percent occurrence; **Rare**, less than or equal 0.006 percent of total abundance and less than or equal 5 percent occurrence]

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Preliminary Study on Development of Biotic Index for Rapid Bioassessment in Mekong II Basin (Thailand)

การศึกษาเบื้องต้นการพัฒนาดัชนีชีวภาพสำหรับการประเมินคุณภาพลำธารแบบเร็ว ในลุ่มน้ำโขง 2 (ส่วนที่อยู่ในประเทศไทย)

Paiboon Getwongsa¹ and Narumon Sangpradub^{2*}

Abstract

Rapid biological monitoring of freshwater is currently an important method as a supplement or even an alternative method to chemical analysis in many developed and some developing countries. We propose a research aimed to develop a standard rapid bioassessment procedure for streams in Thailand by following the Rapid Bioassessment Protocol of USEPA (Barbour et al., 1999). The study areas were conducted in 20 streams in the Mekong II Basin (Thailand) during the cool season of 2005 and the hot season of 2006. Spatial and temporal structure and composition of macroinvertebrate community were considered. Environmental and physicochemical parameters were also measured. Twelve references and 8 test sites were classified by location, surrounding criteria of streams, some physicochemical parameters of the water and habitat assessment. Total scores of habitat assessment in reference sites were higher than those of test sites. During the cool season of 2005, number of orders, families, genera, and individuals of macroinvertebrates in the reference sites were also higher than those of test sites. Whereas, in the hot of 2006 the number of orders, families, and genera of macroinvertebrates in the reference sites were lower than those of test sites, but the abundance in the reference sites was higher than those of test sites. Total number of taxa and number of intolerant taxa are potential candidate metrics. The study will be carried out for 2 years in order to construct a standard monitoring method for streams in the Mekong II Basin, Thailand.

บทคัดย่อ

การติดตามคุณภาพแหล่งน้ำด้วยวิธีการทางชีวภาพเพื่อใช้เป็นวิธีเสริมหรือแทนที่การวิเคราะห์ทางเคมีในประเทศที่พัฒนาแล้วหลายประเทศและประเทศกำลังพัฒนาบางประเทศได้รับความสนใจเพิ่มขึ้นในปัจจุบัน

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การวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการมาตรฐานสำหรับการประเมินคุณภาพลำธารแบบเร็วด้วยวิธีทางชีวภาพ โดยใช้แนวทาง Protocol ของ USEPA (Barbour et al., 1999) เก็บตัวอย่างจากลำธาร 20 แห่ง ในช่วงฤดูร้อน 2548 และฤดูหนาว 2549 บริเวณลุ่มน้ำโขง 2 (ส่วนที่อยู่ในประเทศไทย) ศึกษาโครงสร้างและส่วนประกอบของชุมชนสัตว์ไม่มีกระดูกสันหลังหน้าดิน พร้อมกับตรวจวัดค่าคุณภาพสิ่งแวดล้อม เพื่อเปรียบเทียบระหว่างฤดูกาลและสถานีศึกษา ผลการศึกษาจาก 2 ฤดูกาลที่ผ่านมา สามารถแบ่งลำธารออกเป็นสถานีอ้างอิง 12 แห่งและสถานีทดสอบ 8 แห่ง โดยใช้เกณฑ์ด้านสิ่งแวดล้อมของลำธาร พารามิเตอร์ทางกายภาพ เคมีบางประการของน้ำ และคะแนนประเมินแหล่งอาศัยรวม สถานีอ้างอิงมีค่าคะแนนประเมินแหล่งอาศัยรวมสูงกว่าสถานีทดสอบ ในช่วงฤดูหนาว 2548 จำนวนอันดับ วงศ์ สกุลและจำนวนตัวของสัตว์ไม่มีกระดูกสันหลังหน้าดินในสถานีอ้างอิงมีมากกว่าสถานีทดสอบ ส่วนในฤดูร้อน 2549 มีเพียงจำนวนตัวของสัตว์ไม่มีกระดูกสันหลังหน้าดินจากสถานีอ้างอิงเท่านั้นที่มากกว่าในสถานีทดสอบ ส่วนจำนวนอันดับ วงศ์และสกุลน้อยกว่าในสถานีทดสอบ Metrics ที่มีศักยภาพในการนำมาพัฒนาเป็นดัชนีชีวภาพได้จากฤดูกาลเก็บตัวอย่างที่ผ่านมา ได้แก่ Total number of taxa และ Number of intolerant taxa ขณะนี้งานวิจัยยังอยู่ในช่วงเริ่มต้นยังคงต้องศึกษาให้ครบ 2 ปี เพื่อพัฒนาให้ได้วิธีการมาตรฐานสำหรับติดตาม/เฝ้าระวังคุณภาพแหล่งน้ำจัดด้วยวิธีทางชีวภาพในลุ่มน้ำโขง 2 ต่อไป

Keywords: Biotic Index, Rapid Bioassessment, Mekong II Basin Thailand

คำสำคัญ: ดัชนีชีวภาพ, การประเมินคุณภาพลำธารแบบเร็ว, ลุ่มน้ำโขง 2 ประเทศไทย

INTRODUCTION

Water depletion and degradation of water quality are still serious problems in Thailand (PCD, 2004). The official method for monitoring surface water quality mainly depends on chemical analysis, which cannot detect effects from non-point source of pollution, such as agriculture, silviculture, mining, and urban land use (Barbour et al., 1996). Currently rapid biological monitoring of freshwater is becoming important as a supplement or even an alternative to chemical analysis in many developed and some developing countries (Resh, 1995; Heart et al., 2001). It has proved to be precise and accurate in as assessing potentially, several kinds of the water pollution (Hellowell, 1989; Resh, 1995). Biomonitoring method also has lower cost than physical and chemical techniques. Therefore, it is more suitable for countries which lack the budget

and planning in water management. These results were the same as the biomonitoring researched in Thailand (Throne and Williams, 1997; Sangpradub et al., 1998; Mustow, 2002). Biotic Index in this study means the “metrics”. The Biotic Index assesses biological condition by describing structure and function of aquatic communities. A metric is defined as a characteristic of the biota that changes in some predictable way with increased human influence (Karr, 1991; Barbour et al., 1995). There are 5 categories of metrics (Table 4), including (1) richness measure, (2) composition measure, (3) tolerance/intolerance measure, (4) feeding measure or trophic dynamics, and (5) habit measure. The biological methodology and measure methods (or metrics) need to be calibrated for different regions of the country and include different impact types (stressors) (Barbour et al., 1999). For

the development of a standardized biotic index for freshwater resources, biomonitoring needs to be conducted in many streams and watersheds. Many places of undisturbed or less impacted sites are necessary to serve as reference conditions or reference sites. Recent study conducted in the Loei River and adjacent catchments of northeast Thailand can potentially be used for biomonitoring. In this study 4 potential metrics were found that could be developed in to indexes (Boonsoong et al., 2005). Davison et al. (2006) surveyed the community of macroinvertebrates in the lower Mekong and selected tributaries in 4 countries, Lao PDR, Thailand, Cambodia, and Vietnam. In this study the communities' compositions of benthic macroinvertebrates was used to determine the water quality of the lower Mekong River. Almost all sites belonged to the 'good category'. However, it is necessary to conduct more research to set up basic knowledge. The objective of this study was to develop standard rapid bioassessment procedure for streams in Mekong II Basin (Thailand) by following the Rapid Bioassessment Protocol (RBPs) of USEPA (Barbour et al., 1999).

METHODS

Study area and site selection

The study area is located in upper Northeast of Thailand (Figure 1), in the Mekong II Basin, or by its local name, I-San Mekong Basin. It is 47,502 square kilometers and located at latitude $14^{\circ} 45' - 19^{\circ} 25' N$ and longitude $99^{\circ} 25' - 105^{\circ} 40' E$. The west and south of the basin are range of the Phu Phan Mountain. The landscape of the area is a pan-bowl highland called Sakon Nakhon Plateau, with an altitude between 160 - 250 meters

above sea level (masl). It drains from the north and east of the basin towards the Mekong River which borders between Thailand and Lao PDR. The catchment spreads from Loei to Nakhon Panom Province and along Phu Phan Range which cover 7 provinces of upper Northeast, Thailand. There are 38 sub catchments in the Mekong II Basin. The mean rainfall is 1,537 mm per year. The study sites are situated in 5 preserved areas: (1) Phu Phan National Park, (2) Phu Pha Yon National Park and (3) Phu Pha Lek National Park, which are located closely together totaling an area of 1,537.3 km² and an altitude of approximately 200 - 765 masl. These preserved areas contain a ripple of hills and a plateau, with sand stone as the component of the mountain. The area conserves plants, animals and headwater forests. Dipterocarpus forests are dominant as well as some deciduous and dry tropical forests. Many streams originate from these preserved areas such as the Phung River, the Songkhram River, the Bangsai and the Bang-I River. (4) The Phu Langka National Park, and (5) Phu Wua Wildlife Sanctuary, are two preserved areas that are located to the north of the basin with an area of 235.6 km², altitude 200 - 536 masl, and a mean rainfall of 1,860 mm per year. The rainfall drains directly from the north to the Mekong River and from the east and the south to the Songkhram River. The rock component of mountain is sand stone.

Twenty study sites were examined and divided into 3 groups along sub basin (Figure 1). The first, "Songkhram group, S", the Songkhram River is the main stream, it flows from the north of Phu Pha Lek National Park and joins the Mekong River at Pla Pak District, Nakhon Panom Province.

Ten sites were examined from S group, 4 sites in Phu Pha Lek National Park (Huai Songkhram 1, S1; Huai Hin Lueam, S2; Huai Songkhram 2, S3 (Figure 2 a, b); Lam Phan Chat, S4), 2 sites in Phu Phan National Park (Huai Un 1, S5; Huai Un 2, S6), 2 sites in Phu Langka National Park (Huai Langka, S7; Huai Tat Kham, S8), and 2 sites in Phu Wua Wildlife Sanctuary (Huai Ka-am, S9; Huai Cha Naen, S10). The second, Kam River group, K, the Kam River is the main stream. The headwater is located in Phu Phan National Park and the upper portion is called Phung River.

Its water drains to the Nam Phung Electrical Dam and flows out to the Nonghan Lake, after that it becomes to the Kam River and joins the Mekong

River at That Pha Nom District, Nakhon Panom Province. Five sampling sites were examined from the K group; Huai Phung 1, K1 (Figure 2 c, d); Huai Yai, K2; Huai Phakkha, K3; Huai Som, K4 (Figure 2 e, f); Huai Phung 2, K5. The third, “Bang Sai-Bang I group, B”, the Bang Sai Noi River and the Bang-I River are the two main streams, the flow from Phu Pha Yon National Park, and drain to the Mekong River in Muang District and Don Tan District, Muk Da Han Province, respectively. Five sites were collected from these areas (Huai Bang I 1, B1; Huai Kaphung, B2 (Figure 2 g, h); Huai Bang I 2, B3; Huai Bang Sai Noi 1, B4; Huai Bang Sai Noi 2, B5.

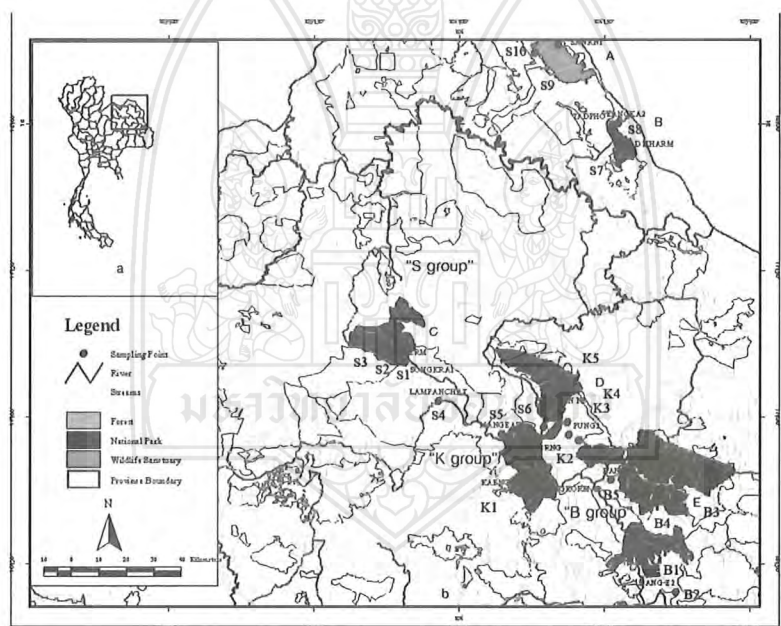


Figure 1. a) Map of study areas showing the distribution of sampling sites. b) Location of Mekong II Basin (Thailand) and the locations of sampling sites (S group = Songkhram River group, K group = Kam River group, B group = Bangsai-Bang-I group), National Park and Wildlife Sanctuary are also shown (A = Phu Wua Wildlife Sanctuary, B = Phu Langka National Park, C = Phu Pha Lek National Park, D = Phu Phan National Park, E = Phu Pha Yon National Park).

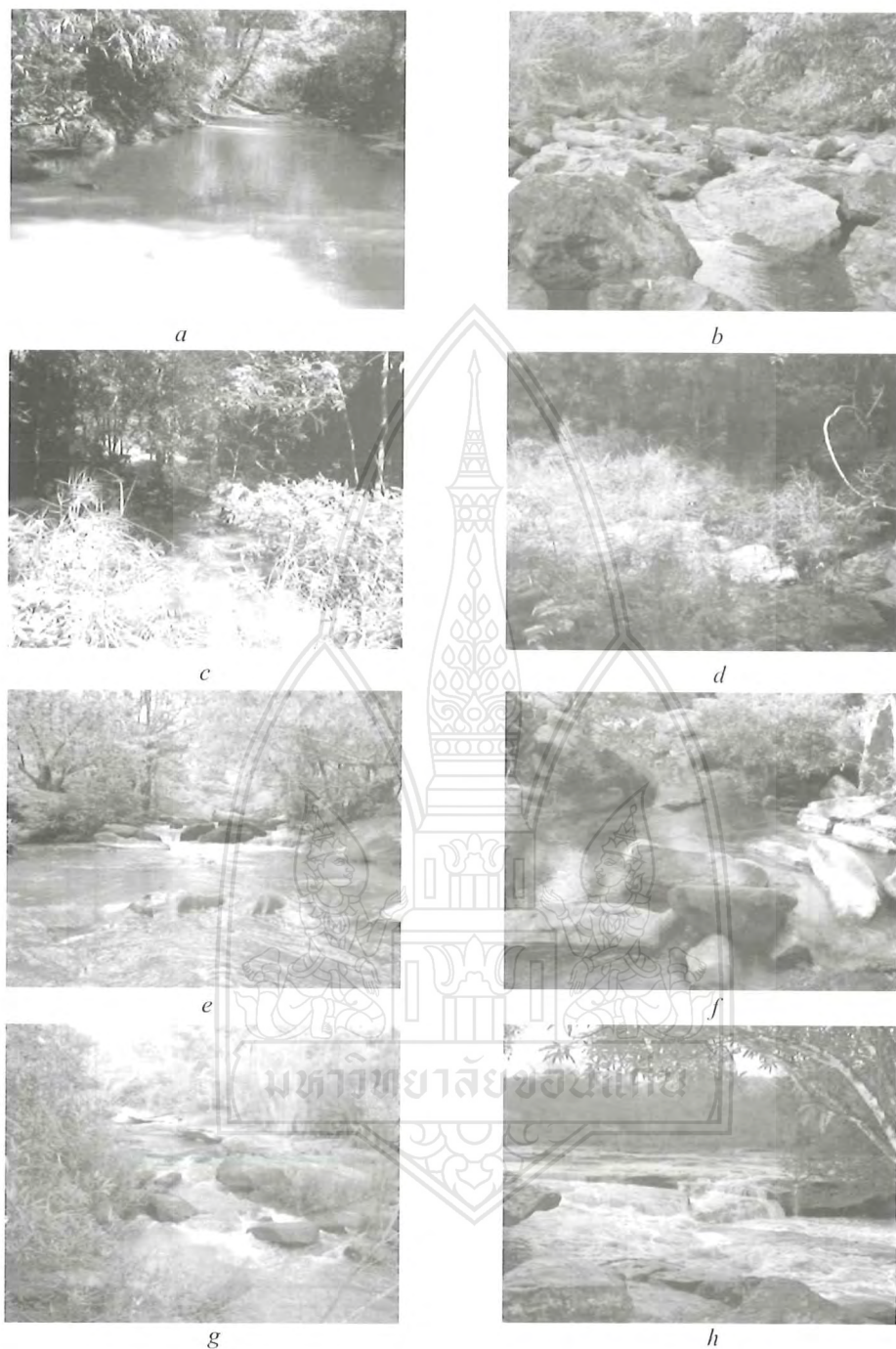


Figure 2. Characteristic of sampling site. a (cool 2005), b (hot 2006) Site S3, test site (200 masl, mean width 5 m). c (cool 2005), d (hot 2006) Site K1, reference site (305 masl, mean width 3 m). e (cool 2005), f (hot 2006) Site K4, test site (130 masl, mean width 3 m). g (cool 2005), h (hot 2006) Site B2, reference site (140 masl, mean width 2 m).

Field and laboratory methods

Fourteen physicochemical parameters of water quality were measured at the same macroinvertebrate sampling sites during the cool season of 2005 (November) and hot season of 2006 (March). The measurements were done prior to the sampling collection. Dissolved Oxygen (DO) (mg/L) and water temperature (°C) were measured by a YSI Model 57, pH with the *sensionTM 1* Portable pH meter, conductivity (µs/cm) and total dissolved solids (TDS) (mg/L) with Fisher Scientific method 09-326-2. Nitrate (mg/L NO_3^- -N), Phosphate (mg/L PO_4^{3-}) and Turbidity (FAU) were measured using the Hach DR/2010 spectrophotometer model 49300-00. BOD5 was determined as the difference between initial and 5-day oxygen concentrations in bottles after incubation at 20 °C. Chlorophyll a (µg/L) was measured with extracted-methanol method. The physical properties of habitat at each sampling site were assessed using a format recommended by the USEPA (High and Low Gradient Streams-EPA 841-B-99-002-; Barbour et al., 1999). Benthic macroinvertebrates were sampled using the multi-habitat approach (Rapid Bioassessment Protocol-EPA 841-B-99-002 Barbour et al., 1999). In the laboratory, 300 individuals fix count sub sampling were used along with Boonsoong et al. (2005). All organisms were identified to the lowest feasible taxonomic level.

Data analysis

Univariate and Multivariate were applied to analyze site distribution based on data of macroinvertebrates, habitat assessment score, and physico-chemical measurement. Metrics were tested for discrimination efficiency of reference and test

sites. EDAS (Ecological Data Application System, version 3.3 MS Access 2000 (Tetra Tech, 2000)) and SPSS Version 11 were used for data analysis. Present or absent transformed format of benthic macroinvertebrate from site samplings were conducted to analyze cluster analysis (Jaccard distance) and ordination (NMDS) by PC-ORD version 4.

RESULTS AND DISCUSSION

Physicochemical parameters

Environmental conditions at the study sites are summarized in Table 1. It's exhibited within the range of surface water quality in natural water system (PCD, 2004). Width, velocity, air and water temperatures, turbidity, conductivity, total dissolved solid, biological oxygen demand, orthophosphates and chlorophyll a were not significantly different between the reference and test sites during the cool season of 2005 and the hot season of 2006, with the exception of depth of water, pH, dissolved oxygen and nitrates. The depth of water in the reference sites was significantly lower than those of test sites during the cool season of 2005 ($p < 0.05$) because all reference sites were located in the headwater with shallow water, while agricultural activities should use around, near, or at the test sites created deeper water. The pH in the reference sites was significantly higher than those of test sites during the cool season of 2005 ($p < 0.01$) but ionic concentration in the test sites tends to rise significantly during the hot season of 2006 ($p < 0.05$) which may be caused by temporary water dry out that may change in pH (Williams, 1996). Dissolved oxygen in the test sites was significantly higher than those of the reference sites during the hot season of 2006 ($p < 0.01$)

which may be caused by algae with addition of chlorophyll a (Table 1) and almost all test sites also received nutrient runoff from waste water from intensive agriculture, crop, rubber plant nursery, and rice fields, which may enhance the growth of algae. Nitrates at the test sites were significantly higher than those of reference sites during the cool season of 2005 ($p < 0.01$).

Table 1. Mean $\pm$ Standard Deviation (SD) of physicochemical parameters of water quality in reference and test sites.

Parameters	Unit	Cool 2005			Hot 2006		
		Reference Site (Mean $\pm$ SD)	Test Sites (Mean $\pm$ SD)	T-test	Reference Site (Mean $\pm$ SD)	Test Sites (Mean $\pm$ SD)	T-test
Width	m	2.4 $\pm$ 1.8	4.3 $\pm$ 4.4	0.30	2.6 $\pm$ 1.4	3.7 $\pm$ 2.8	0.41
Depth	cm	24.0 $\pm$ 11.8	39.1 $\pm$ 12.8	0.03*	25.5 $\pm$ 16.4	22.3 $\pm$ 18.0	0.74
Velocity	m/s	0.4 $\pm$ 0.3	0.5 $\pm$ 0.3	0.41	0.04 $\pm$ 0.08	0.4 $\pm$ 0.5	0.08
Air temperature	°C	22.9 $\pm$ 1.3	23.4 $\pm$ 2.6	0.64	30.1 $\pm$ 3.4	32.8 $\pm$ 3.7	0.20
Water temperature	°C	21.7 $\pm$ 1.7	22.3 $\pm$ 1.6	0.48	26.3 $\pm$ 2.1	28.3 $\pm$ 1.8	0.09
Turbidity	FAU	9.45 $\pm$ 8.2	9.53 $\pm$ 6.3	0.98	22.0 $\pm$ 28.7	18.0 $\pm$ 33.7	0.82
Electricity conductivity	μ S/cm	83.7 $\pm$ 49.1	65.7 $\pm$ 38.8	0.43	94.3 $\pm$ 52.1	84.6 $\pm$ 71.9	0.78
Total dissolved solid	mg/L	55.8 $\pm$ 32.7	43.7 $\pm$ 25.8	0.43	63.8 $\pm$ 33.7	56.4 $\pm$ 47.8	0.75
pH	-	7.7 $\pm$ 0.2	7.4 $\pm$ 0.2	0.00**	6.7 $\pm$ 0.3	7.1 $\pm$ 0.2	0.04*
Dissolved oxygen	mg/L	7.3 $\pm$ 0.9	7.2 $\pm$ 1.1	0.81	4.0 $\pm$ 1.8	6.7 $\pm$ 1.0	0.00**
Biological oxygen demand	mg/L	1.3 $\pm$ 0.3	1.6 $\pm$ 0.6	0.21	1.04 $\pm$ 0.6	1.05 $\pm$ 1.0	0.99
Nitrate	mg/L	0.3 $\pm$ 0.2	0.7 $\pm$ 0.2	0.00**	0.4 $\pm$ 0.2	0.2 $\pm$ 0.1	0.11
Orthophosphates	mg/L	0.04 $\pm$ 0.02	0.05 $\pm$ 0.03	0.31	0.10 $\pm$ 0.04	0.11 $\pm$ 0.05	0.50
Chlorophyll a	μ g/L	0.82 $\pm$ 0.5	0.89 $\pm$ 0.3	0.75	2.30 $\pm$ 2.3	4.17 $\pm$ 6.7	0.50

* = $p < 0.05$, ** = $p < 0.01$

Habitat assessment and characteristics

Figure 3 shows that mean values of habitat assessment of the reference sites were significantly higher than those of test sites during either the cool season of 2005 or hot season of 2006 ($p < 0.05$). The reference sites (S1, S2, S5,

S7, S8, S9, S10, K1, K2, K3, B1 and B2) had a variety of suitable surrounding environment or undisturbed aquatic microhabitats such as substrate or microhabitat, current velocity, riparian vegetation and forest buffer zone for benthic macroinvertebrates survived. It is important to select the reference sites

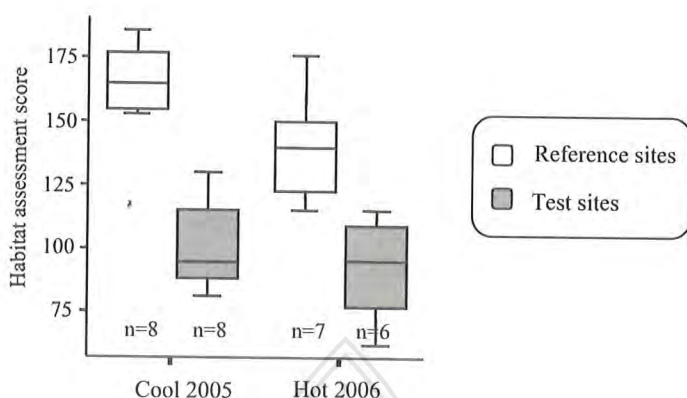


Figure 3. Box plot of habitat assessment score in reference and test sites. The central horizontal line represents the median values, the outer horizontal lines are the interquartile values.

located in undisturbed habitat in order to seek reference community conditions.

Whereas the test sites (S3, S4, S6, K4, K5, B3, B4 and B5) were surrounded agricultural activities such as rice fields, crops and residential areas with good current velocity and fair microhabitat and less riparian vegetable and forest buffer zone.

Macroinvertebrate assemblages

Sixteen orders, 76 families and 144 taxa, 8,986 macroinvertebrates were quantified from laboratory sub-sampling during a 1-y study (cool season of 2005 to hot season of 2006). Most taxa were immature aquatic insects (134 taxa, 93.06 % of total) and the remaining were represented by mollusks (8 taxa, 5.56 % of total), annelid (1 taxon, 0.69 % of total) and nematode (1 taxon, 0.69 % of total), respectively (Table 2). Overall, taxonomic compositions of aquatic insect fauna were dominated numerically by Diptera (2,919, 34.69 %), Ephemeroptera (2,532, 30.09 %), Trichoptera (2,015, 23.6 %). Hemiptera (441, 5.24 %) and Coleoptera (228, 3.42 %) were also relatively abundant (Table

3). Taxa richness is shown in Table 3. The most abundant taxa was Hemiptera (25) and was followed by Diptera (24), Coleoptera (18) and Odonata (11), and the rest were relatively less abundant.

Based on numbers of families (Figure 4 a) and genera (Figure 4 b) of macroinvertebrates during the cool season of 2005, values were higher in the reference sites than those of test sites, but did not differ significantly ($p > 0.05$). Whereas the data from the reference sites were lower than those of test sites during the hot season of 2006, indicating that density patterns in the reference and test sites did not vary between seasons. Macroinvertebrate compositions from this survey were slightly different from those in the study done by Boonsoong et al. (2005) from headwater streams of the Loei River and adjacent catchments, northeast Thailand, were only 16 orders, 78 families and 132 genera were found. Boonsoong and Sangpradub (2006) found that the more diverse orders were Trichoptera, Ephemeroptera and Diptera, respectively, while Chironomidae (Diptera) became the most abundance family (55.03 %), followed by Cheumatopsyche

(Trichoptera) (48.94 %) and Elmidae (Coleoptera) (26.84 %). Davison et al. (2005) also reported in a macroinvertebrate survey, the finding of 19 orders, 113 families and 180 genera in the lower Mekong River and selected tributaries. Two hundred and eleven taxa of littoral macroinvertebrates were found; Trichoptera was the most diverse order and

followed by Coleoptera, Hemiptera and Ephemeroptera, respectively, whereas 77 taxa were found from grab collection of benthic macroinvertebrates. The study has done by Davison et al (2006) shows that species richness of the littoral macroinvertebrates depends on habitat types and environmental factors.

Table 2. Total number of phyla and individuals of macroinvertebrates counted from sub sampling.

Phylum	Number of taxa	Individuals
Annelida	1	1
Nematoda	1	33
Mollusca	8	420
Arthropoda	134	8,532
Total	144	8,986

Table 3. Aquatic insects composition were found from sub-sampled.

Order	Family	Taxa	Individuals
Collembola	1	1	3
Coleoptera	16	18	288
Diptera	15	24	2,919
Ephemeroptera	7	22	2,532
Hemiptera	10	25	441
Lepidoptera	1	3	5
Odonata	8	11	121
Orthoptera	2	2	6
Plecoptera	1	1	85
Trichoptera	10	22	2,015
Total	71	129	8,415

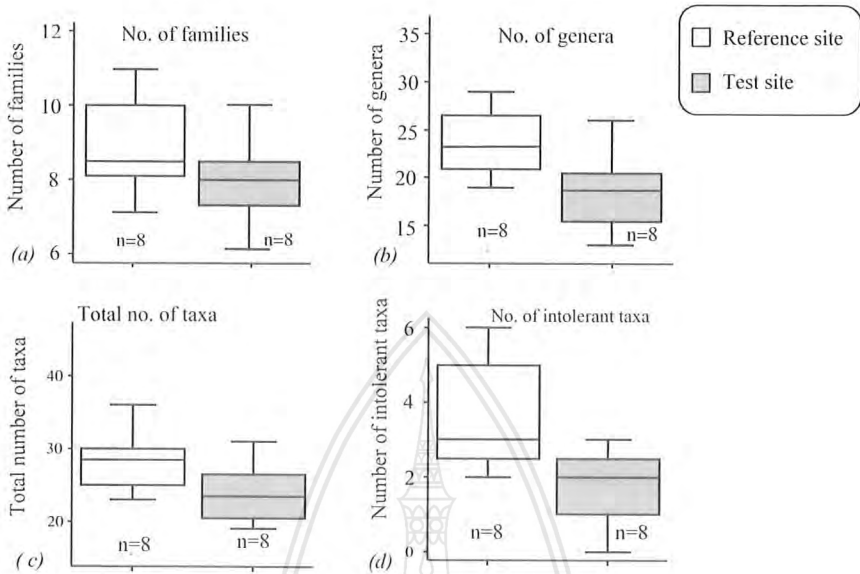


Figure 4. Box plots of multivariate analysis comparing the number of families (a) and genera (b) between the reference and test sites in cool 2005; and box plots of multimetric analysis comparing total number of taxa (c), and number of intolerant taxa (d) between the reference and test sites in cool 2005 (n = numbers of study sites)

Evaluation of metrics and index development

In the present study, the 14 RBPs metrics were used for the testing of discriminatory ability between the reference and test sites (Table 4). As only total number of taxa (Figure 4 c) and number of intolerant taxa (Figure 4 d) increased higher in the reference sites than in the test sites during the cool season of 2005. It is possible to determine the total number of taxa and number of intolerant taxa for a develop index, whereas the remaining metrics did not discriminate between the reference and test sites. According to Karr and Chu (1999), most multimetric biological indexes for aquatic systems comprise of 8 to 12 metrics, each selected because

it reflects an aspect of the system's biological condition. It may suggest that the data from the 1-y study (cool season of 2005 to hot season of 2006) is insufficient to support the development of an index. Therefore, more data is needed from future samplings in order to develop a more robust index. Richness measures reflect the diversity of the aquatic assemblage (Resh et al., 1995). According to Barbour et al. (1996), increasing diversity correlates with increasing health of the assemblage and suggests that niche space, habitat, and food source are adequate to support survival and propagation of many species.

Table 4. Definitions of candidate metrics and expected direction of metrics response to increasing perturbation in the Mekong II streams (followed by Barbour et al., 1999).

Category	Metrics	Definition	Expected response to increasing perturbation
Richness measures	Total No. Taxa	Measures the overall variety of the Macroinvertebrate assemblage	Decrease
	No. EPT Taxa	Number of taxa in the insects orders Ephemeroptera (E, mayflies), Plecoptera (P, stoneflies), and Trichoptera (P, caddishflies)	Decrease
	No. Ephemeroptera Taxa	Number of mayflies Taxa	Decrease
	No. Plecoptera Taxa	Number of stoneflies Taxa	Decrease
	No. Trichoptera Taxa	Number of caddishflies Taxa	Decrease
Composition measures	% EPT	Percent of the composition of mayfly, stonefly, and caddishfly larvae	Decrease
	% Ephemeroptera	Percent of mayfly nymphs	Decrease
Tolerance/Intolerance measures	No. of Intolerant Taxa	Taxa richness of those organisms considered to be sensitive to perturbation	Decrease
	% Tolerant Organisms	Percentage of macrobenthos considered to be tolerant of various types of perturbation	Increase
	% Dominant Taxon	Measures the dominance of the single most taxon	Increase
Feeding measures	% Filterers	Percent of the macrobenthos that filter FPOM from either the water column or sediment	Variable
	% Grazers and Scrapers	Percent of the macrobenthos that scrape or graze upon periphyton	Decrease
Habit measures	Number of Clinger Taxa	Number of taxa of insects	Decrease
	% Clingrers	Percent of insects having fixed retreats or adaptations for attachment to surfaces in flowing water.	Decrease

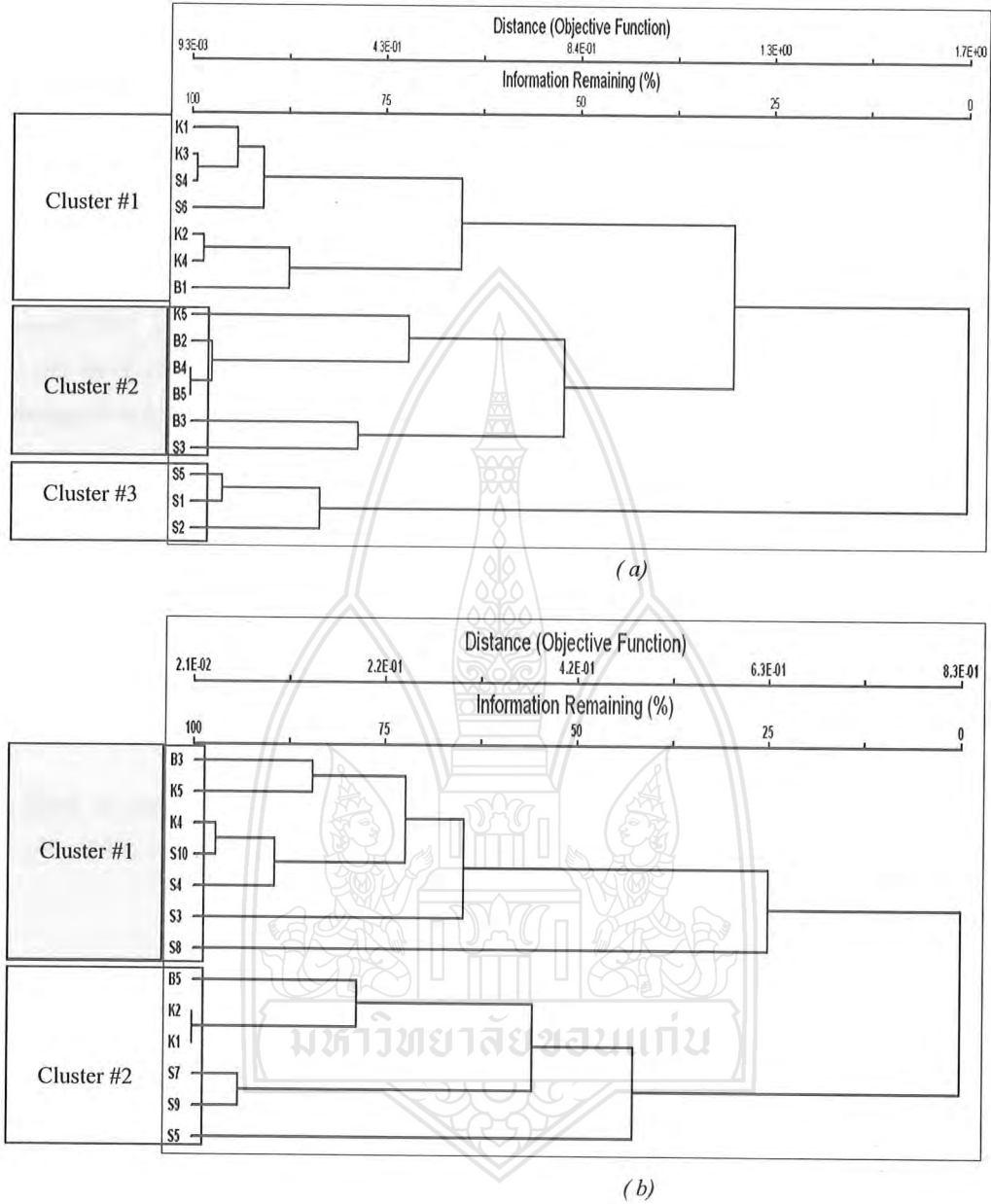


Figure 5. Dendrogram of the reference and test sites, using group average clustering from Jaccard distance measure an presence/absence 300 organism counting data in genus level; a) cool 2005; b) hot 2006.

Multivariate analysis

Cluster analysis of sampling sites was based on the presence/absence of benthic macroinvertebrates during 2 seasons. Three clusters of sampling sites were identified during the cool season of 2005, at 30 % similarity threshold (Figure 5 a). Site cluster #1 was the reference sites consisting of K1, K3, K2, B1 and S4, S6, K4 from the test sites by characteristic of stream microhabitat and velocity regimes. The site of cluster #2 was the test sites with high water velocity, and it was comprised of K5, B2 (from the reference sites), B4, B5, B3 and S3. The other cluster (#3), were the reference sites with very low or standing water were consisted of S5, S1 and S2.

In the hot season of 2006, the cluster analysis produced 2 groups of sampling sites at 25 % similarity threshold (Figure 5b). Cluster #1 was the test sites with very high water velocity comprising of B3, K5, K4, S10, S4, S3 and S8. Whereas the cluster #2, the reference sites with mostly of bed rock and low level of water, consisted of B5, K2, K1, S7, S9 and S5.

Multivariate approaches are statistical analysis used to compare between the test, and reference sites (or non human impact sites) by using macroinvertebrates information. This method is widely used in Europe, Australia, and Canada, e.g. RIVPACS, AUSRIVAS, BEAST, and ANNA (AURIVAS, 2000; Clarke et al., 2003; Linke and Norris, 2005; Reynoldson et al., 1997). The multivariate approaches are complex and difficult to communicate to water managers (Fore et al., 1996). Meanwhile, the multimetric approaches are the most frequently used by water resource agencies in USA because it provides ecological information that is readily

translated to water quality manager (Barbour and Yoder, 2000). The strength of the multimetric approach is its ability to integrate information from individual, population, community, and ecosystem levels and to allow evaluation with reference to biogeography as a biologically based on indicator of water resource quality (Barbour et al., 1995). The multimetric approach is more widely used around the world (Throne and Williams, 1997; Boonsoong et al, 2005; Bonada et al., 2006). From this study, the multivariate analyze (Figure 5 a, b) appeared to simultaneously separate the site group of reference and test sites. Whereas the multimetric approaches of the reference sites (Figure 4 a, b, c, d) showed no significant from the test sites. More sampling data is required for evaluation on seasonal variable and consistency of the analyses.

ACKNOWLEDGMENTS

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Hydrochemical Characteristic of Brines and Bitterns in the Northeast, Thailand

ลักษณะทางเคมีอุทกวิทยาของน้ำเกลือและน้ำเกลือขม ในภาคตะวันออกเฉียงเหนือของประเทศไทย

Sarunya Promkotra¹

Abstract

Local salt industries in Sakon Nakhon, Udon Thani and Khorat Basin were investigated to study the chemical compositions in brines and bitterns. Brines and bitterns compared to evaluate and distribute the chemical compositions in the area of interest. The samples were randomly collected from Nakhon Ratchasima, Mahasarakham, Udon Thani, Sakon Nakhon and Nong Khai province. Hydrochemical parameters and also chemical compositions of major elements of brines and bittern were examined. Hydrochemical facies of brines were generally Na-Cl type. However, only one area in Nakhon Ratchasima differed from the others, consisting of Na-Ca-Cl-HCO₃ type as opposed to Na-Cl type. The typical facies of brines depended on the hydrogeochemistry and hydrodynamics of the regional areas. The Na/Cl ratios of brines and bitterns were approximately 0.627 and 0.638, respectively, which was close to the combined ratio of Na and Cl in pure crystals of halite, 0.65. The chemical compositions of K/Cl, K, Ca/(SO₄+HCO₃) and Mg/SO₄ of bitterns were mostly greater than brines. The Mg/SO₄ ratios in bitterns showed remarkably intensive results.

บทคัดย่อ

การศึกษาลักษณะทางเคมีของน้ำเกลือและน้ำเกลือขมจากการสำรวจอุตสาหกรรมการทำเกลือท้องถิ่นในแอ่งสกลนคร อุตร และโคราช เพื่อเปรียบเทียบและประเมินการกระจายตัวขององค์ประกอบทางเคมีในบางพื้นที่ โดยเก็บตัวอย่างจากจังหวัดนครราชสีมา มหาสารคาม อุรธานี สกลนคร และ หนองคาย ได้ทำการตรวจวัดค่าพารามิเตอร์ทางเคมีอุทกวิทยาและองค์ประกอบทางเคมีที่เป็นธาตุหลักของน้ำเกลือและน้ำเกลือขมพบว่า น้ำเกลือมีรูปแบบทางเคมีอุทกวิทยาเป็น โซเดียม-คลอไรด์ (Na-Cl) แต่มีแหล่งเดียวที่จังหวัดนครราชสีมาเป็นแบบ โซเดียม-แคลเซียม-คลอไรด์-ไบคาร์บอเนต (Na-Ca-Cl-HCO₃) ลักษณะที่แตกต่างกันนี้เกิดจากธรณีเคมีอุทกวิทยาและพลวัตอุทกวิทยาของพื้นที่ อัตราส่วนของโซเดียมต่อคลอไรด์ (Na/Cl) ของน้ำเกลือและน้ำเกลือขมมีค่าประมาณ 0.627 และ 0.638 ซึ่งใกล้เคียงกับอัตราส่วน 0.65 ของโซเดียมต่อคลอไรด์ในผลึกแร่บริสุทธิ์แฮไลต์ องค์ประกอบ

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ทางเคมีของ K/Cl , K , $Ca/(SO_4+HCO_3)$ และ Mg/SO_4 ของน้ำเกลือขมโดยส่วนใหญ่แล้วมีค่ามากกว่าน้ำเกลืออัตราส่วนของแมกนีเซียมต่อซัลเฟต (Mg/SO_4) ของน้ำเกลือขมมีปริมาณมากอย่างมีนัยสำคัญ

Keywords: hydrochemistry, hydrochemical facies, brines and bitterns

คำสำคัญ: เคมีอุทกวิทยา, รูปแบบเคมีอุทกวิทยา, น้ำเกลือและน้ำเกลือขม

INTRODUCTION

Salt Production: Solar salt can be produced by the action of the sun on seawater or natural brines from underground. When water on salt ponds evaporates, salt solutions are gradually concentrated, and salts crystallize on the ground as salt floors.

Salt productivity from the solar salt production can yield an abundance of salts and have a lower cost than the mining solution. Solar salt fields in Northeast Thailand (Figure 1) mostly begin to produce salts in the summer and winter, when evaporation rates are high and there is low rainfall.

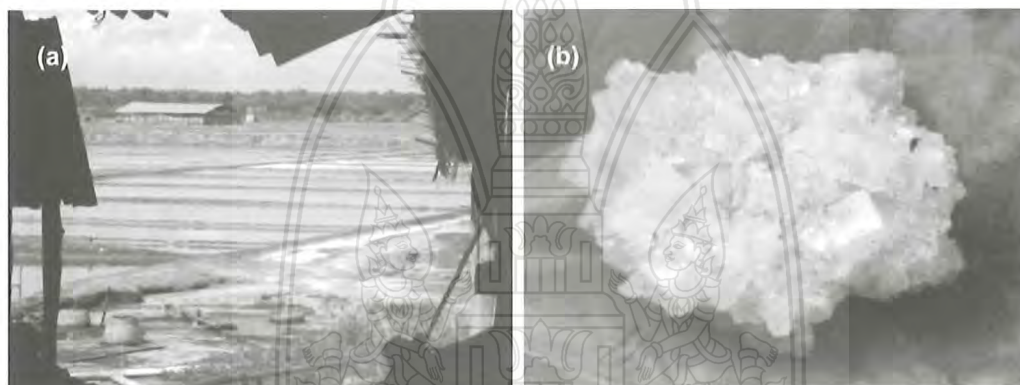


Figure 1. (a) Solar salt field in Sakon Nakhon and (b) an agglomerated salt crystal.

Other saltwork processes include boiling or evaporating of natural brines by heat (Figure 2). This process can be achieved at home as a “local salt industry”, which presently is very extensive in the Northeast, Thailand. Evaporated salts from this method can be produced all year round. Both solar salt and evaporated salt production are very important for salt production of the community. There are currently many brine used for the local salt industry. Salt crystals are crystallized and separated

from the concentrated brines. Concentrated solutions after salt separation, called bitterns, will be collected and mixed with the new brines before continuing the salt production processes. Finally, bitterns are discarded out of the process which still contains many residual ions. For this reason, the salinity of bitterns rapidly disperses to the surroundings.



Figure 2. Evaporated salt productions in Nong Khai (a) storage tank of brines before pumping to the process, (b) rice husk pile which used for boiling brines, (c) evaporated salts in a pan and (d) dried salts in buckets.

STUDY AREAS

Rock salts are mainly found in two regions in the Northeast, Thailand, North and South basin (Figure 3). The North basin consisted of Sakon Nakhon and Udon Thani Basins while the South basin was consists of Khorat Basin. Brines aquifers in Sakon Nakhon and Udon Thani Basins are of shallow level, approximately 40 - 70 meters deep. However, brines in Khorat Basin flow in deep brine aquifers, roughly 70 - 180 meters deep.

Salt industries in Sakon Nakhon, Udon Thani and Khorat Basins were investigated to study the chemical compositions in brines and bitterns. The samples were used to compare with the evaluation and distribution of the chemical composition in the area of interest. The samples were randomly collected from Nakhon Ratchasima, Mahasarakham, Udontani, Sakon Nakhon and Nong Khai provinces.

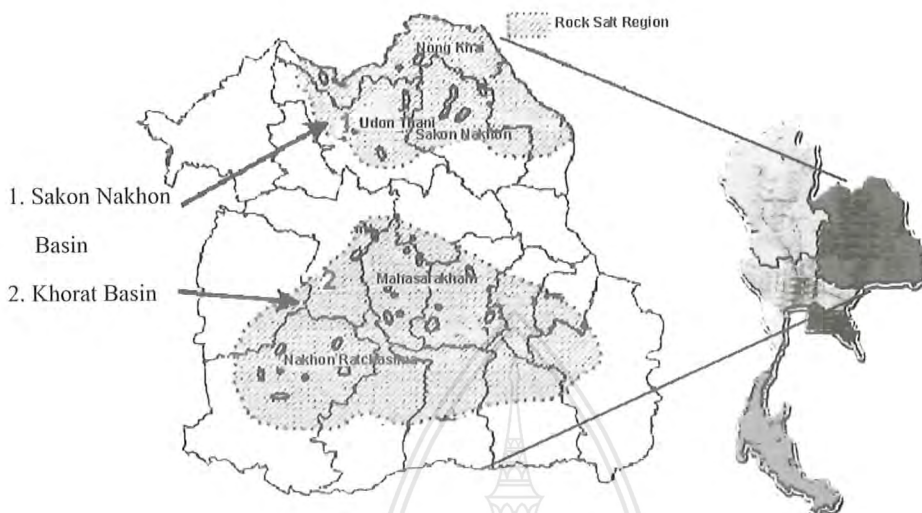


Figure 3. Rock salt regions in the Northeast, Thailand (after Department of Mineral Resources, 2005).

MATERIAL, METHODS AND RESULTS

Brines and biterms of both solar salt and evaporated salt production were collected from the local salt industries during November 2005. The parameters of EC (electrical conductivity), pH, Eh (redox potentials) and O_{Baume} were measured on site at the study areas (Table 1). The samples were carried to the geochemical laboratory. The major ion parameters were examined including Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, NO₃⁻, SO₄²⁻ and HCO₃⁻. All major cations were analyzed by Atomic Absorption Spectrometry. Other major anions, the Cl⁻, NO₃⁻ and SO₄²⁻ were measured by Flow Injection Analysis System (FIAS). Moreover, the HCO₃⁻ was examined by titration. The analytical results were shown in Table 2 and Table 3.

Hydrochemical diagrams were interpreted in combination with distribution parameters. The “relationship of differing hydrochemical water types was first evaluated hydrochemical distributions of brines and biterms (Bassett and Bentley, 1982). The Piper diagram can present a large number of analyses in trilinear diagrams (Lloyd and Heathcote, 1985). Graphical plots can be achieved using AquaChem computing software program (Waterloo Hydrogeologic, Inc., 1999). Piper diagrams of brines and biterms are indicated in Figure 4. The X-Y plots allow a preliminary assessment of hydrochemical parameters. Comparisons with data between biterms and brines were considered in pair of ions as followed K^+/Cl^- , $Ca^{2+}/(SO_4^{2-}+HCO_3^-)$ and Mg/SO_4^{2-} (Drever, 1982).

Table 1. Parameter measuring on the locations at the study areas.

No.	Location Site	Grid References	EC ($\mu\text{S/cm}$)		pH		Eh (mV)		°Baume'	
			Brines	Bitterns	Brines	Bitterns	Brines	Bitterns	Brines	Bitterns
1	Somrong, Nakhon Ratchasima	821305 1676160	618,000	632,000	6.21	6.09	45	54	23.50	24.10
2	Pra Thong Kham, Nakhon Rachasima	814650 167457	537,000	647,000	6.07	6.24	55	41	24.10	25.00
3	Kantharawichai, Mahasarakham	319454 1807242	584,000	673,000	5.94	5.80	63	71	22.10	23.10
4	Ban Dung, Udon Thani	314050 1958997	538,000	573,000	6.23	6.23	46	45	22.10	23.00
5	Ban Phon Sung, Udon Thani	314052 1958999	524,000	668,000	6.24	3.29	45	220	23.00	24.00
6	Ban Fang, Udon Thani	318753 1955886	511,000	641,000	6.14	5.75	51	74	21.50	23.50
7	Ban Thung, Udon Thani	318753 1955886	455,000	699,000	6.02	5.81	58	71	19.50	25.00
8	Ban Kut Ruea Kham, Sakon Nakhon	342155 1964947	632,000	676,000	5.97	6.00	57	59	25.00	25.50
9	Ban Bo Daeng, Sakon Nakhon	341851 1969844	630,000	640,000	6.23	6.15	46	50	26.00	26.10
10	Ban Nong Kwang, Sakon Nakon	341606 1966206	658,000	690,000	6.21	6.20	47	47	28.60	29.70
11	Phonphisai-1, Nong Khai	642276 1821018	595,000	622,000	6.24	6.28	41	43	25.00	25.10
12	Phonphisai-2, Nong Khai	320356 1984575	543,000	634,000	6.31	6.22	41	46	24.50	25.00

Table 2. Major cation parameters (Na^+ , K^+ , Mg^{2+} and Ca^{2+}) of each location.

No.	Grid References	Na^+ (mg/L)		K^+ (mg/L)		Mg^{2+} (mg/L)		Ca^{2+} (mg/L)	
		Brines	Bitterns	Brines	Bitterns	Brines	Bitterns	Brines	Bitterns
1	821305 1676160	118,028.30	127,924.53	1,803.27	2,643.33	2,890.15	3,065.33	1,560.24	871.79
2	814650 167457	76,650.94	97,287.74	1,174.81	1,615.37	2,969.37	7,001.50	47,705.16	52,025.44
3	319454 1807242	117,924.53	130,235.85	257.28	624.41	1,178.09	3,853.20	4,483.16	8,508.61
4	314050 1958997	112,028.30	119,028.30	649.08	906.37	987.33	945.37	1,880.77	5,300.57
5	314052 1958999	126,768.87	132,028.30	2,790.18	744.81	967.98	1,289.08	3,565.18	1,870.79
6	318753 1955886	104,339.62	120,872.64	340.11	313.09	1,834.73	5,196.09	4,676.98	3,117.98
7	318753 1955886	91,391.51	136,872.64	214.40	1,551.13	1,072.40	2,481.20	1,898.23	2,320.10
8	342155 1964947	118,028.30	126,132.08	3,766.59	8,076.83	3,310.18	5,951.47	1,901.17	2,128.16
9	341851 1969844	117,924.53	114,976.42	5,349.62	8,664.24	2,055.24	3,929.50	1,748.57	9,289.35
10	341606 1966206	107,287.74	100,235.85	27,608.08	31,132.52	2,632.38	7,744.54	6,235.97	4,365.18
11	642276 1821018	120,872.64	136,132.08	2,259.82	3,083.88	2,194.96	3,242.79	1,492.89	2,806.19
12	320356 1984575	114,976.42	132,665.09	1,057.33	4,992.95	903.50	2,784.98	1,529.06	3,741.58

Table 3. Major anion parameters (Cl^- , NO_3^- , SO_4^{2-} and HCO_3^-) of each location.

No.	Grid References	Cl^- (mg/L)		SO_4^{2-} (mg/L)		NO_3^- (mg/L)		HCO_3^- (mg/L)	
		Brines	Bitterns	Brines	Bitterns	Brines	Bitterns	Brines	Bitterns
1	821305 1676160	177,575.0	171,987.5	3,960.6	2,965.0	17.5200	23.8000	2,379.37	1,329.48
2	814650 167457	163,425.0	198,425.0	3,644.9	2,877.5	34.0600	40.3400	72,750.37	89,838.80
3	319454 1807242	193,512.5	204,712.5	3,105.7	2,359.0	0.0123	0.0121	11,411.82	81,250.62
4	314050 1958997	166,175.0	163,362.5	2,713.8	2,527.5	0.0128	0.0126	2,868.17	8,083.37
5	314052 1958999	182,350.0	169,825.0	3,071.9	3,556.3	0.0123	0.0122	6,656.90	2,852.96
6	318753 1955886	162,212.5	185,750.0	3,338.9	2,537.8	0.0121	16.0200	7,132.39	4,754.93
7	318753 1955886	123,860.0	202,300.0	4,289.6	3,924.8	14.9700	23.0800	2,894.80	2,470.66
8	342155 1964947	214,125.0	236,237.5	25,069.0	5,926.3	19.4200	16.9300	2,594.29	2,940.45
9	341851 1969844	173,862.5	187,887.5	2,825.6	2,583.4	16.6700	25.6300	2,666.56	15,691.26
10	341606 1966206	182,700.0	190,400.0	2,805.5	2,146.1	7.3200	24.6500	9,509.85	6,656.90
11	642276 1821018	174,375.0	230,924.0	3,496.3	3,790.1	0.0123	0.0124	2,276.66	4,279.43
12	320356 1984575	179,637.5	201,500.0	3,614.0	3,850.3	0.0121	28.5700	2,331.82	5,705.91

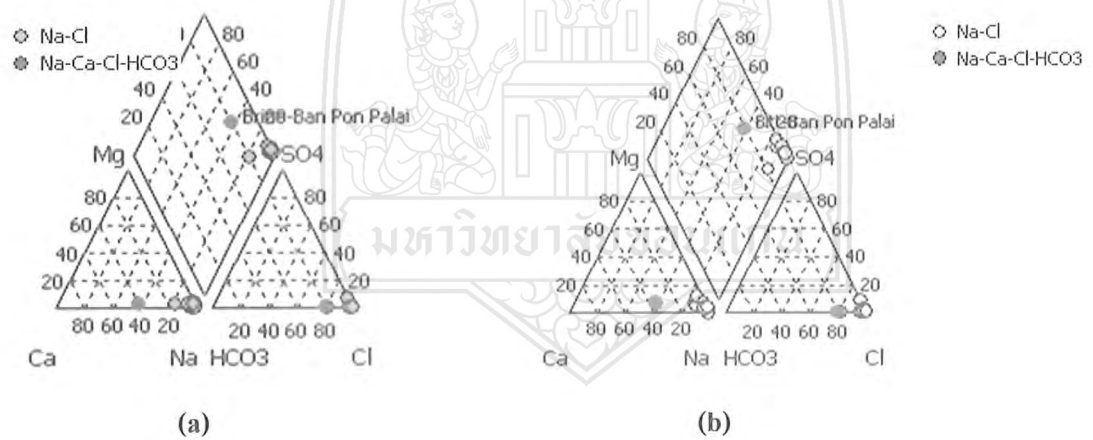


Figure 4. Trilinear diagrams of (a) brines and (b) bitterns, which all hydrochemical facies indicated water type of Na-Cl, except Ban Pon Palai, King Amphoe Pra Thong Kham, Nakhon Ratchasima showing Na-Ca-Cl-HCO₃.

DISCUSSION

Hydrochemical facies were generally the Na-Cl type (Figure 4). However, a sample of Ban Pon Palai, King Amphoe Pra Thong Kham, Nakhon Ratchasima (sample No. 2 in Table 1) indicated a facies of the Na-Ca-Cl-HCO₃, which differed from the others. The calcium (Ca²⁺) and bicarbonate (HCO₃⁻) ions, which dissolved in a sample, may come from dissolution of meteoric water in an aquifer. Both facies in Piper diagrams of brines and bitterns (Figure 4a and 4b) were similar to each other. A few sulphate ions of bitterns were more distributed than in brines. Nevertheless, the ratios of Na/Cl in both brines and bitterns were 0.6370 and 0.6353, respectively, as shown in Table 4. The ratio of Na/Cl of pure crystals of halite was generally the rationalized value of 0.65, which is greater than that of seawater, 0.556. This result implied that bitterns

still had much sodium chloride (NaCl) remaining in the concentrated solution. The Na/Cl ratio of brines was close to the ratio of the pure crystal halite. Therefore, rock salts underground gradually dissolved.

Although, the Piper diagrams or trilinear diagrams can identify the major distributions of different type of ions, they are very difficult to make a distinction of hydrochemical facies in detail in brines and bitterns. Thus, X-Y plots can be applied to evaluate the ion ratios instead.

The relationship between bitterns and brine according to the ratios of K/Cl and potassium ions was plotted and illustrated in Figure 5. Potassium ion (K⁺) in bitterns (Figure 5b) evidently increased greater than brines, compared to K/Cl ratios. The chloride, which is an anion, can bond with sodium ions and also the other cations, such as magnesium ions. Thus, the distributions of chloride fluctuated.

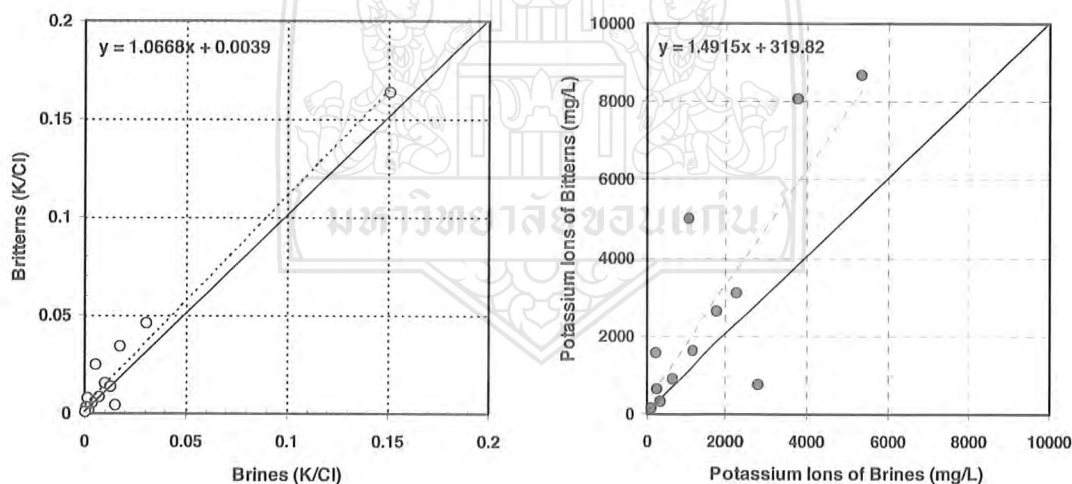


Figure 5. Bitterns related to brines as a (a) K/Cl ratio and (b) potassium ion.

Other anions, which were highly distributed, included sulphates and bicarbonates (Figure 6). These anions can be interchanged in ion exchange with the surroundings, especially clay minerals. The ratios of $\text{Ca}/(\text{SO}_4 + \text{HCO}_3)$ in bitters and brines were very dispersed, which depended upon the

hydrogeology of aquifers (Figure 6a). On the other hand, the ratios of Mg/SO_4 of bitters and brines can be distinguished with a linear trend (Figure 6b). The relationship between bitters and brines according to the ratios of Mg/SO_4 showed significantly growth.

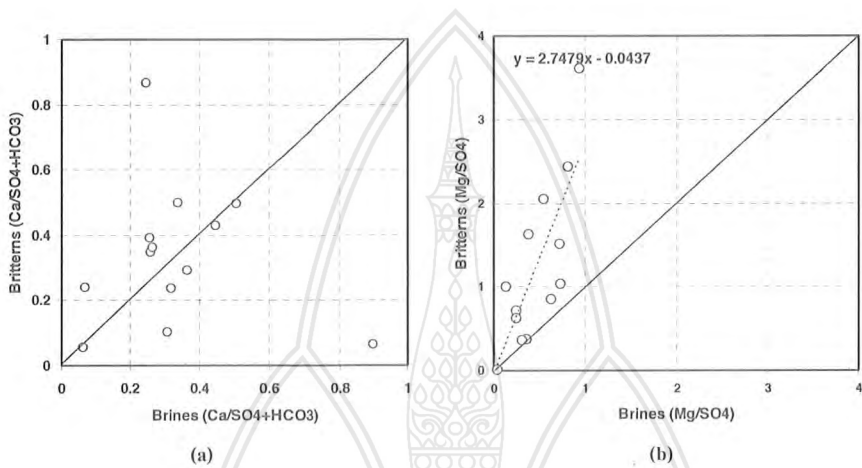


Figure 6. Bitters related to brines as a (a) $\text{Ca}/(\text{SO}_4 + \text{HCO}_3)$ and (b) Mg/SO_4 ratios.

Table 4. The Na/Cl ratios of brines and bitters.

No.	Location	Na/Cl	Na/Cl
		Brines	Bitters
1	Somrong, Nakhon Ratchasima	0.6647	0.7438
2	Pra Thong Kham, Nakhon Rachasima	0.4690	0.4903
3	Kantharawichai, Mahasarakham	0.6094	0.6362
4	Ban Dung, Udon Thani	0.6742	0.7286
5	Ban Phon Sung, Udon Thani	0.6952	0.7774
6	Ban Fang, Udon Thani	0.6432	0.6507
7	Ban Thung, Udon Thani	0.7379	0.6766
8	Ban Kut Ruea Kham, Sakon Nakhon	0.5512	0.5339
9	Ban Bo Daeng, Sakon Nakhon	0.6783	0.6119
10	Ban Nong Kwan, Sakon Nakon	0.5872	0.5264
11	Phonphisai-1, Nong Khai	0.6932	0.5895
12	Phonphisai-2, Nong Khai	0.6400	0.6584
Average (Na/Cl)		0.6370	0.6353

Chemical compositions of bitterns were greater than brines in the following ratios: Mg/SO_4 , 62.33 %, Ca/SO_4 28.96 %, and K/Cl , 20.72 %, respectively.

The nitrate-nitrogen ratio of bitterns and brines was indicated in Figure 7. All data of bitterns

mostly showed higher results than brines. Most of them can be plotted above the 1:1 line. These results imply that bitterns have a higher level of dissolved solid than brines. Nitrates may be harmful to human beings if they contaminate in ground water at levels over 45 mg/L.

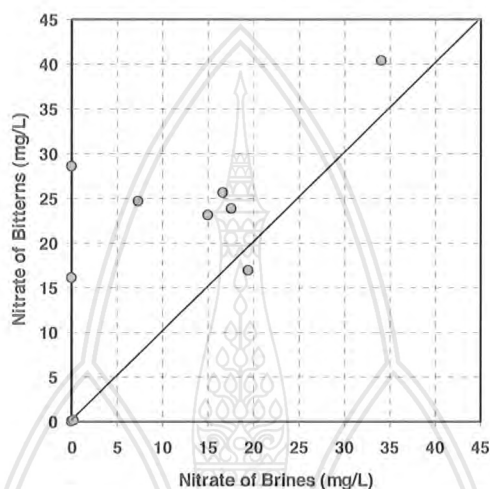


Figure 7. Bitterns related to brines as the NO_3^- -N.

CONCLUSION

Hydrochemical facies of bitterns and brines were generally Na-Cl type. Some areas in Nakhon Ratchasima showed the type of Na-Ca-Cl- HCO_3 . The difference in hydrochemical facies depended on the hydrogeology of the brine aquifers. The Na/Cl ratios of bitterns and brines were similar to the ratios of the pure crystal of halite, which is 0.65. Bitterns were similar to brines in regards to potassium ions and Mg/SO_4 .

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Predicting the Recurring Flood Extent and Damage Assessment in Chi Watershed, Northeast Thailand Using Multi-temporal RADARSAT and Landsat Data

การคาดการณ์พื้นที่ที่ประสบอุทกภัยซ้ำซากและการประเมินความเสียหายในลุ่มน้ำชีภาคตะวันออกเฉียงเหนือ ด้วยข้อมูลจากภาพถ่ายดาวเทียมเรดาร์แซทหลายช่วงเวลา และข้อมูลจากแลนด์ซัท

Charat Mongkolsawat^{1*} and Tussaporn Thanajaturon²

Abstract

With the cloud penetrating capability of RADARSAT, a new geo-informatics-based approach for flood prediction was developed. This approach has the objectives of predicting the recurring flood extent with an analysis using multi-temporal RADARSAT data and the damage assessment. Chi watershed is the study area selected, with an area of about 49,477 sq.km that covers extensively in Northeast Thailand. The study was based on multi-temporal RADARSAT data acquired in 2001, 2002 and 2003. The flood inundation areas of the 3 years were delineated and then digitally encoded in the GIS Databases. The pre-flood land cover was prepared, using Landsat data and co-registered to the same coordinates resulting in a single data set. The overlay analysis was then performed based on the historical flood extent at the combination of the 2001, 2002 and 2003 floods to establish the recurring flood extent. In addition to the overlay operation, performed on the recurring flood layer and land cover, the flood damages in terms of land use and villages affected could be assessed. The land cover classes and villages during the flood event were digitally analyzed. The result obtained provided the basis for an alternative to conventional hydrologic-based flood prediction and damage assessment.

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บทคัดย่อ

ด้วยสมรรถนะของภาพถ่ายจากดาวเทียมเรดาร์แซท (RADARSAT) ที่สามารถถ่ายภาพทะลุทะลวงเมฆได้ ทำให้มีแนวทางทางด้านภูมิสารสนเทศที่ทันสมัยในการคาดการณ์พื้นที่ที่ประสบอุทกภัย ในการศึกษานี้มีวัตถุประสงค์เพื่อคาดการณ์พื้นที่น้ำท่วมซ้ำซากและประเมินความเสียหายจากการใช้ภาพถ่ายจากดาวเทียมเรดาร์แซทหลายช่วงเวลา การดำเนินการในครั้งนี้ได้ใช้ภาพถ่ายจากดาวเทียมเรดาร์แซทในปี พ.ศ. 2544, 2545 และ 2546 จากข้อมูลเหล่านี้ได้ทำการวิเคราะห์ภาพและจัดทำขอบเขตพื้นที่ที่ประสบอุทกภัยทั้ง 3 ปี และได้สร้างฐานข้อมูลเชิงพื้นที่ของพื้นที่ที่ประสบอุทกภัยด้วยระบบสารสนเทศภูมิศาสตร์ในขณะเดียวกันได้จัดทำฐานข้อมูลการใช้ที่ดินจากภาพถ่ายดาวเทียมในพื้นที่เดียวกันในช่วงเวลาก่อนเกิดอุทกภัย และได้ประมวลผลให้อยู่ในพิกัดสอดคล้องกับภาพถ่ายจากดาวเทียมเรดาร์แซท ได้ดำเนินการวิเคราะห์แบบซ้อนของพื้นที่ที่ประสบอุทกภัยทั้ง 3 ช่วงเวลาเพื่อได้มาซึ่งพื้นที่น้ำท่วมซ้ำซาก และได้ประเมินความเสียหายจากพื้นที่น้ำท่วมซ้ำซากจากการวิเคราะห์แบบซ้อนทับพื้นที่น้ำท่วมซ้ำซากกับพื้นที่การใช้ที่ดินผลที่ได้รับได้แสดงพื้นที่การใช้ที่ดินและหมู่บ้านที่มีผลกระทบจากอุทกภัย ข้อมูลที่ได้รับเป็นฐานสำหรับกำหนดทางเลือกเพิ่มจากการคาดการณ์อุทกภัยและความเสียหายที่ใช้ฐานด้านอุทกวิทยา

Keywords: recurring flood, RADARSAT, Chi Watershed

คำสำคัญ: น้ำท่วมซ้ำซาก, เรดาร์แซท, ลุ่มน้ำชี

1. BACKGROUND

Floods are annual events in the Chi watershed, Northeast Thailand and usually occur during the second peak of the rainy season around September and October. It has been well recognized as the most recurring and widespread in the downstream of the watershed. The economic and social impacts of flooding are significant, floods cause crop failures and property damage every year. Most of the flooding found is difficult to prevent, however the associated impacts can be minimized through flood preparation strategies. The strategies require the extent of risk of flooding particularly in the recurring areas of flood. For years, the recurring flood in the Chi watershed was reported in terms of crop damage and communities and villages affected. Several programs of government rescues were launched at the time of flooding but no reliable spatial information of flood was available after the

events. Traditionally, flood prediction has been approached using hydrological models that base preliminarily on ground-based data for inputs. Yet the information available can be obtained as description reports. These require a number of themes and long term data that there exist some difficulties in its acquisition and analysis. So far the government calls for support in assisting the people in the recurring flood areas. There still exists no measure of managing the flood in advance and no spatial information of the recurring flood events available. With the advent of remote sensing technology, the use of SAR imagery has become well developed since the launch of ERS-1 in 1991. The SAR imagery offers additional advantages of cloud penetration capability and day and night data acquisition (Brown et al., 1996). The identification and estimation of the flood extents using SAR data was undertaken with successful results (Chen et al., 1999;

Mongkolsawat et al., 2003; Desnos et al., 1996). Bourgeau-Chavez et al. (2005) developed the methods to derive information on the spatial and temporal changes in hydrologic condition from ERS SAR data collected over southern Florida wetlands. This included flooding and relative water depth. A set of multi-temporal ERS-2 SAR PRI was successfully used to assess the flood extents and the affected paddy field in Mekong River Delta (Vietnam) in 1996 (Nguyen Thanh et al., 2001). As results of the historical flood extent identification the recurring flood areas can be predicted using multi-temporal RADARSAT data. In addition, based on the spatial information obtained, flood damages can be estimated and the decision makers can be used for better preparation for disaster in advance. The compensation for damages should be made based on reliable information, not only by ground investigation methods. The objectives of this study are to explore and predict the repeating pattern of flood areas using multi-temporal RADARSAT data and to assess the flood damages in terms of cropping areas, villages affected and road networks.

2. STUDY AREA

The study area, Chi Basin, is a sub-catchment of Mekong watershed and covers an area of about 9,477 sq.km (Figure 1). The Chi River drained eastwards to the Mun River which finally flows into the Mekong at the Thai-Laos border in

the east. Average annual rainfall varies from 1,000 mm to 1,500 mm and is higher in the west. The rainfall is unevenly distributed during the rainy season (May to October), with over 60 % occurring during August and September. The elevation of the basin ranges from 1,100 m in the west to 100 m in the east. Physiographically, the main area is formed by the so called Korat Plateau and characterized by small hills for the high land in the west and gently undulating alluvial plains for the lower land in the east.

Geologically, the area is mainly underlain by the thick sequences of Mesozoic rocks of Maha Sarakham Formations which consist of sandstone, siltstone and interbedded rock salts. (Department of Mineral Resource, 1982). The remaining forest in the area, mainly dipterocarp forest type, is found in the upper stream in the west and subsists about 15 % of the area. The majority of soil is inherently low in fertility and is salinized in lower terrace of the area where there exists rock salts. Most soil types have a relatively narrow range in landform, a given soil is generally recognized in a definite land form. (Scholten et al., 1973) The main land forms in the Northeast are predominantly determined by the Mekong river and its tributaries. (Moormann et al., 1964).

Figure 2 illustrates the flood event and the area affected in 2001.



Figure 1. The study area.



Figure 2. Flood event in 2001.

3. METHODOLOGY

The study of predicting the recurring flood areas and flood damage assessment in the Chi watershed is based on the flood events in 2001, 2002 and 2003 in together with the land cover using Landsat TM acquired in 2002.

Multi- temporal RADARSAT data (C-band, W1-mode) acquired in 2001, 2002 and 2003 as described in Table 1.

Topographic maps of the Royal Thai Survey Department at the scale 1: 50,000 which were used for geo-referencing.

LANDSAT TM data acquired in 2002 used for establishing land use.

3.1 Data Sources

The flood detection in the Chi watershed was derived from the following sources:

Table 1. Scenes of RADARSAT data used in the study.

Imagery scene	Imagery scene	Center (Lat/ Long)	Acquisition date	Acquisition time
	450016	15:32:32.30N/ 104:32:16.80E	20- Sep- 01	11:05:37
	450017	16:49:32.50N/ 104:15:28.80E	20- Sep- 01	11:04:59
	450018	14:47:42.70N/ 104:42:00.00E	20- Sep- 01	11:04:24
	450108	15:14:25.80N/ 102:54:57.60E	14- Sep- 01	23:06:37
	450206	16:24:51.80N/ 103:18:14.45E	13- Sep- 01	11:09:05
	450264	15:06:27.88N/ 103:34:49.17E	08- Sep- 02	11:08:25
	450265	16:25:22.44N/ 103:17:38.33E	08- Sep- 02	11:08:47
	450269	17:52:59.19N/ 102:31:36.74E	09- Sep- 02	23:05:59
	460001	16:14:19.31N/ 104:29:27.40E	06- Sep- 02	22:53:40
	450270A	15:18:03.27N/ 101:58:20.05E	09- Sep- 02	23:06:43
	47140	16:04:37.75N/ 101:17:22.64E	13- Sep- 03	11:16:29
	47141	17:24:36.99N/ 100:59:53.88E	13- Sep- 03	11:16:51
	47142	15:21:14.39N/ 103:12:20.59E	20- Sep- 03	11:12:19
	47143	17:58:13.38N/ 102:38:38.39E	20- Sep- 03	11:13:03
	47147	17:44:25.81N/ 103:45:01.25E	27- Sep- 03	11:08:47

3.2 Pre-processing of data

The RADARSAT data was digitally performed to provide improved data for visual interpretation and subsequent analysis. The geometric correction of the images (2001) was performed by registering the images to the topographic map (1:50,000). The Nearest Neighbor resampling was applied. The images of 2002 and 2003 were digitally co-registered to those of 2001 by referencing image to image. The individual image scenes were then mosaiced to cover more than the study area and subsequently defining a window that corresponded the watershed area of interest.

3.3 Recurring flood areas

The overlay analysis was then performed using GIS with criteria based on the historical flood extent of the combination of the 2001, 2002 and 2003 floods (Figure 3). Flood patterns in the terms of spatial extent vary year by year depending upon the amount and duration of rainfall. The recurring areas of floods were recognized and recorded as a result of rainfall and overflow of the rising water. The areas vulnerable to 3 years, 2 years and 1 year floods are defined as the results of its combinations. The 3, 2 and 1 year floods were defined as very high, high and moderate risk areas respectively.

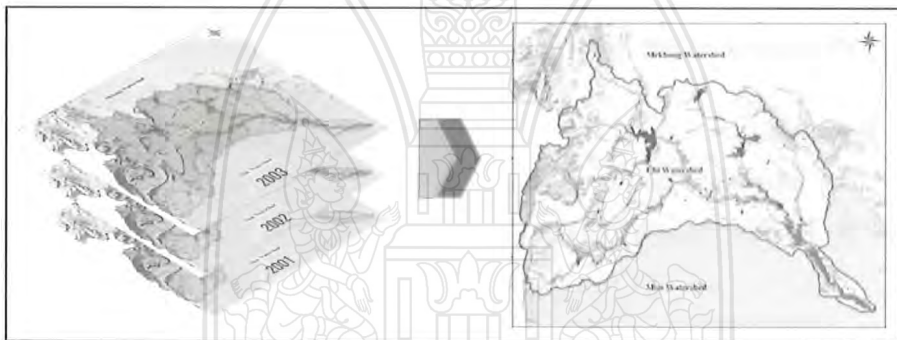


Figure 3. The process of establishing the recurring flood areas.

3.4 The recurring flood area and land use

The land use maps of the Chi were prepared at the 1:50,000 scale using the series of Landsat TM. The maps were digitally encoded in GIS database with geo-reference to UTM projection. The overlay process of the flood

recurring area and land use was digitally performed with a selection criteria of the recurring flood areas over the different types of land use (Figure 4). As a result of this task, the different types of land use under the recurring flood area were identified including village sites and transportation network.

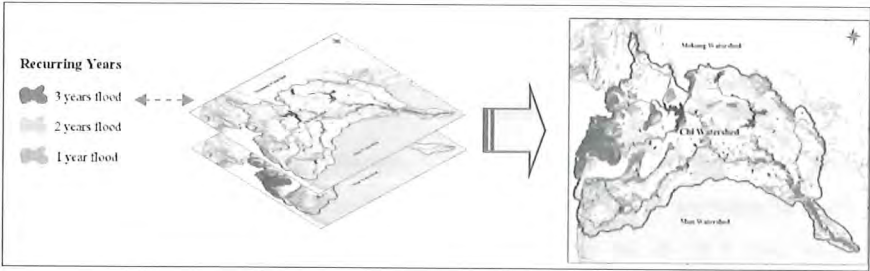


Figure 4. Overlay of the recurring flood area on land use.

4. RESULTS AND DISCUSSION

4.1 The flood extent

The series of flooding extent in 2001, 2002 and 2003 as detected by RADARSAT were illustrated in Figure 5. The areas of inundation for

the 2001, 2002 and 2003 were presented in Table 2. It was found that the intensity of flood in 2002 was the highest and covered an area of about 2,756.51 sq km. or 5.71 % of the total area of the Chi watershed.

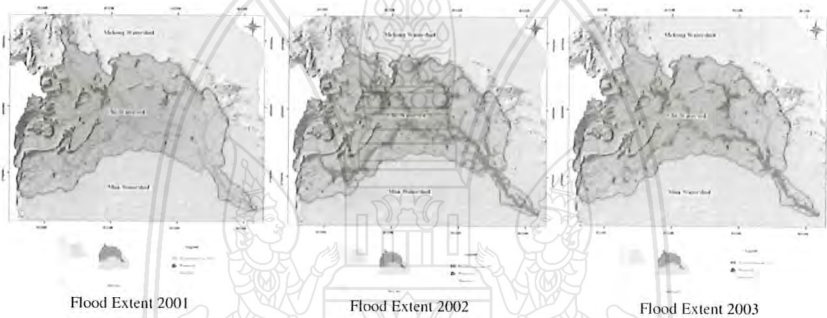


Figure 5. The flood extent of the Chi watershed

Table 2. Flood areas in the Chi basin.

Year	Flood area (km ²)	Percent
2001	2,158.43	4.47
2002	2,756.51	5.71
2003	1,149.10	2.38

Total area = 49,477 Sq km.

Table 3. The recurring flood areas.

Class	Area	
	sq km.	Percentage
3 years flood (Very high risk area)	752.05	1.52
2 years flood (High risk area)	831.21	1.68
1 years flood (Moderate risk area)	23,25.42	4.70
Non flood (Low risk area)	45,568.32	92.10

4.2 The recurring flood area

The recurring flood areas for 3 years record were presented in Table 3. The spatial distribution of the recurring flood was shown in Figure 5. The 3, 2 and 1 years of recurring floods cover areas of about 1.5, 1.68 and 4.70 % of the total area respectively. The recurring flood areas for the 3

years extended along low-lying plains adjacent to the river bank mostly in the downstream areas connecting to the Mun river. Figure 6 provided the spatial patterns of the recurring floods based on the historical flood extent of the combination of the 2001, 2002 and 2003 floods.

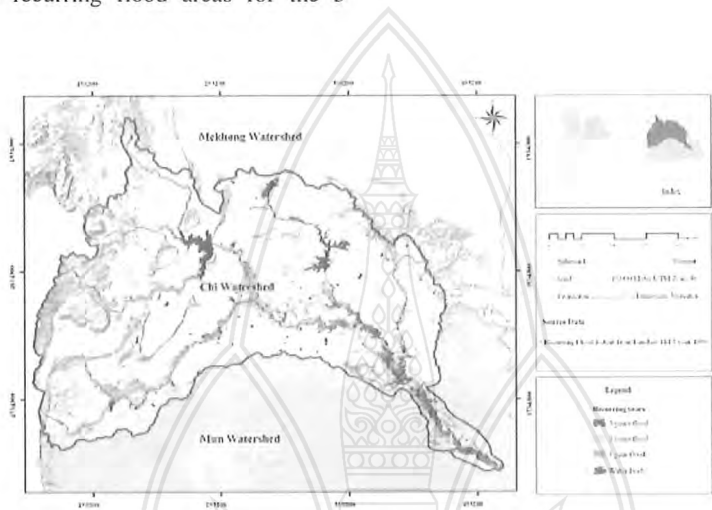


Figure 6. The Recurring flood extent.

4.3 The recurring flood extent and land use

The analysis of land use in relation to the recurring flood areas was presented in Figure 7 and 8. The vulnerable flood areas were restricted to paddy fields and cover areas of about 1.26, 1.39 and 3.93 % for 3, 2 and 1 years of the recurring flood respectively. The analysis of land use in relation to the recurring flood areas was presented in Figure 8. The very high risk areas were found in the downstream of the watershed and in the flood plains and low terraces which were mostly restricted to paddy fields and riparian vegetation. The flood risk areas are illustrated in Table 3. The areas of 752.05 sq.km (1.52% of the total area) of rice crop

were severely damaged and at very high risk for the flood event in the watershed. These areas were increased to 831.21 sq.km for the high risk or in case of increasing rainfall. Estimation of rice crop damage within the risk area, based on the minimum cost for paddy fields, may reach 8,000 - 10,000 million baht for the affected areas of about 1,500 sq.km. As the results of the damage, the budget allocation for protection in advance should be suggested. This is to minimize the damage which may result from the poor drainage system within the areas due to sediment deposits, flow obstruction of infrastructure construction and human activities, misuse of land and etc.

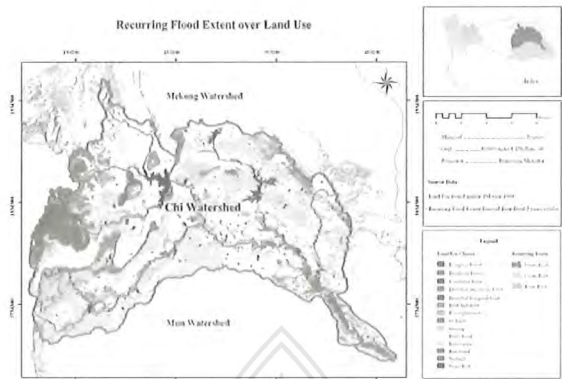


Figure 7. The Recurring flood extent and Land use.

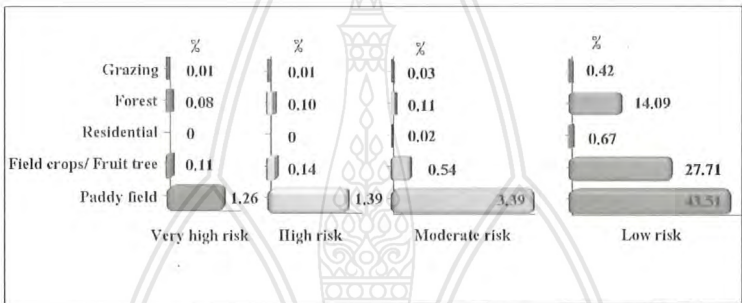


Figure 8. The Land use types as affected by the flood risks

4.4 Villages affected

The model predicted that the villages affected in the watershed will be 495, 575 and 128 villages for very high, high and moderate risk of the recurring floods respectively. The very high risk covers mainly in downstream areas as shown in Figure 9. Table 4 and 5 represents the number of

villages affected in each province within the Chi. This study also provides an overview of the village distribution resulting from integration of the 3 historical floods and the village locations. The village names within the administrative boundaries and their locations were restored in the database and could be easily retrieved.

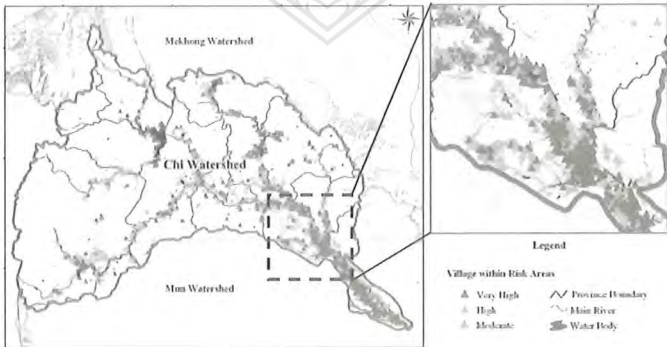


Figure 9. Villages affected within the risk areas.

Table 4. The number of villages affected in each province.

Province	No. of Affected Villages		
	Very High Risk	High Risk	Moderate Risk
Kalasin	111	104	5
Khon Kaen	38	79	32
Chiyaphum	22	67	33
Nakhon Ratchasima	3	10	1
Maha Sarakham	38	38	2
Yasothon	42	16	23
Roi Et	144	153	25
Si Sa Ket	4	3	-
Nong Bua Lamphu	29	34	2
Udon Thani	44	50	3
Ubon Ratchathani	20	21	2
Total	495	575	128

Table 5. Flood and transportation network (number of district affected).

Province	No, road affected
Kalasin	5
Khon Kaen	1
Chaiyaphum	2
Mahasarakam	3
Yasothon	3
Roi-Et	2
Nongbualamphu	1
Udonthani	1

4.5 Roads and flood extent

The map represents the flood areas and transportation networks by province within the watershed. The roads affected by floods were mostly located in Roi-Et, Kalasin, Khon Kaen and Chaiyaphum (Figure 10). The number of roads in the districts affected could be reported from the GIS layers established. With the information established, decision makers could identify the roads affected in relation to the severity of the floods.

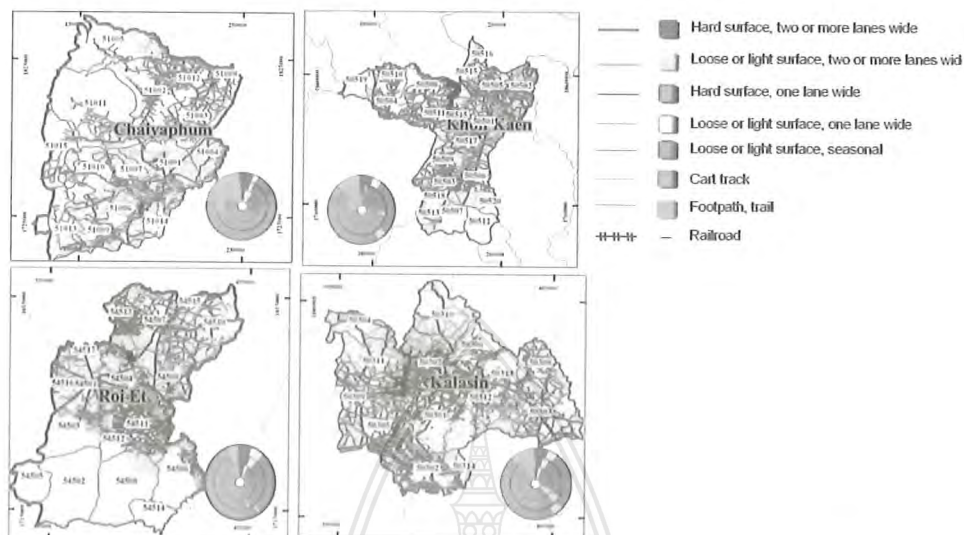


Figure 10. Transportation networks and flood.

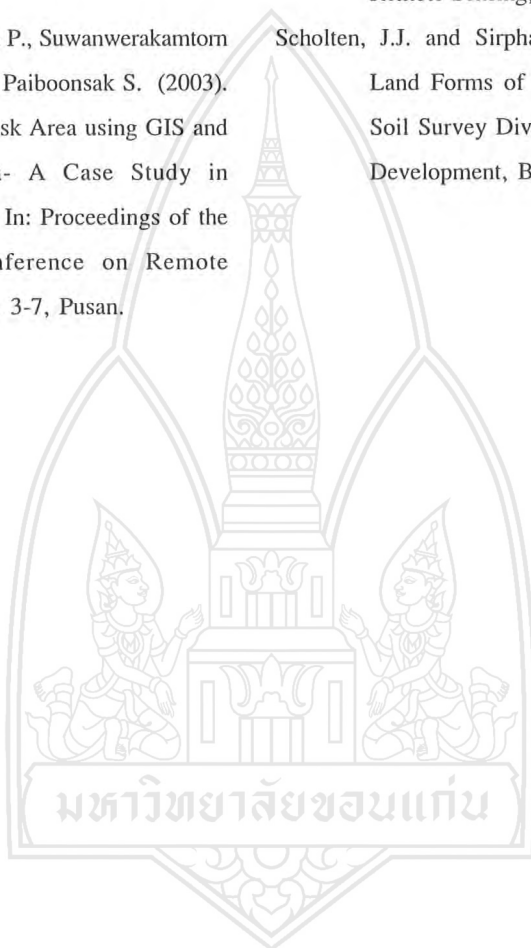
5. CONCLUSIONS

In conclusion, the results obtained by the prediction of the recurring flood areas can support the strategy plans for regional development. The information offers not only locational areas of flooding but also their related quantitative data. The government enables allocation of budgets at the right place for protection in advance. There are still some limitations in predicting the duration of floods in relation to the duration and amount of rainfall. However, this study greatly enhances the method of rapidly accessing information by providing the areas at risk in advance in case of no real time satellite data. The GIS-based-information established can help support the flood mitigation and the synthesis of flood scenarios in the future if necessary.

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The Multi-agent-based Model for Community Forest Management

แบบจำลองสำหรับการจัดการป่าชุมชนโดยใช้หลายเอเจนต์

Thanawat Thongtan¹ and Somjit Arch-Int^{2*}

Abstract

Community forests are essential to the local population of Thailand. The problem is that a community forest is an ecosystem with much complexity involving many for stakeholders; moreover, stakeholders are in conflict over community forest usage. The guide for solving the problem uses multi-agent system (MAS) for community forest model construction in order to simulate the dynamics of community forest resources caused by villager behavior using the community forest resources in the time period. The stakeholders can address and solve the problems; in addition, they could collaboratively manage community forest for sustainable yields. The results of the Khaonoi-napang community forest case study, Khon Kaen province, showed that there were 68,888 trees, and the tree birth rate was 0.06 (trees/year), the tree dead rate was 0.03 (trees/year), and the illegal tree cutting rate was 0.59 (trees/person/year). The simulation results showed that the tree cutting rate, which will generate a sustainable community forest in the future, was less than 7.47 (trees/person/year).

บทคัดย่อ

ป่าชุมชน มีความสำคัญต่อประชากรในชนบทของประเทศไทย เพราะเป็นระบบนิเวศชนิดหนึ่งที่มีความสลับซับซ้อน เกี่ยวข้องกับผู้มีส่วนได้เสียจำนวนมาก และเกิดความขัดแย้งกันในการใช้ประโยชน์ป่าชุมชน แนวทางในการแก้ไขปัญหาแบบหนึ่ง คือ ใช้ทฤษฎีแบบหลายเอเจนต์สำหรับการสร้างแบบจำลองสำหรับการจัดการเพื่อที่จะจำลองสถานการณ์การเปลี่ยนแปลงของทรัพยากรป่าชุมชน ซึ่งมีสาเหตุมาจากพฤติกรรมของชาวบ้านในการใช้ประโยชน์ทรัพยากรในช่วงระยะเวลาต่างๆ ผู้มีส่วนได้เสียสามารถทำความเข้าใจและแก้ไขปัญหา ตลอดจนร่วมมือกันในการจัดการป่าชุมชนให้ได้ผลผลิตที่ยั่งยืนต่อไป ผลจากการทดลองกรณีศึกษาป่าชุมชนบ้านเขาน้อย

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นาแพ่ง อำเภอกุเวียง จังหวัดขอนแก่น พบว่ามีจำนวนต้นไม้ทั้งหมด 68,888 ต้น โดยอัตราการเกิดของต้นไม้เท่ากับ 0.06 ต้นต่อปี อัตราการตายของต้นไม้เท่ากับ 0.03 ต้นต่อปี และอัตราการตัดต้นไม้แบบผิดกฎหมายของชาวบ้านเท่ากับ 0.59 ต้นต่อคนต่อปี ผลการจำลองสถานการณ์พบว่า อัตราการตัดต้นไม้ของชาวบ้านที่จะทำให้ป่าชุมชนที่ยั่งยืนในอนาคตจะเท่ากับหรือน้อยกว่า 7.47 ต้นต่อคนต่อปี

Keywords: Community forest, Multi-agent system

คำสำคัญ: ป่าชุมชน, ระบบหลายเอเจนต์

1. INTRODUCTION

The community forests effects the local villagers in the country of Thailand, supplying the four basic needs of villagers: housing, foods, cloths, and herbs. They are a part of the way of life and customs of the villagers. There are two problems in the community forest management. First, the community forests are ecosystems with much complexity involving many stakeholders. Second, stakeholders have conflicts over forest usage, with each having a different aspect of forest usage. There are two problem solving solutions. The first solution is the analysis and design of a multi-agent-based model for community forest management. The second solution is the simulation of community forest resource dynamics caused by individual behaviors of stakeholders in forest resource usage. The result of this research is a suitable multi-agent-based model for community forest management. The simulation result can be taken to the stakeholders for discussion in workshops. As a result, the forest offices and other stakeholders can conduct a sustainable community forest management plan for future operation and guiding. A sustainable community forest management plan for guiding and usage of the forest could be done by forest office and other stakeholders. The research used a case study at

Khaonoi-napang community forest, Puwieng district, Khon Kaen province.

This paper will discuss the following topics: the body will dissert theory and methodology while the conclusion will include results analysis and recommendations.

2. THEORY AND RELATED WORK

2.1 Theory

The use of this methodology and the associated tools accompanies the shifts in various paradigms on the study of ecological complexity. Behavior and interactions are now key issues for understanding and modeling ecosystem organization. The models can then be used in constructivist ways. Multi-agent simulation (MAS) is introduced conceptually and is compared with individual-based modeling approaches. Various architectures of agents are presented, the role of the environment is emphasized, and some computer tools are presented.

A discussion follows on the use of MAS for ecosystem management. The strength of MAS has been discussed for social sciences and for spatial issues such as land-use change. The research argues here that MAS is useful for problems involving the integration of integrating social and spatial aspects. The research then discusses how

MAS can be used for several purposes, from theorization to collective decision-making support. The research proposes some research perspectives on individual decision making processes, institutions, scales, the credibility of models, and the use of MAS. In the conclusion, the paper argues that researchers in the field of ecosystem management can use multi-agent systems to go beyond the role of the individual and to study more deeply and more effectively the different forms of organization (spatial, networks, hierarchies) and interactions among different organizational levels. For that objective there is considerably more fruit to be had on the tree of collaboration between social, ecological, and computer scientists than has so far been harvested (Bousquet, 2003).

2.2 The related work

A successful sustainable development strategy requires that forest management be carried out in a participatory way, which includes the involvement of local communities. The importance of community participation has been written into Indonesian Law No. 41 on Forestry (1999). However, the implementation of this law in areas already allocated to a concession holder is still unclear. The state-owned company, Inhutani II Sub Unit Malinau, has managed a forest area in Malinau District, East Kalimantan, for over 10 years. Forest-dependent communities located in the managed area are Long Seturan, Long Loreh and Langap villages. The company managed the area based on plans approved by the local and central government, which established permanent sample plots for measuring the stand growth and yield data in their area, and demanded the improvement and well-being of local

communities. However, the schemes did not give the company sufficient space to manage the area creatively, or provide a systematic way to involve the communities in the management of the forest.

This research is aimed at seeking scenarios of sustainable forest management (SFM) that addressed the above limitations. To reach this aim, two research hypotheses were proposed:

(1) Local forest stakeholders can define their own SFM Criteria and Indicators (C&I) for specific sites where they live, or which concern them;

(2) Collaborative management of forest by all relevant stakeholders will achieve better forest management outcomes.

The research results showed that local communities that lived in the area of Inhutani II were able to define C&I of SFM. The local C&I are not different from the generic or scientific C&I of SFM except that these C&I are formulated with different structures and argumentations. Collaboration between concessionaires and communities appeared to be the most suitable alternative for SFM, particularly for improving communities' incomes without decreasing the quality of the forest. An appropriate decentralization policy is a condition for implementing a collaborative forest management (Purnomo, 2003).

The participatory approach to development in general, and natural resource management in particular, is now a widely accepted management strategy. Multi-agent system (MAS), a computer-based tool, offers a promising approach for multi-stakeholder management systems such as the case involving community-managed resources. A MAS provides a framework where stakeholders (or agents)

individual actions, behaviors, and rational decisions can be analyzed in the context of other stakeholders' actions and decisions. This robust approach offers a convenient analytical framework that can be used to simulate agents' actions, reactions, and interactions. The approach also provides an environment where strategies or multi-stakeholder forest management scenarios can be developed and analyzed. This paper describes a MAS model developed for a forest management unit located in East Kalimantan, Indonesia. Results and experience gained from the case study suggest that MAS is a suitable approach for developing multi-stakeholder forest management strategies (Purnomo et al., 2005)

3. METHODOLOGY

This research used the system development methodology for analysis of community forest system. The framework of system development process management is called the systems development life cycle (SDLC), which is a project management framework organized into phases and activities. The research used the object-oriented approach. The systems development life cycle (SDLC) consists of 4 phases.

(1) Project Planning Phase

The objective of the phase is to identify the scope of the community forest system and define the problem activities.

(2) Analysis Phase

The objective of the analysis phase is to understand and document the system needs, and the

processing requirements of the new system. Analysis is essentially a discovery process. The key words that drive the activities during analysis are discovery and understanding. The activities are the field survey and modeling which are the conceptual model, event table, object analysis, domain class diagram, use case model, system sequence diagram, and sequence diagram.

(3) Design Phase

The objective of the design phase is to design a solution system based on the requirements defined, and decisions made during analysis. The activities are design class diagram, and package diagram.

(4) Implementation Phase

The final system was built, tested, and installed. The objectives of the activities of this phase are to produce a reliable, fully functional information system. The activities are programming, simulations, and model testing.

In this systems development life cycle, the approach applies the notion of iteration across phases. Iteration means that work activities are done once, then again, and yet again (they are repeated). With each iteration, the results are refined so that it is closer to what is needed. Iteration assumes that no one gets the right result the first time. System development process is done incrementally and iteratively in order to accomplish its goals (see Figure 1). More analysis and design are then needed to make improvements.

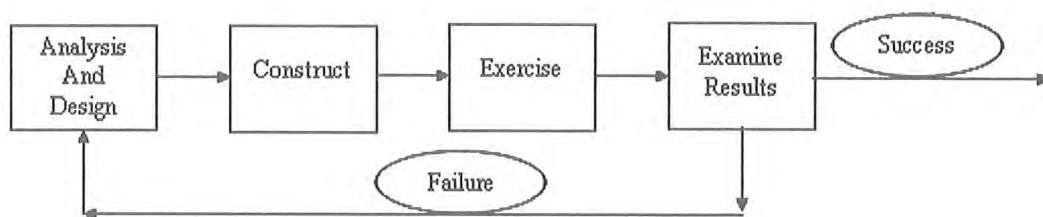


Figure 1. Model development process.

3.1 Defining the problem

In this activity, the community forest problems are defined by interviewing village leaders and other stakeholders, such as a head of village, head of a group of villages (Kamnan), head of Tambon administration organization (TAO), members of TAO, monks, forest officers, and representative of villagers. The objective of this activity is to gather primary data at the top level in order to identify the problems and to set the hypothesis of the research.

3.1.1 The interviews of stakeholders involving with community forest

The stakeholders of the Khaonoinapang community forest were interviewed, these include the village heads, head of a group of villages (Kamnan), head of Tambon administration organization (TAO), members of TAO, monks, forest officers, and representative of villagers. Two approaches were defined from the gathered data.

(1) The first problem is the illegal tree cutting by some villagers due to demand for timber used for household needs.

(2) The second problem is a conflict of interest in uses of community forest resource by stakeholders. In the history of this community forest, the leaders of the community and

the community forest committees set strict rules for the use of forest resource by prohibiting the cutting of tree unless the tree has death. This prohibition was practiced since 1994 until now. There are currently two different interests in forest use. Some stakeholders want to permit villagers cut trees for timber for basic household use. Other stakeholders don't want to permit the cutting of trees because they need to conserve the forest for sustainable future.

3.1.2 Hypotheses

After defining the problems, a hypothesis was determined: "Villagers and community forest could coexist sustainably if villagers do not cut down trees at a rate that exceeds forest productivity."

3.2 Field Survey

Primary data and secondary data are investigated and collected.

3.2.1 Secondary data collection

Secondary data from Tombon Administration Organization, office of community forest committees, office of head of village, office of Kamnan (head of a group of villages), office of forest officer and office of Puwieng district are collected.

3.2.2 Primary data collection

Primary data via in depth interviewing of stakeholders and collecting some community forest resources are gathered.

- (1) Forest officers, community forest committees, and monks for their roles, duties, and activities are interviewed; in addition, the representatives of villagers for their fundamental data and community forest resource use data are collected.
- (2) Community forest resource data by a forest resource inventory method are collected in an area of 87.20 hectares. Data collection included trees, saplings, and undergrowths, was 25 sample plots each plot is 1,000 square meters.

3.3 Modeling

The data gathered from the field about villager activities of forest use, forest resources, and other stakeholder data will be analyzed.

3.3.1 The conceptual model

A conceptual model of Khaonoi-napang community forest system was set. The conceptual model shows the stakeholders and resources of the community forest in its current situation and the indicators observed (see Figure 2).

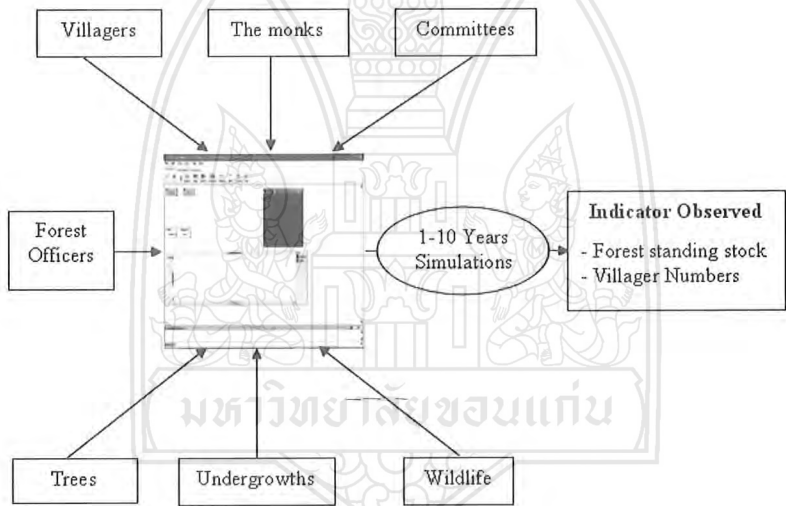


Figure 2. Conceptual model.

3.3.2 Event table

The event table was set from data collected in the field survey as shown in Table 1. There are 7 events of villager activities: tree cutting

needs, ant egg needs, planting tree needs, patrolling needs, wildlife hunting needs, livestock feeding needs, and Pakwan collecting needs.

Table 1. The event table of Khaonoi-napang community forest system.

Koanoi-napang Community Forest Event Table					
Event	Trigger	Source	Activity/ Use Case	Response	Destination
1.Villagers want timbers for using.	Villagers cut the trees in the forest.	Villagers	Cut Tree	Amount of Timbers	Villagers
2.Villagers want ant egg for food.	Villagers collect ant egg in the forest.	Villagers	Collect Ant Egg	Amount of Collected Ant Egg	Villagers
3.Monks want plant trees in the forest.	Monks plants trees in the forest.	Monks	Plant Trees	Amount of planted Trees	Forest
4.Committees want to patrol the forest.	Committees patrol the forest.	Committees	Patrol	Frequency of Patrols	Committees
5.Villagers want to hunt wildlife for food.	Villagers hunt wildlife in the forest.	Villagers	Hunt Wildlife	Amount of hunted wildlife	Villagers
6.Villagers want to feed livestock in the forest.	Villagers feed livestock in the forest.	Villagers	Feed Livestock	Amount of Livestock	Villagers
7.Villagers want to collect Pakwan for food.	Villagers collect Pakwan in the forest.	Villager	Collect Pakwan	Amount of Collected Pakwan	Villagers

3.3.3 Things analysis

system or that come up in discussions with

In the object-oriented approach, these stakeholders. In case study of Khaonoi-napang things are the objects that interact in the system. community forest system, there are 12 things: Types of things, as with events, an analyst should villagers, monks, forest committees, forest officers, ask the users to discuss the types of things that they community forest, trees, undergrowths, animals, work with routinely. A useful procedure to follow is wildlife, livestock, land and land cover.

to begin by listing all of the nouns that users mention when talking about the system. Consider 3.3.4 Domain Class Diagram In this activity, things or special the events, the activities or use cases, the external objects in real world of Khaonoi-napang community forest system are analyzed. The result of the from the event table as potential things, for example. Then add to the list any additional nouns analysis is a domain class diagram as shown in Figure 3.

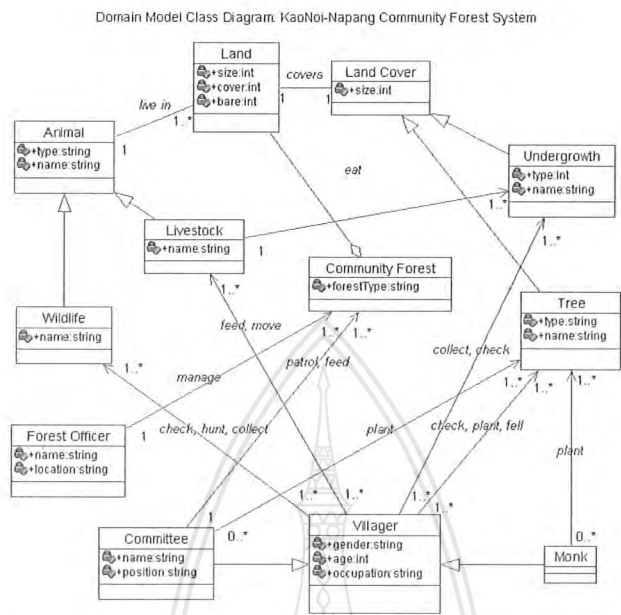


Figure 3. Problem Domain Model (Domain Class Diagram).

3.3.5 Use Case Diagram

From the event table, use case diagram was conducted, which includes 7 use cases such as patrolCommunityForest, plantTree, fellTree, huntWildlife, feedLivestock, collectAntEgg, collectPakwaan (see Figure 4).

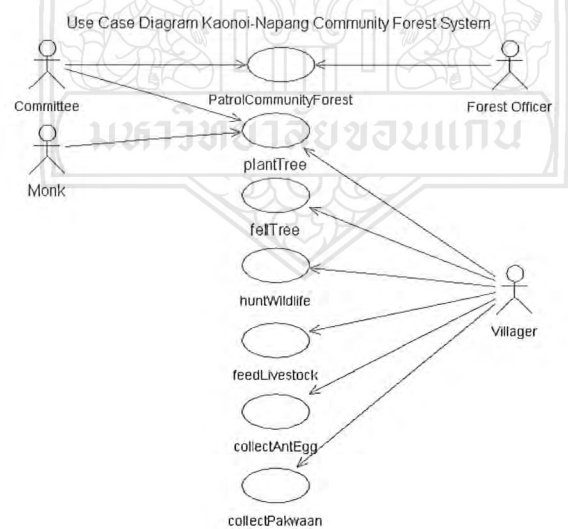


Figure 4. Use Case Diagram in community forest system.

3.3.6 Use Case Description

Every use case is then described in more details as a use case description or a use case scenario as shown in Figure 5.

Use Case Name:	Fell Tree	
Senario:	Fell trees in the community forest'	
Triggering Event:	Villagers fell trees in the community forest.	
Brief Description:	Villagers go to community forset, survey tree types and need sizes, fell trees, take timbers to their home.	
Artoris:	Villagers	
Related Use Case:		
Stakeholders:		
Precondition:		
Postcondition:		
Flow of Events	Actor	System
	1. Villagers go to forest.	
	2. Villagers survey tree types sizes, quantities.	2. Show types, sizes, amount of trees.
	3. Villagers fell trees.	
	4. Villagers take timbers to their house.	4. Show amount of timbers.
Exception		
Condition:		

Figure 5. Use Case Description of tree cutting activity in community forest system.

3.3.7 System Sequence Diagram

From the use case descriptions system sequence diagrams were created (see Figure 6).



System Sequence Diagram: Fell tree

Figure 6. System Sequence Diagram of tree cutting activity in community forest system.

3.3.10 Package Diagram

From the design class diagram, a package diagram was built in order to illustrate the connection among classes (see Figure 9).

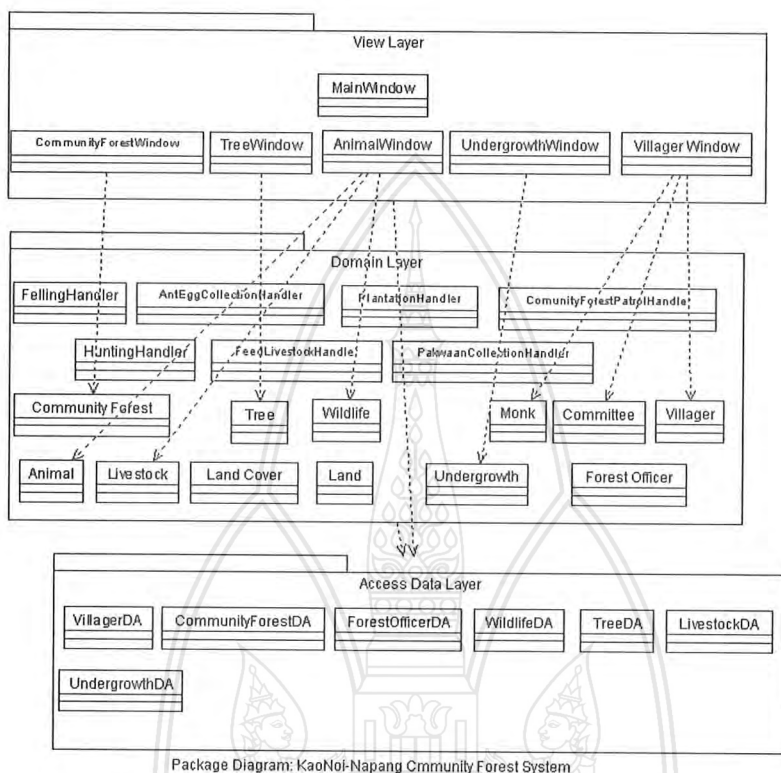


Figure 9. Package Diagram in community forest system.

3.3.11 The critical event selection

In this research, because of limited time not all 7 use cases were implemented. Therefore, the research selected the tree cutting event, which is the most influential for a sustainable community forest. Because of logging in the past 13 years, Khaonoi-napang community forest has been destroyed and resources such as wildlife and herbs decreased. Since then committees of the community forest have been prohibiting logging until the present. As a result, the community forest has regrowth and other resources have been increasing.

3.3.12 Programming

At this stage, NetLogo by The NetLogo System Dynamics Modeler was used for coding and simulating forest resource change caused by villager behavior in forest resource use.

NetLogo is a multi-agent programming language and modeling environment for simulating complex phenomena. It is designed for both research and education and is used across a wide range of disciplines and education levels. It is particularly well suited for modeling complex systems evolving over time. Modelers can give

instructions to hundreds of independent “agents” all operating concurrently, in order to explore connections between micro-level behaviors of individuals and macro-level patterns that emerge from their interactions (NetLogo, 2006)

The NetLogo System Dynamics Modeler

System Dynamics is a modeling method used to understand how things relate to one another. With the agent-based approach usually used in NetLogo, the behavior of individual agents is programmed into the model and the result of the examined interactions among agents. With the system Dynamics Modeler, the behavior of individual agents is not programmed. Instead, how populations of agents behave as a whole is programmed. The System Dynamics Modeler allows developers to draw a diagram that defines these populations, or “stocks”, and how they affect each other. The Modeler reads developed diagram and generates the appropriate NetLogo code-global variables, procedures and reports-to run the System Dynamics model inside of NetLogo.

Basic Concepts of the NetLogo System Dynamics Modeler

A System Dynamics diagram is made of four kinds of elements: Stocks, Variables, Flows and Links.

A Stock is a collection of things, an aggregate. For example, a Stock can represent a population of sheep, the water in a lake, or the number of widgets in a factory.

A Flow brings things into, or out of a Stock. Flows are like pipes with a faucet because the faucet controls how much stuff passes through the pipe.

A Variable is a value used in the diagram. It can be an equation that depends on other Variables, or it can be a constant.

The Aggregate Model Programming

In the System Dynamic Modeler Diagram Window, the parameters involving the tree cutting activity in the button of Diagram consist of new tree rate, tree death rate, tree cutting rate, tree amount, villager birth rate, villager death rate and villager amount are chosen to be parameters of the model.

Tree and villager equations

In the tree cutting done by villagers, tree and villager equations with the system dynamics method is constructed.

Tree equation

$$Y_t = Y_1 + r_n Y_1 - (r_d Y_1 + r_c X_1)$$

Where

- Y_t is the amount of trees in the time period t years.
- Y_1 is the amount of trees in the first year, 68,888 trees.
- X_1 is the amount of villagers in the first year, 1,198 villagers.
- r_n is the new tree rate, 0.16.
- r_d is the death tree rate, 0.03.
- r_c is the tree cutting rate of villagers, 0.59, 0, 7.0 and 7.47 trees/person/year.

Villager equation

$$X_t = X_1 + r_b X_1 - r_d X_1$$

Where

- X_t is the amount of villagers in the time period t years.
- X_1 is the amount of villagers in the first year, 1,198 villagers.
- r_b is the villager birth rate, 0.03.
- r_d is the villager death rate 0.01.

The model can be represented as a diagram using NetLogo System Dynamic Modeler Diagram Window (see Figure 10).

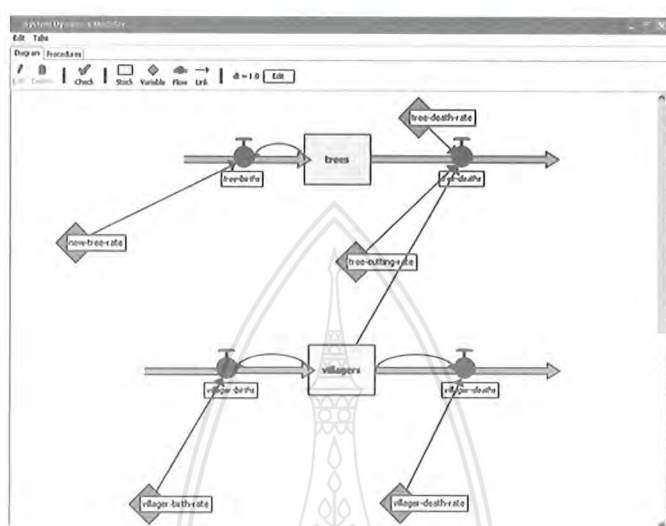


Figure 10. System Dynamic Modeler Diagram Window.

3.4 Simulations

In this step, a plot is built in NetLogo to create a graph diagram for presenting the variations of trees and villagers uses the time of a year, and a monitor is built to show the variation of trees and villagers. From the results of the simulations can be the following concluded.

(1) If tree cutting rate is 0.59 trees/person/ year, the number of trees in the next 10 years will be 151,368, and the number of villagers will be 1,438.

(2) If tree cutting rate is 0 trees/person/ year, the number of trees in the next 10 years will be 158,438, and the number of villagers will be 1,438.

(3) If tree cutting rate is 6.0 trees/person /year, the number of trees in the next 10 years will be 86,558, and the number of villagers will be 1,438.

(4) If tree cutting rate is 7.47 trees/person/ year, the number of trees in the next 10 years will be 68,947, and the number of villagers will be 1,438. This tree cutting rate is a balance between the number of new trees added and number of dead trees and trees cut by villagers. This balancing line is beginning of sustainable community forest as shown in Figure 11.

(5) If the tree cutting rate is 15 trees/person /year, the number of trees in the next 7 years will be 5,783, and the number of villagers will be 1,366.

Simulation results

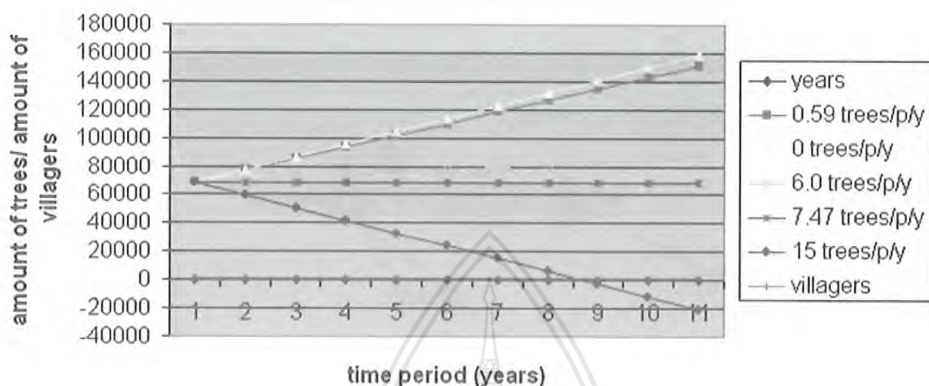


Figure 11. Simulation results.

3.5 Model testing

The model was used for simulating the trees of Khaonoi-napang community forest. The change in number of forest caused by the individual tree cutting rates of 0.59, 0, 6.0, 7.47, and 15 trees/person/ year. The simulation showed that the number of trees in the next 10 years will be 151,368, 158,438, 86,558, 68,947, and 5,783 (in the next 7 years). These results are the expected numbers of the tree dynamics in the forestry of Dry Dipterocarp forest which has an area of 87.20 hectares.

The model was tested by putting a large number of parameters: 1,000,000 trees; and 100,000 villagers. The time periods of the simulation were normal.

4. RESULTS

The results of the research is a suitable multi-agent-based model for community forest management and community forest resource usage simulations in the case of tree cutting activity of villagers.

Hypotheses testing

In this activity, the hypothesis will be the hypothesis testing "Villagers and community forest can coexist sustainable at a rate which exceeds if villagers do not cut trees forest productivity."

The hypothesis has been tested by simulations as shown in Figure 11. In conclusion, if villagers cut down trees at a rate of less than 7.47 trees/person/year, the villagers and community forest can coexist sustainably.

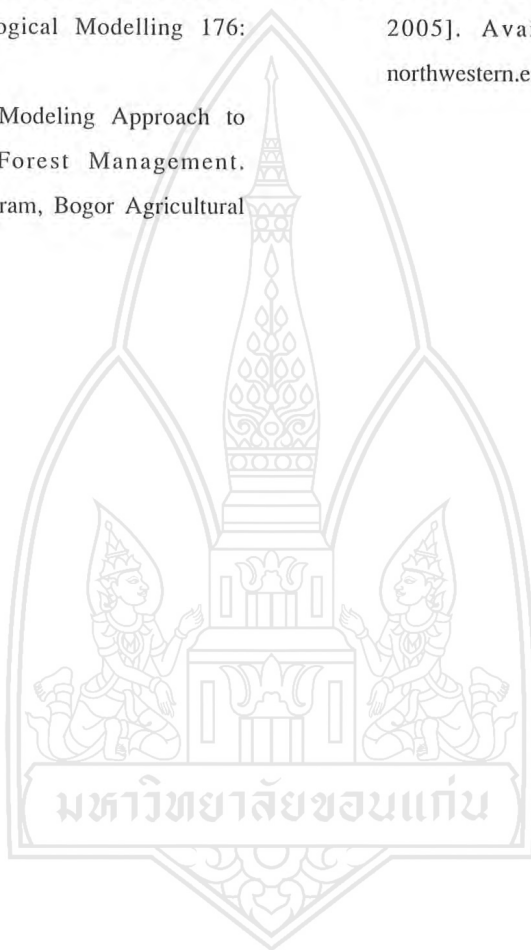
5. CONCLUSIONS AND RECOMMENDATIONS

In this research, the suitable multi-agent-based model for community forest management and community forest resource usage simulations in the case of tree cutting activity of villagers was obtained. The simulation results can be used in workshops for Khaonoi-napang community forest stakeholders in order to make a collaborative community forest management plan. For future work, other activities such as patrolling community forest,

planting tree, hunting wildlife, feeding livestock, collecting ant egg, collecting Pakwaan can be researched.

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Forecasting Models for Monthly Patient Movements in Admissions and Discharges of Rajavithi Hospital

ตัวแบบการพยากรณ์การเคลื่อนไหวการรับและการจำหน่าย ผู้ป่วยรายเดือนของโรงพยาบาลราชวิถี

Faungludda Sopa^{1*} and Lily Ingsrisawang²

Abstract

The objective of this study was to find the appropriate forecasting model for monthly patient admissions and discharges. A data set on monthly patient admissions and discharges was collected from the Rajavithi hospital, consisting of 72 monthly observations from October 1998 to September 2004. The analyses were conducted by using intervention analysis and the Box-Jenkins (BJ) methods. The four different data sets of 36, 48, 60 and 72 monthly series that ran backwards from September 2004 for patient admissions and discharges were used to develop in BJ models. Results showed that the BJ approach was a more appropriate way for both monthly patient admissions and discharges using the 60 monthly historical data. However, the accuracy of the forecasting depended upon the variation within the data.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อหาตัวแบบการพยากรณ์ที่เหมาะสมสำหรับจำนวนการรับและการจำหน่ายผู้ป่วยรายเดือนด้วยวิธีการพยากรณ์ทางสถิติ 2 วิธี คือ วิธีวิเคราะห์อินเตอร์เวนชัน และวิธีบ็อกซ์และเจนกินส์ โดยใช้ข้อมูลอนุกรมเวลารายเดือนจำนวนการรับและการจำหน่ายผู้ป่วยของโรงพยาบาลราชวิถี ตั้งแต่เดือนตุลาคม 2541 ถึงเดือนกันยายน 2547 รวม 72 เดือน การวิเคราะห์ใช้วิธีอินเตอร์เวนชันและสร้างตัวแบบการพยากรณ์ด้วยวิธีบ็อกซ์และเจนกินส์ โดยใช้ข้อมูลย้อนหลังจากเดือนกันยายน 2547 ด้วยขนาดอนุกรมเวลาต่างๆ คือ 36, 48, 60 และ 72 เดือน ตามลำดับ ผลการศึกษาพบว่า วิธีบ็อกซ์และเจนกินส์ที่ใช้ข้อมูลรายเดือนย้อนหลัง 60 เดือนจะให้ตัวแบบที่เหมาะสมสำหรับการพยากรณ์จำนวนการรับและการจำหน่ายผู้ป่วยของโรงพยาบาลราชวิถีแต่อย่างไรก็ตามความถูกต้องแม่นยำของการพยากรณ์ยังขึ้นอยู่กับความผันแปรภายในของชุดข้อมูลเองที่มีการเปลี่ยนแปลงในพฤติกรรมจำนวนการรับและการจำหน่ายผู้ป่วยที่ไม่เหมือนในอดีต

Keywords: forecasting model, Intervention analysis, Box-Jenkins method

คำสำคัญ: ตัวแบบการพยากรณ์, การวิเคราะห์อินเตอร์เวนชัน, วิธีบ็อกซ์และเจนกินส์

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1. INTRODUCTION

Most public hospitals in Thailand have faced the increasing number of patients in each year, but the number of medical staff did not increase as it should have. Moreover, the limitation of hospital equipment, manpower, laboratory support, laundry needs, and other service resources have affected the management of health care services and patients' satisfaction. In February 2001, the Thai government announced the policy of "Universal of Health Care Project (UHP)" or "30 baht health policy" to all public hospitals in which all Thai people have the same right to access quality health care services and the health care expenditure is not a constraint in obtaining the health services. The UHP policy should have affected the increasing number of patients in hospitals. Hospitals planning to move forward to universal health care coverage would be affected by the monthly changes in the number of patients. The issues of modeling and forecasting from month to month movements of patients are an important input for planning and making a decision in health care services. A case study of Rajavithi hospital in Bangkok was focused because it is a big public hospital that adopted and started the intervention "the 30 baht health policy" for health care services since October 2001. Therefore, the objective of this study was to find the appropriate forecasting model for monthly patient admissions and discharges of the Rajavithi hospital.

2. MATERIAL AND METHODS

A data set on monthly patient in admissions and discharges was collected from the Rajavithi hospital, consisting of 72 monthly observations from October 1998 to September 2004,

using two statistical forecasting methods of intervention analysis and Box-Jenkins (BJ) approach.

The intervention analysis was first conducted to model the historical data taking into account a new intervention policy of the universal of health care project (UHP). Given the intervention, the null hypothesis that the intervention event caused a change in the series of the actual number of patients was tested. If there was statistically significant evidence, the intervention model would be developed to explain the magnitude of the change in the time series of monthly patients in admissions and discharges. If no significant evidence was found, the BJ approach would be applied to develop the forecasting models using the past data of 36, 48, 60 and 72 monthly series that ran backward from September 2004 for each individual series of monthly patient admissions and discharges. The forecasting performances of the BJ models were evaluated using the MAD, MSE and MAPE, and the appropriate forecasting model was considered from the minimum values of these indices. Finally, the chosen model was used to make forecasts for six months ahead for monthly patient admissions and discharges. After that, the forecast values were compared with the actual values for the period of October 2004 to March 2005.

2.1 Intervention Model

The general intervention models are defined as follows:

$$Y_t = f(X_t) + N_t$$

where

Y_t denotes the outcome variable at time t ,
 $f(X_t)$ denotes the intervention function,

X_t denotes the dummy variable whose values prior to the UHP are zero and whose subsequent values are one, and N_t denotes the noise component of the ARIMA model.

The parameter estimates in the model can be obtained by using either the least square or the maximum likelihood method. The intervention procedures are detailed in various books and articles (Bowerman and O'Connell, 1993; Makridakis et al., 1998; Taesombut, 2006).

2.2 Box-Jenkins (BJ) Approach

The BJ can only be applied to stationary series or series which have been made stationary by differencing. The BJ model is also called the autoregressive integrated moving average (ARIMA) model which consists of four steps of analysis, including identification, estimation, diagnostic checking, and forecasting (Farnum and Stanton, 1989; Bowerman and O'Connell, 1993; Gaynor and Kirkpatrick, 1994). However, the minimum number of successive observations per series required for conducting the BJ model is in question. Taesombut (2006) and Gateacem (2003) suggested that at least 30 observations were required for the BJ analysis, while Box et al. (1994) stated that *"It is normally supposed that successive values of the time series under consideration or of the input-output data are available for analysis. If possible, at least 50 and preferably 100 observations or more should be used. In the cases where a past history of 50 or more observations are not available, one proceeds by using experience and past information to yield a preliminary model. This model may be updated from time to time as more data become available"*.

Identification:

The tentative ARIMA model of order p , d , and q for the stationary time series is identified by analyzing autocorrelation and partial autocorrelation functions from the correlogram plots. The ARIMA (p , d , q) model is defined as follows:

$$Z_t = \theta_0 + \phi_1 Z_{t-1} + \phi_2 Z_{t-2} + \dots + \phi_p Z_{t-p} + \varepsilon_t - \theta_1 \varepsilon_{t-1} - \theta_2 \varepsilon_{t-2} - \dots - \theta_q \varepsilon_{t-q}$$

where

Z_t = observation at time t of stationary time series

p = AR order

q = MA order

d = number of differencing

θ_0 = constant

ϕ_i = autoregressive (AR) parameters; $i = 1, 2, \dots, p$

θ_i = moving average (MA) parameters; $i = 1, 2, \dots, q$

ε_t = error at time t

Estimation

The parameters of the tentative ARIMA model can be estimated by the method of least square or maximum likelihood.

Diagnostic checking

To check the adequacy of the tentatively identified model, two diagnostics are used.

1) Testing model parameters: the hypotheses of $H_0: \theta_i = 0$ (θ_i is MA parameters) vs $H_1: \theta_i \neq 0$; $i = 1, 2, \dots, q$; and $H_0: \phi_i = 0$ (ϕ_i is AR parameters) vs $H_1: \phi_i \neq 0$; $i = 1, 2, \dots, p$ are tested with the t -statistics:

$$t = \frac{\hat{\theta}_i}{\hat{\sigma}_{\hat{\theta}_i}} \quad \text{and} \quad t = \frac{\hat{\phi}_i}{\hat{\sigma}_{\hat{\phi}_i}}$$

Reject H_0 if $|t| > t_{\alpha/2, n-1}$, conclude that the parameter being tested (θ_i or ϕ_i) is nonzero at significant level α

2) Testing autocorrelation for residuals: the hypotheses of $H_0: \rho_1 = \rho_2 = \dots = \rho_m = 0$ vs H_1 : at least one $\rho_i \neq 0$ are tested with the Ljung-Box statistics:

$$Q_m = n(n+2) \sum_{k=1}^m \frac{r_k^2}{n-k}$$

where

n is the number of observations in the series

m is the number of autocorrelation coefficients being tested

r_k is the sample autocorrelation function of the k th residual term

Reject H_0 if $Q_m > \chi_{\alpha, m-n_p}^2$ such that n_p

is the number of estimated parameters in the model.

Forecasting

After the fitted model is obtained, it will be used to make forecasts for future time periods. The forecasting performances can be evaluated using the mean absolute deviation (MAD), the mean square error (MSE), or the mean absolute percentage error (MAPE). The minimum values of these indices are preferred to be a criterion for indicating the appropriate forecasting model (Gaynor and Kirkpatrick, 1994). The computation of these measures is shown here;

1) The mean squared error (MSE)

$$= \frac{\sum_{t=1}^n e_t^2}{n}$$

2) The mean absolute deviation (MAD)

$$= \frac{\sum_{t=1}^n |e_t|}{n}$$

3) The mean absolute percentage error (MAPE)

$$= \frac{\sum_{t=1}^n \left| \frac{e_t}{Y_t} \right|}{n} 100$$

where

e_t is the forecast error in time period t ,

Y_t is the actual value in time period t ,

and n is the number of forecast observations in the estimation period.

3. RESULTS

3.1 Results for intervention analysis

Table 1 presented the results of intervention analysis. It turned out that the intervention parameter estimates (ω_0) for patient admissions ($\omega_0 = 31.68$, t-statistics = 0.50 and P-value = 0.6161) and discharges ($\omega_0 = 24.24$, t-statistics = 0.58 and P-value = 0.5640) were no differ from zero at significance level .05. In conclusion, the UHP didn't affect the change in the number of monthly patient admissions and discharges of Rajavithi hospital at significant level 0.05.

Table 1. Models and parameter estimates of intervention analysis for patients admissions and discharges.

Time series	Model	Parameter estimates	t - stats	P - value
Admissions	$Z_t^y = \omega_0 Z_t^X + \frac{\varepsilon_t}{(1 - \phi_1 B - \phi_2 B^2)}$	$\omega_0 = 31.68$ $\phi_1 = -0.71$ $\phi_2 = -0.53$	0.50 -6.18 -4.65	0.6161 0.0001* 0.0001*
Discharges	$Z_t^y = \omega_0 Z_t^X + (1 - \theta_1)\varepsilon_t$	$\omega_0 = 24.24$ $\theta_1 = 0.85$	0.58 10.17	0.5640 0.0001*

Notes: 1) $Z_t^x = (1-B)(1-B^{12}) X_t$ and $Z_t^y = (1-B)(1-B^{12}) Y_t$
2) * significant at a level of 0.05

3.2 Results of the BJ model

Consider the trends of monthly patients in admissions and discharges from Figure 1 and Figure 2, respectively. It indicated that there might be an appearance of a trend or seasonal variation in the data. This could be detected by analyzing the autocorrelations function and partial autocorrelation function from correlogram plots using different sizes of monthly historical data, as shown in Table 2.

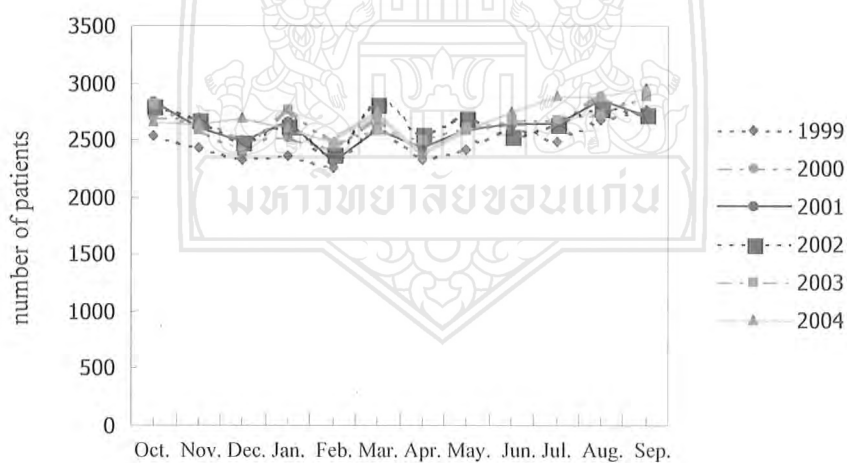


Figure 1. Trends of monthly patients in admissions from fiscal year 1999 to 2004.

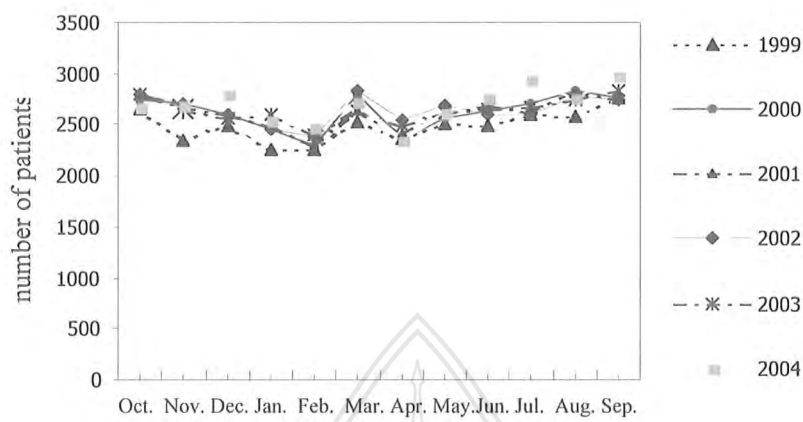


Figure 2. Trends of monthly patients in discharges from fiscal year 1999 to 2004.

Table 2. Results of trend and seasonal effects for each individual time series of monthly patients in admissions and discharges.

Size of monthly historical data		Admissions		Discharges	
		Trend	seasonal	Trend	seasonal
36 months		×	✓	×	✓
48 months		✓	✓	✓	✓
60 months		✓	✓	✓	✓
72 months		✓	✓	✓	✓

The final fitted Box-Jenkins (BJ) forecasting models for admissions and discharges using the past data of 36, 48, 60 and 72 monthly series were summarized in Table 3.

Table 3. Box-Jenkins models for admissions and discharges.

Time series	Models			
	36 months	48 months	60 months	72 months
	(Oct. 2001-Sep. 2004)	(Oct. 2000-Sep. 2004)	(Oct. 1999-Sep. 2004)	(Oct. 1998-Sep. 2004)
Admissions	SARIMA(0,1,0) ₁₂	ARIMA(2,1,0) × SARIMA(0,1,0) ₁₂	ARIMA(2,1,0) × SARIMA(0,1,1) ₁₂	ARIMA(2,1,0) × SARIMA(0,1,0) ₁₂
Discharges	SARIMA(0,1,0) ₁₂	ARIMA(0,1,1) × SARIMA(0,1,0) ₁₂	ARIMA(2,1,0) × SARIMA(0,1,0) ₁₂	ARIMA(0,1,1) × SARIMA(0,1,0) ₁₂

Additionally, the results of the forecasting performance were measured by MAD, MSE and MAPE of the BJ models for monthly patients in admissions and discharges using different sizes of past monthly series data and presented in Table 4 and Table 5, respectively. It was indicated that the admission series using 60 monthly historical data

was a more appropriate forecasting model that yielded the smallest values of MAD (85.45), MSE (11,681.83) and MAPE (3.22 %), respectively. Similarly, the monthly patient in discharges using the 60 monthly historical data was a more appropriate forecasting model that yielded smallest values of two indices of MAD (83.13) and MAPE (3.13 %), respectively.

Table 4. Comparison of MAD, MSE and MAPE of Box-Jenkins models for admissions.

Accuracy measure	Time series			
	36 months (Oct. 2001-Sep. 2004)	48 months (Oct. 2000-Sep. 2004)	60 months (Oct. 1999-Sep. 2004)	72 months (Oct. 1998-Sep. 2004)
MAD	111.17	100.71	85.45	88.75
MSE	17,372.42	17,565.29	11,681.83	13,452.54
MAPE	4.15%	3.78%	3.22%	3.36%

Table 5. Comparison of MAD, MSE and MAPE of Box-Jenkins models for discharges.

Accuracy measure	Time series			
	36 months (Oct. 2001-Sep. 2004)	48 months (Oct. 2000-Sep. 2004)	60 months (Oct. 1999-Sep. 2004)	72 months (Oct. 1998-Sep. 2004)
MAD	91.13	87.09	83.13	86.59
MSE	12,969.13	11,427.49	11,667.21	12,075.98
MAPE	3.39%	3.27%	3.13%	3.28%

The chosen BJ models obtained from table 3 using 36, 48, 60 and 72 monthly historical data were used to make forecast for six months ahead from October 2004 to March 2005 of monthly patient in admissions and discharges. The forecast values were compared with the actual values for the corresponding periods. The MAD, MSE and MAPE were calculated and compared again from the data

of six periods ahead. It appeared that the monthly patients in admissions using 60 monthly historical data yielded the smallest values of MAD, MSE and MAPE with 67.83, 8,219.50 and 2.39 %, respectively. Similarly, the model of monthly patients in discharges using the 60 monthly historical data yielded the smallest values of MAD, MSE and MAPE were 77.00, 9,737.33 and 2.65 %, respectively.

respectively. These measures confirmed that the most appropriate forecasting models for both admissions and discharges were the models that used 60 months historical data.

The 95 % confidence limits of the six forecast values using 60 monthly historical data in

the BJ models covered the actual values of admissions and discharges, as shown in Figures 6 and 7. That is, the BJ forecasting model using 60 monthly historical data is appropriate for monthly patient movements in admissions and discharges of Rajavithi hospital.

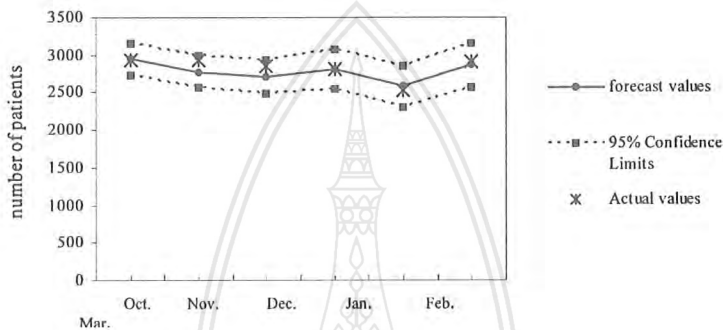


Figure 6. The 95 % confidence limits of the forecast values and the actual values of admissions for the period of October 2004 to March 2005.

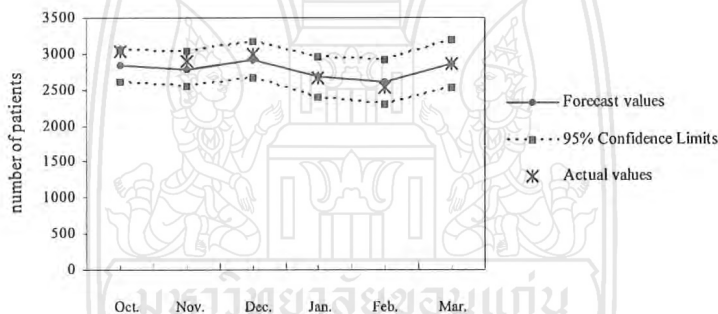


Figure 7. The 95% confidence limits of the forecast values and the actual values of discharges for the period of October 2004 to March 2005.

4. CONCLUSION AND DISCUSSION

The results of this study indicated that the appropriate forecasting models for monthly patient admissions and discharges of the Rajavithi hospital during the period of October 1999 to September 2004 were the BJ models using 60 monthly historical

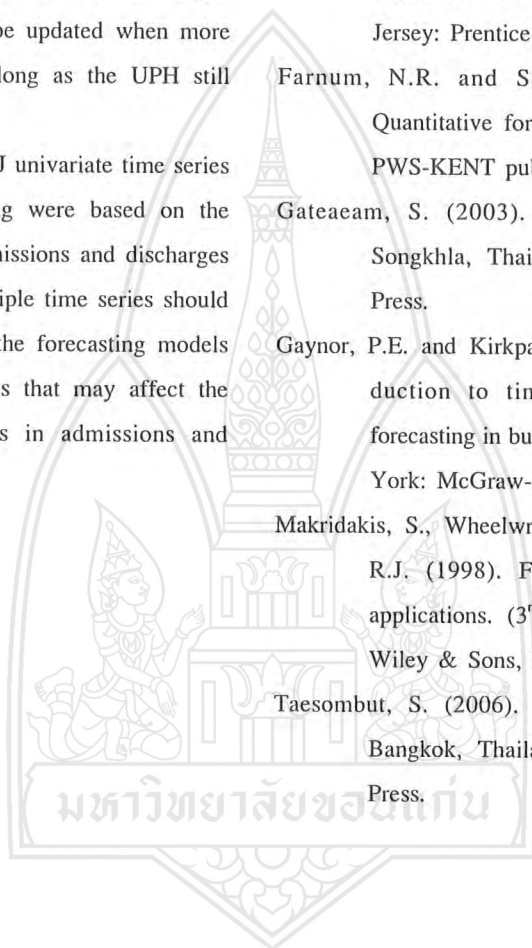
data. This might support the statement of Box et al. (1994) that the time series analysis should use at least 50 observations. As mentioned, the Rajavithi hospital has joined the UHP and started data collection since October 2001. The complete time series data were available from October 2001 to

September 2004 during the period of study. Then, the preliminary forecasting models obtained would benefit to the Rajavithi hospital in planning of health care services and hospital management. Moreover, the models can be used for a guideline of other public hospitals in conducting forecasting models for their own hospitals. However, these developed preliminary models should be updated when more data becomes available as long as the UPH still continues.

In this study, the BJ univariate time series that were used for modeling were based on the behavior of past data of admissions and discharges only. For further study, multiple time series should be considered to formulate the forecasting models by including more covariates that may affect the monthly patient movements in admissions and discharges.

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Tangent Linear Model for a Barotropic Model

แบบจำลองเทนเจนต์เชิงเส้นสำหรับแบบจำลองบาโรโทรปิก

Thawatchai Sirikantisophon^{1*} and Dusadee Sukawat²

Abstract

In this paper, a linear tangent model is applied to a barotropic model. The barotropic model is a simple model for the atmosphere. The tangent linear model (TLM) is a method which is used to generate several initial conditions from a single observed initial conditions which is very helpful in reducing uncertainty in the forecast. The results of the tangent linear model for the barotropic model are compared with the analytic solution.

บทคัดย่อ

ในงานวิจัยครั้งนี้ แบบจำลองเทนเจนต์เชิงเส้นได้ถูกนำมาประยุกต์ใช้กับแบบจำลองบาโรโทรปิก ซึ่งแบบจำลองบาโรโทรปิกถือได้ว่าเป็นแบบจำลองที่ง่ายที่สุดสำหรับในทางบรรยากาศ ส่วนวิธีการแบบจำลองเทนเจนต์เชิงเส้นเป็นแบบจำลองที่ถูกใช้ในการสร้างเงื่อนไขในการเริ่มต้นหลายหลายเงื่อนไขจากภายใต้เงื่อนไขเริ่มต้นเพียงเงื่อนไขเดียวซึ่งวิธีการนี้ถือได้ว่ามีประโยชน์อย่างมากในการลดความไม่แน่นอนของการพยากรณ์สำหรับการทดลองของแบบจำลองเทนเจนต์เชิงเส้นสำหรับแบบจำลองบาโรโทรปิกได้ถูกเปรียบเทียบกับผลจากการวิเคราะห์

Keywords: Tangent Linear Model (TLM), Barotropic Model

คำสำคัญ: แบบจำลองเทนเจนต์เชิงเส้น, แบบจำลองบาโรโทรปิก

1. INTRODUCTION

At present, forecasting of the atmosphere is always uncertain, especially the movement of tropical cyclones. This uncertainty can cause a lot of damage to human life and property. It happens

because the atmosphere is sensitive to initial conditions. Even though there are now highly complex models available as weather forecast models, the results have not been satisfactory. The concept of numerical weather prediction emphasizes

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the environment, which is large scale and does not require a high resolution model. However, to use a high resolution weather prediction model for operational forecasts, a very large, fast and expensive computer is required. Another option to improve the accuracy of the weather forecast is to reduce uncertainty in the output from numerical models by performing several forecasts of the same event from several initial conditions. One way to get more initial condition values is to generate an initial condition based on the observed initial conditions by using the "tangent linear model" method.

2. TANGENT LINEAR MODEL (TLM) METHOD

Consider a nonlinear model

$$x(t) = M[x(t_0)], \quad (2.1)$$

where $x(t)$ is the state at time t , $x(t_0)$ is the state at initial time and M is the time integration of the numerical scheme from the initial condition to time t . The tangent linear model (TLM) is defined such that:

$$L = \frac{\partial M}{\partial x}, \quad (2.2)$$

where L is a tangent linear model of (Kalnay, 2003).

The tangent linear model (TLM) thus evolves a perturbation in time,

$$y(t) = L(t_0, t)y(t_0), \quad (2.3)$$

where $y(t)$ is the perturbation state at time t and $y(t_0)$ is the perturbation state at initial time $t=0$ (Kalnay, 2003).

3. BAROTROPIC MODEL

The barotropic model (Holton, 1992) is a simple model for the atmosphere. This model is based on the assumptions that (1) the horizontal equation of motion is sufficient, (2) the atmosphere is non-divergent, (3) the density is constant and (4) there is no friction. So the horizontal equations of motion governing the non-divergent barotropic flow is

X-Momentum:

$$\frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} = -g \frac{\partial z}{\partial x} + fv, \quad (3.1)$$

Y-Momentum:

$$\frac{\partial v}{\partial t} + u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} = -g \frac{\partial z}{\partial y} - fu. \quad (3.2)$$

The continuity equation for the system is

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0, \quad (3.3)$$

where u and v are horizontal velocity components, g is gravity, $f = 2\Omega \sin \phi$ is the Coriolis parameter where ϕ is latitude, and Ω is the Earth's angular velocity. Differentiating equation (3.2) by $\partial/\partial x$ and equation (3.1) by $\partial/\partial y$ and taking the difference between the two equations, we obtain the vorticity equation for the non-divergent barotropic flow as

$$\frac{d}{dt} \zeta_a = 0, \quad (3.4)$$

where $\zeta = \frac{\partial v}{\partial x} - \frac{\partial u}{\partial y}$ is the relative vorticity and

$\zeta_a = \zeta + f$ is the absolute vorticity. The absolute vorticity of a fluid is conserved following the motion.

In Cartesian coordinates, the non-divergent part of the flow can be expressed in terms of a streamfunction, ψ , such that;

$$u_\psi = -\frac{\partial \psi}{\partial y} \quad \text{and} \quad v_\psi = \frac{\partial \psi}{\partial x}. \quad (3.5)$$

In streamfunction form, the relative vorticity, ζ , may be expressed as follows;

$$\zeta = \frac{\partial v}{\partial x} - \frac{\partial u}{\partial y} = \nabla^2 \psi \text{ .} \tag{3.6}$$

The barotropic model is based on one equation and one unknown, the streamfunction ψ ,

$$\frac{\partial}{\partial t} \nabla^2 \psi = -J(\psi, \nabla^2 \psi) - \beta \frac{\partial \psi}{\partial x} \text{ ,} \tag{3.7}$$

where J is the Jacobian operator, $\beta = \frac{\partial f}{\partial y}$ is the beta parameter and f is the coriolis parameter. Equation (3.7) forms the basis of the non-divergent barotropic model. The equation has one unknown, ψ (streamfunction). The integration procedure consists of replacing the derivatives of the prediction equation (3.7) with finite difference approximations at a discrete set of points in space and time.

4. THE EXPERIMENT

For this research, a simple track of a tropical cyclone (Typhoon LINGLING during 06 - 12 November 2001) is used in this experiment. This track moves like a straight line which is easy to study as shown in Figure 1.

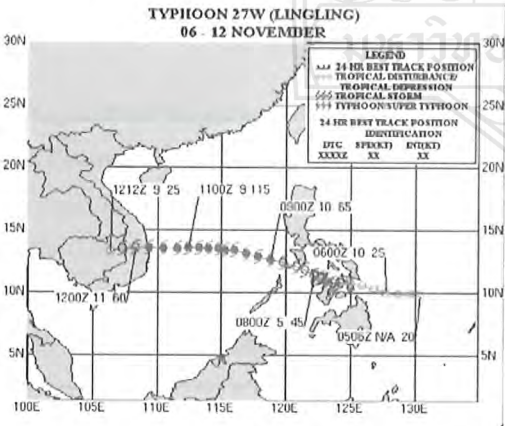


Figure 1. Simple track of tropical cyclone (Typhoon LINGLING during 06 - 12 November 2001).

The data for the model are prepared using data from the European Center for Medium Range Weather Forecasting (ECMWF). These data were taken from http://data.ecmwf.int/data/era40_daily, which can be classified as in Table 1.

Table 1. Data from the ECMWF (2004).

	Simple Track
Type	Pressure levels
Date	06-11-2001 to 12-11-2001
Time	00:00,06:00,12:00,18:00
Parameter	U,V velocity
Level	500 mb
Resolution	2.5 degree

There are two models used in the experiment which are (1) Barotropic model and (2) Tangent linear model for barotropic model. Both models were used. The domain for running the model is 100°E - 140°E, 0°N - 30°N and the time step is half an hour. The experiment was run for 72 hours (3 day from 09 November 2001, 00 UTC to 12 November 2001, 00 UTC) and the output interval from the model forecast is 6 hours. The results are displayed by the GrADS (Grid Analysis and Display System) program (Institute of Global Environment and Society, 2005).

5. THE RESULTS

5.1 The Results of the Barotropic Model

The domain for running the model is 100°E - 140°E, 0°N - 30°N, and the time step is half an hour. The experiment was run for 72 hours (from 09 November 2001, 00 UTC to 12 November 2001,

00 UTC) and the output interval from the model forecast is 6 hours. The outputs shown below are examples of all outputs in Figures 2 - 5.

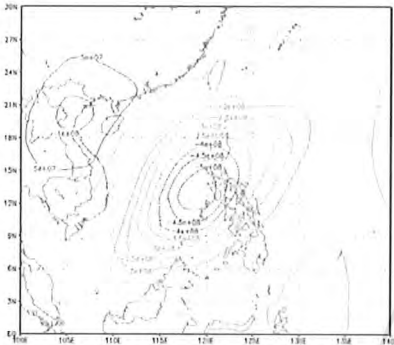


Figure 2. Initial Streamfunction, 9 November 2001, 00 UTC ($t + 0$).

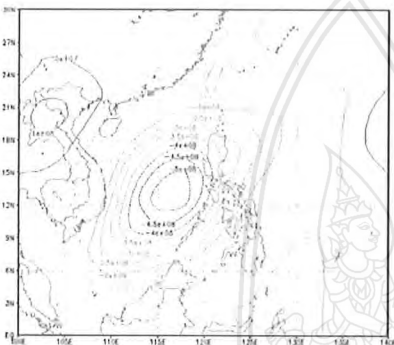


Figure 3. Forecast Streamfunction, 10 November 2001, 00 UTC ($t + 24$).

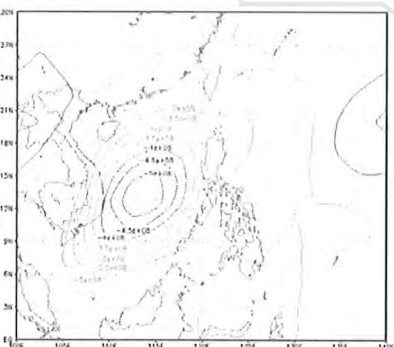


Figure 4. Forecast Streamfunction, 11 November 2001, 00 UTC ($t + 48$).

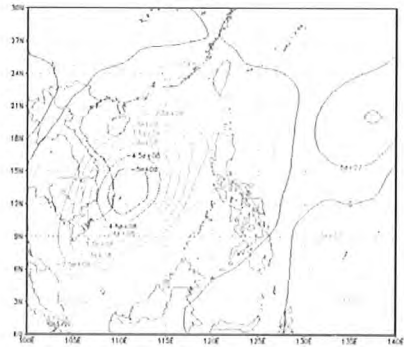


Figure 5. Forecast Streamfunction, 12 November 2001, 00 UTC ($t + 72$).

Figures 2 - 5 show results from running the barotropic model by using observed initial conditions as input. Figure 2 shows the initial streamfunction (9 November 2001, 00 UTC) as input for forecasting streamfunction from the barotropic model run. Figures 3 - 5 show forecast streamfunction at 24 (10 November 2001, 00 UTC), 48 (11 November 2001, 00 UTC) and 72 (12 November 2001, 00 UTC) hours. From the results, as time changes, the system moves from right to left as in the analysis.

5.2 The Result of Tangent Linear Model for Barotropic Model

The domain for running the model is $100^{\circ}\text{E} - 140^{\circ}\text{E}$, $0^{\circ}\text{N} - 30^{\circ}\text{N}$, and the time step is half and hour. The experiment was run for 72 hours (from 09 November 2001, 00 UTC to 12 November 2001, 00 UTC) and the output interval from the model forecast is 6 hours. The outputs shown in Figures 6 - 9 correspond to output from the barotropic model in Figures 2 - 5.

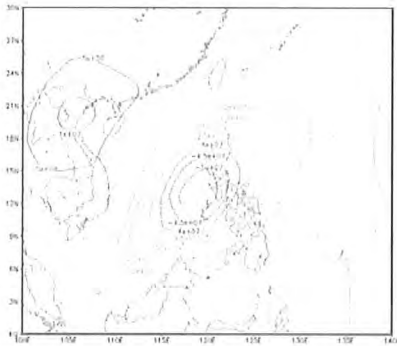


Figure 6. Initial Streamfunction, 9 November 2001, 00 UTC ($t + 0$).

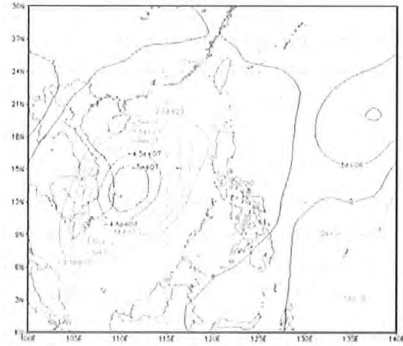


Figure 9. Forecast Streamfunction, 12 November 2001, 00 UTC ($t + 72$).

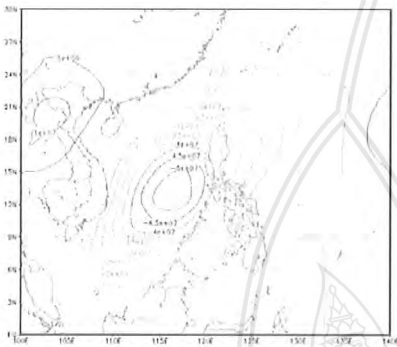


Figure 7. Forecast Streamfunction, 10 November 2001, 00 UTC ($t + 24$).

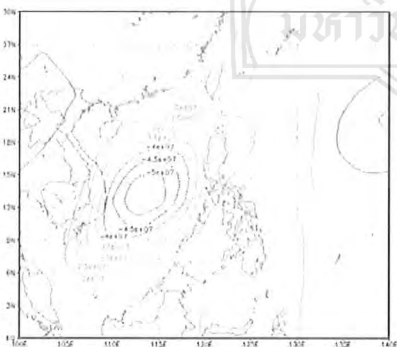


Figure 8. Forecast Streamfunction, 11 November 2001, 00 UTC ($t + 48$).

Figures 6 - 9 show results from running the tangent linear model by using generated initial conditions based on the observed initial conditions as input. Figure 6 shows the initial streamfunction (9 November 2001, 00 UTC) as input for forecasting streamfunction from the barotropic model run. Figures 7 - 9 show the forecast streamfunction at 24 (10 November 2001, 00 UTC), 48 (11 November 2001, 00 UTC) and 72 (12 November 2001, 00 UTC) hours. From these 3 figures, as time changes, the system moves from right to left.

6. DESCRIPTION OF THE RESULTS

From the results of the barotropic model, the center of the cyclone obtained from the control run is different from analysis data but the track of the cyclone moves along the same direction which is right to left as shown in Figure 10.

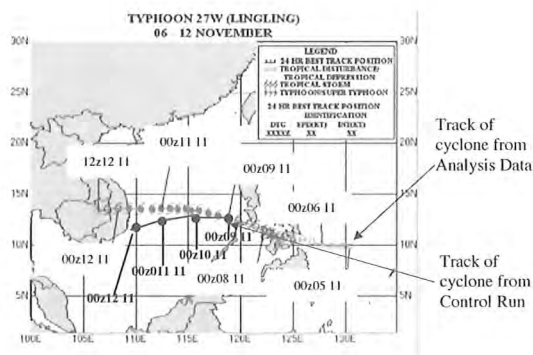


Figure 10. The comparison of 2 cyclone tracks between analysis data and control run.

From the results of the tangent linear model for the barotropic model, the center of the cyclone obtained from the tangent linear model is different from the analysis data but the tangent linear model track moves as a straight line along the same direction which is right to left as shown in Figure 11.

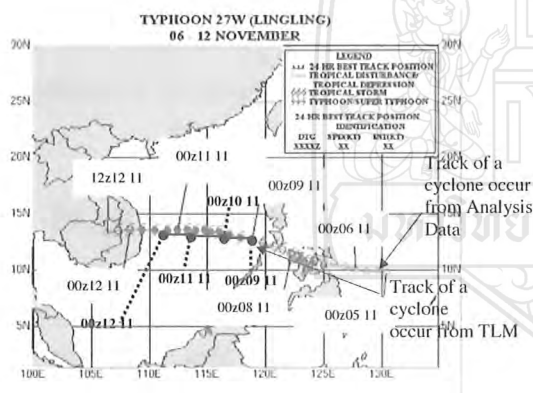


Figure 11. The comparison of 2 cyclone tracks between cyclone from analysis data and TLM.

From Figure 10 and Figure 11, the center of the cyclone obtained from the control run and the tangent linear model moved along the same direction as the analysis data, but the tangent linear

model track is closer to the analysis data than the track of the control run, while the movement of the tangent linear model track is much slower than the control run.

7. CONCLUSION

From the experiment, the tangent linear model method can be used in generating initial conditions based on the observed initial conditions which is very helpful in reducing uncertainty in the forecast.

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Round Down Technique for Solving an Integer Linear Programming

เทคนิคการปัดเศษลงเพื่อการหาคำตอบของปัญหาโปรแกรมเชิงเส้นจำนวนเต็ม

Sirirat Wongprakornkul¹

Abstract

The One-dimensional cutting stock problem (1D-CSP) is a classical NP-hard problem which cannot be solved with the polynomial computation time. The finite number of cutting patterns may be very large. Gilmore and Gomory (1963) claimed that if the stock to be cut has length $l = 200$ inches and there are demands for 40 different lengths from 20 to 80 inches, the number of possible patterns could be exceed 10 to 100 million. The more cutting patterns these are, the more columns of constraints are involved. The most powerful algorithm for solving linear programs with many columns is Column-Generation Procedures. To due with Column-Generation Technique, the 1D-CSP had to be relaxed and a sub-problem was created, called a knapsack problem, for generating all possible essential cutting patterns. Although, the decomposition principle was applied to decrease the number of Integer variables, this number may be still very large which causes increase expenses because of computation time. Therefore, the heuristic method was raised to solve the Integer Linear Programming within reasonable computation time. The principle of the heuristic method applied the round-down technique. Hence, three more groups of constraints were added to the full model.

In this paper, One-dimensional cutting stock problem with multiple facilities and multiple commodities was investigated. The particular problem was formed into a mathematical model. Then the initial cutting patterns were created and the mathematical model was relaxed for applying Column-Generation Technique. When the optimal solution had been reached, all possible essential cutting patterns were generated. Now the problem was formed in a full model of an Integer Linear Programming. After that, the proposed heuristic method was raised to solve the problem.

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บทคัดย่อ

ปัญหาการตัดหนึ่งมิติ (ID-CSP) เป็นปัญหาที่ทราบกันว่าเวลาที่ใช้ในการหาคำตอบไม่อยู่ในรูปของโพลีโนเมียล เนื่องจากจำนวนรูปแบบการตัดแบบจำกัดมีจำนวนมาก Gilmore and Gomory กล่าวว่า ถ้าสิ่งที่เราต้องการตัดยาว (l) 200 นิ้วและความยาวของขนาดที่ต้องการตัดแตกต่างกัน 40 ขนาดโดยมีความยาวอยู่ระหว่าง 20 - 80 นิ้วแล้วจำนวนรูปแบบการตัดที่เป็นไปได้ทั้งหมดอาจเกิน 10 - 100 ล้านรูปแบบ และยังจำนวนรูปแบบการตัดมากหมายความว่าจำนวนตัวแปรจำนวนเต็มก็เพิ่มจำนวนมากไปด้วย เทคนิคที่มีประสิทธิภาพที่สุดในการหาคำตอบกรณีที่จำนวนตัวแปรมีจำนวนมากคือ เทคนิคการสร้างสมมติ เทคนิคดังกล่าวนี้นำมาใช้โดยการแบ่งรูปแบบปัญหาออกเป็นปัญหาหลัก และปัญหาย่อย โดยปัญหาหลักคือการหาคำตอบของปัญหาโปรแกรมเชิงเส้น จากนั้นนำผลลัพธ์ที่ได้ใช้ในปัญหาย่อยเพื่อการสร้างรูปแบบการตัดที่สำคัญส่งผลให้ได้คำตอบที่ดีขึ้นเรียกปัญหานี้ว่าแนปแซค ถึงแม้ว่าจะนำเทคนิคการแบ่งปัญหามาใช้เพื่อลดจำนวนรูปแบบการตัด แต่จำนวนรูปแบบการตัดที่ยังคงมีจำนวนมาก ในการหาคำตอบปัญหาเชิงเส้นจำนวนเต็มโดยตรงจึงอาจทำให้เวลาในการหาคำตอบใช้เวลานานมากเพื่อแก้ปัญหาดังกล่าว การวิจัยครั้งนี้เสนอเทคนิคฮิวริสติกส์โดยประยุกต์หลักการของเทคนิคการปิดเศษลงทำให้ในในรูปแบบปัญหาจึงมีสามกลุ่มของข้อจำกัดเพิ่มเติมขึ้นมา

ในการวิจัยนี้ปัญหาการตัดหนึ่งมิติด้วยขนาดความยาวของสิ่งที่ต้องการตัดหลายขนาด และขนาดที่ต้องการจากการตัดหลายขนาด เช่นเดียวกัน รูปแบบปัญหาได้สร้างในรูปแบบปัญหาทางคณิตศาสตร์จากรูปแบบการตัดเริ่มต้นที่สร้างขึ้นมาเชิงปัญหาโปรแกรมเชิงเส้น จากนั้นประยุกต์เทคนิคการสร้างสมมติเพื่อสร้างรูปแบบการตัดที่สำคัญทุกรูปแบบสุดท้ายประยุกต์เทคนิคการปิดเศษลงเพื่อหาคำตอบของปัญหาโปรแกรมเชิงเส้นจำนวนเต็ม

Keywords: Column-Generation Technique, One-dimensional cutting problem

คำสำคัญ: เทคนิคการสร้างสมมติ, ปัญหาการตัดหนึ่งมิติ

1. INTRODUCTION

More than four decades ago, mathematicians studied ways to solve large scale problems. The One-dimensional cutting stock problem (ID-CSP) with multiple cutting facilities, which is intractable, involves a large number of variables. This problem is a classical combinatorial problem, which is solvable in non-polynomial time, also known as an NP-hard problem. Dantzig and Wolfe (1961) initiated the extensive work on large-scale mathematical programming. The most efficient technique when applied to linear programs is the Column-Generation Technique. It is applied for solving problems with few rows and much more

columns. There are wide varieties of problems, which can be solved by this approach.

The ID-CSP can be addressed by finding the best process with the minimum cost to cut stock material (stock) to meet the demand for small pieces (retail size) of prescribed dimensions. This problem is very difficult; since all variables must be integers and the number of columns can be very large. For example, if the stock to be cut has a length of 200 inches and the required demands for 40 different lengths from 20 - 80 inches, the number of possible patterns could easily exceed from 10 to 100 million (Gilmore and Gomory, 1963). However, some patterns are not needed to solve the problems. Hence,

the important concept for solving the problems faster is to find out the technique to originate the effective patterns. That technique is the column-generation technique. This technique is successful for solving the large number of variables in the linear programming formulation of the problem (Gilmore and Gomory, 1961; 1963). In addition, the 1D-CSP is broadly applied in industries such as in the aluminum industry (Stadtler, 1990) and the clothing industry (Gradišar et al., 1997). Typical cutting stock problems implicitly assume that the stocks are cut at a single facility. Holthaus (2002) raised decomposition approaches based on the Column-Generation Technique for solving the 1D-CSP with different types of standard lengths or multiple cutting facilities.

Although, Column-Generation Technique was applied to decrease the number of cutting patterns, this problem with integer variables still took time to solve. Therefore, the heuristic method was raised to solve the Integer Linear Programming within reasonable computation time.

2. LITERATURE REVIEWS

There are many papers working on the 1D-CSP. This paper may express some of them as follows:

2.1 The Delayed Column-Generation Technique

The first attempt to solve this problem was accomplished in 1961 by the Delayed Column-Generation Technique (Gilmore and Gomory, 1961). Let D_m be the required number of retail items l_m where $m \in \{1, \dots, M\}$ to be cut from stock length L_k where $k \in \{1, \dots, K\}$ and the number of stock k is unlimited, C_k be the cost per unit of the stock

length k , X_{kp} be the number of times at stock k and pattern p where $p \in \{1, \dots, P\}$. For a stock size k with length L_k a cutting pattern p indicates how often each order length l_m has to be cut off from this stock size. Any feasible cutting pattern p for stock size k can be represented by M -dimensional non-negative integer vector, $a_{kp} = (a_{1kp}, \dots, a_{Mkp})^T$ fulfilling $\sum_{m=1}^M l_m a_{mkp} \leq L_k$. This model can be formulated as Integer Linear Programming (ILP) model with the objective that minimizes the total cost of all stock lengths as follows:

$$\text{Minimize } \sum_{p=1}^P \sum_{k=1}^K c_k x_{kp} \quad (1)$$

$$\text{Subject to } \sum_{p=1}^P \sum_{k=1}^K a_{mkp} x_{kp} \geq D_m, \quad m=1, \dots, M, \quad (2)$$

$$x_{kp} \geq 0, \quad k=1, \dots, K, p=1, \dots, P \quad (3)$$

From equation (2), if the demand and different sizes of retail items are large, the overall patterns to be generated are not practical. Therefore, the duality theorem can be used to relax this model by starting basis columns with a minimum number of patterns that can be solved (1) - (3). These columns are referred to initial patterns. From the weak duality theorem, any pattern is not to be a basis column, if $\sum_{m=1}^M u_m D_m \leq c_k$, $\forall k$ where u_m be dual variables, Otherwise, one pattern that, $\sum_{m=1}^M u_m D_m > c_k$, $\exists k$ can be entered as a basis column. The sub-problem to be determined for the basis column is called the unbounded knapsack problem (UKP) with a mathematical model as follows:

Maximize $\sum_{m=1}^M u_m a_{mk(P+1)}$, (4)

Subject to $\sum_{m=1}^M l_m a_{mk(P+1)} \leq L_k$, (5)

$a_{mk(P+1)} \in I^+ , \forall m.$ (6)

The UKP can be solved as a branch-and-bound procedure because of the restriction of $a_{mk(P+1)}$ to be integer variables of (6) or an algorithm based on dynamic programming as such

$F_m(l) \geq \max \{F_m(l-l_m) + u_m, F_{m-1}(x)\}$ for $m > 1$ (7)

where $L \geq 1 > \sum_{m=1}^M l_m a_{mk(P+1)}$.

The UKP is to be solved for each different stock size by the single dynamic programming as described earlier. When the constraints, $\sum_{m=1}^M u_m D_m > c_k$, could not be detected, an optimal solution is verified.

2.2 A branch-and-bound procedure for solving the 1D-CSP exactly with single stock length

One possible method for solving the 1D-CSP if all patterns are known under a single stock length is the branch-and-bound method (Scheithauer and Terno, 1995). Consider at each stock length k, three lower bounds can be computed for each branching nodes after solving the continuous relaxation of the 1D-CSP as follows:

Lowerbound₁ = $Z = \sum_{p=1}^P c_k \lfloor x_{kp} \rfloor$, (8)

where $Z = \sum_{p=1}^P c_k \lfloor x_{kp} \rfloor$,

$d_m^r = D_m - \sum_{p=1}^P a_{mkp} \lfloor x_{kp} \rfloor$.

The second lower bound will be suggested as followed:

$Z_c = \text{Minimize } \sum_{p=1}^P c_k x_{kp}$, (9)

Subject to $\sum_{p=1}^P a_{mkp} x_{kp} \geq d_m^r , \forall m,$ (10)

$x_p = 0$, if $a_{mkp} > D_m , \forall m.$ (11)

From equations (9) - (11) the obtained lower bound is

Lowerbound₂ = $Z + Z_c$ (12)

The third lower bound is obtained from the solution of a knapsack problem with weight $q_{km} = \lfloor L_k / l_m \rfloor$ and suggested as follows:

$\mu = \text{maximize } \sum_{m=1}^M \frac{a_{mk(P+1)}}{q_{km}}$, (13)

Subject to

$\sum_{m=1}^M l_m a_{mk(P+1)} \leq L_k$, (14)

$a_{mk(P+1)} \leq d_m^r , \forall m$ (15)

$a_{mk(P+1)} \in I^+ , \forall m$ (16)

From equations (13) - (16) the obtained lower bound is

Lowerbound₃ = $Z + \left[\frac{1}{\mu} \sum_{m=1}^M \frac{d_m^r}{q_{km}} \right]$ (17)

The branch-and-bound procedure uses these three lower bounds to decide when to exclude or include a_{mkp} for each branching node. The number of X_{kp} variables will be increased by one.

2.3 A branch-and-price procedure for solving the 1D-CSP exactly with the number of single stock length

A branch-and-price procedure (Vance, 1998) suggested for solving 1D-CSP exactly with a single stock length that the upper bound of number of stock lengths is S . The mathematical model is as follows:

$$\text{Minimize } \sum_{s=1}^S y_s, \quad (18)$$

Subject to

$$\sum_{s=1}^S x_{ms} \geq D_m, \forall m, \quad (19)$$

$$\sum_{m=1}^M l_m x_{ms} \leq L y_s, \forall s, \quad (20)$$

$$y_s \in \{0,1\}, \forall s, \quad (21)$$

$$x_{ms} \in I^+, \forall m,s. \quad (22)$$

The equations (18) - (21) can be relaxed to the following formulation by assigning from:

$$\text{Minimize } \sum_{s=1}^S \sum_{p=1}^P \lambda_{sp}, \quad (23)$$

Subject to

$$\sum_{s=1}^S \sum_{p=1}^P x_{msp} \lambda_{sp} \geq D_m, \forall m, \quad (24)$$

$$\sum_{p=1}^P \lambda_{sp} \leq 1, \forall s, \quad (25)$$

$$\sum_{p=1}^P x_{msp} \lambda_{sp} \in I^+, \forall m,s, \quad (26)$$

$$\lambda_{sp} \geq 0, \forall s,p. \quad (27)$$

From equations (23) - (27) if all feasible patterns can be generated for obtaining X_{msp} , therefore, the solution is exact by branching rules that relaxed equation (26) as:

$$\sum_{p=1}^P x_{msp} \lambda_{sp} \geq \lceil \alpha \rceil, \quad (28)$$

$$\sum_{p=1}^P x_{msp} \lambda_{sp} \leq \lfloor \alpha \rfloor, \quad (29)$$

where λ_{kp} is fractional and not greater than 1 and α is a specified value. The goal of this branching is to restrict the integer condition of X_{kp} .

2.4 The Sequential Heuristic Procedure

The Sequential Heuristic Procedure (SHP) (Gradišar et al., 1999) is a neighborhood search for local optimum defined as $z(C)$ in a set of feasible solutions defined as $N(C)$. The step can be described as follows:

1. Generate a initial feasible solution C^{init} with objective $z(C^{\text{init}})$
2. Let $C^* = C^{\text{init}}$ and $z(C^*) = z(C^{\text{init}})$
3. Find $z(C^s)$ such that $z(C^s) = \min\{z(C) : C \in N(C)\}$
4. If $z(C^s) < z(C^*)$ then $C^* = C^s$ and $z(C^*) = z(C^s)$ else terminate the procedure and C^* and $z(C^*)$ are the solution and the objective of SHP, respectively.

In this case, to determine C_s from the step3 of the above procedure, solving the continuous relaxation of 1D-CSP is determined and searching for a cutting pattern that $c_k - \sum_{m=1}^M u_m D_m$ is minimized

in an ascending order. Hence, the cutting patterns must be added until the demands of all required items are fulfilled.

2.5 Rounding procedure from the continuous relaxation for multiple stock lengths

From the solving of 1D-CSP exactly for equation (1) - (3) with the restriction of X_{kp} to be integer, it cannot be guarantee that the problem can be solved within a deterministic computed time. Therefore, the heuristic approach such as a rounding method must be used. Three initial procedures for rounding the solutions are suggested after solving equation (1) - (3). They are shown as below:

Procedure 1: The round-up procedure (RU) suggests to equal $\lceil x_{kp} \rceil$.

Procedure 2: The optimizing rounding procedure (ORP) suggests X_{kp} to equal $\lceil x_{kp} \rceil$ or $\lfloor x_{kp} \rfloor$ by a branch-and-bound method.

Procedure 3: The decomposition procedure (DP) based on rounding down X_{kp} to equal $\lfloor x_{kp} \rfloor$ and applying ILP solver or other sequential heuristic procedures.

From the three initial procedures above, DP is clearly superior to other rounding procedures [10] Then, Holthaus (2002) has proposed the R1 and R2 procedures for DP that $d_m^r = D_m - \sum_{p=1}^P a_{mkp} \lfloor x_{kp} \rfloor$.

The R1 method

The R1 method will be used if there is a pattern a_{mkp} that is chosen from the existed patterns that $a_{mkp} \leq d_m^r, \forall m$, otherwise this method is

terminated. For all pattern $p=1, \dots, P$, choose a

pattern that $\sum_{m=1}^M a_{mkp}$ is maximized. Then, increases

X_{kp} by one and decrease d_m^r by $a_{mkp}, \forall m$. and

repeat this step until the method is terminated.

The R2 method

The R2 method will be used if solving the UKP sub-problems obtained a pattern a_{mkp} that

$a_{mkp} \leq d_m^r, \forall m$, and $\sum_{m=1}^M u_m D_m = c_k$, then increases

X_{kp} by one and decreases by d_m^r by $a_{mkp}, \forall m$.

Otherwise, this method is terminated. This step will be repeated until this method is terminated.

The hybrid R1 + R2 method

If the terminated criterions were not found, instead of repeating the steps of R1, the steps of R2 are repeated and on the way round, instead of repeating the steps of R2, the steps of R1 are repeated.

Holthaus (2002) suggested that DP + R1 + ILP, DP + R2 + ILP, and DP + R1 + R2 + ILP are successful for some instances and could not determine whether one of these procedures is superior to the others or not.

3. COLUMN-GENERATION TECHNIQUE

Consider the procedure to apply the Column-Generation Technique to the 1D-CSP so that at least X_{kp} pieces of standard length L_k are furnished. First, the problem is relaxed in order to provide the simplex multipliers. The rules for converting PRIMAL problem to DUAL problem are shown in Table1.

Table 1. Rules for converting PRIMAL to DUAL.

* * P R I M A L M I N I M I Z A T I O N P R O B L E M			
If the primal variables are	Then the dual constraints are	If the primal constraints are	Then the dual variables are
≥ 0	$\leq$	$\geq$	≥ 0
≤ 0	$\geq$	$\leq$	≤ 0
Unrestricted	$=$	$=$	Unrestricted
* * P R I M A L M A X I M I Z A T I O N P R O B L E M			
If the primal variables are	Then the dual constraints are	If the primal constraints are	Then the dual variables are
≥ 0	$\geq$	$\geq$	≤ 0
≤ 0	$\leq$	$\leq$	≥ 0
Unrestricted	$=$	$=$	Unrestricted

** If the objective function of the Primal problem is “Min” then the objective function of the Dual problem will be “Max”, in the other way, if the objective function of the Primal problem is “Max” then the objective function of the Dual problem will be “Min”.

After that, the problem is decomposed into I sub-problems, called [new pattern procedure or NPP]: a new-pattern generated sub-problem. These sub-problems applied the Column-Generation Technique to search the essential cutting patterns for the problem.

The ID-CSP model is written in the relaxed form (see also equations (30) - (33)) as the PRIMAL model. Following the rules in Table 1, the DUAL model is written in equations (34) - (37).

- k = index for stock lengths (k = 1,....., K),

m = index for retail items (m = 1,....., M),

L_k = the length of stock k; k = 1,....., K,

l_m = the length of item m; m = 1,....., M,

g_k = the cost of stock k,

R_k = amount of stock k available,

p = index for patterns,

P_k = the number of feasible patterns for stock k,

a_{mkp} = the number of strips of item m cut in pattern p for stock length k, fulfilling $\sum_{m=1}^M l_m a_{mkp} \leq L_k$,

D_m = the demands of item m, and

X_{kp} = decision variables that represent the number of times pattern p of stock k is used.

[PRIMAL]:

$$\text{Minimize } \sum_{k=1}^K \sum_{p=1}^{P_k} g_k x_{kp} \quad (30)$$

$$\text{Subject to } -\sum_{p=1}^{P_k} x_{kp} \geq -R_k, \forall k, \quad (31)$$

$$\sum_{k=1}^K \sum_{p=1}^{P_k} a_{mkp} x_{kp} \geq D_m, \forall m, \quad (32)$$

$$x_{kp} \geq 0, \forall k, p. \quad (33)$$

To develop the DUAL model, the simplex multipliers used throughout this paper are defined as follows:

e_k = the simplex multipliers of the used stock k from constraints (31),

r_m = the simplex multipliers of the item m from constraints (32).

[DUAL]:

$$\text{Maximize } \sum_{k=1}^K e_k (-R_k) + \sum_{m=1}^M r_m D_m, \quad (34)$$

$$\text{Subject to } -e_k + \sum_{m=1}^M r_m a_{mkp} \leq g_k, \forall k, p, \quad (35)$$

$$e_k \geq 0, \forall k, \quad (36)$$

$$r_m \geq 0, \forall m, \quad (37)$$

The simplex multipliers of [DUAL] are used to search a new legitimate cutting pattern. The multi-sub-problems are decomposed by the Column-Generation Technique to find out the effective cutting patterns. The [NPP] can be modeled as:

[NPP]:

$$\text{Maximize } V_{k^*} = \sum_{m=1}^M r_m a_{mk^*p^*} - g_{k^*} - e_{k^*}, \quad (38)$$

$$\text{Subject to } \sum_{m=1}^M l_m a_{mk^*p^*} \leq \sum_{k=1}^K L_k q_k, \quad (39)$$

$$\sum_{k=1}^K q_k = 1, \quad (40)$$

$$a_{mk^*p^*} \geq 0 \text{ and integer, } m = 1, \dots, M, \quad (41)$$

$$q_k \geq 0 \text{ and binary, } k = 1, \dots, K \quad (42)$$

where

e_{k^*} = the simplex multipliers of the used stock k^* for cutting a new pattern from constraints (31),

$a_{mk^*p^*}$ = the number of strips of item m cut in the new pattern p^* for stock length k^* , fulfilling $\sum_{m=1}^M l_m a_{mkp} \leq L_k$, and

q_k = the used stock length for stock k ; $q_k \in \{0,1\}$, if $q_k = 1$ then the stock length k is used. Otherwise, the stock length k is not used.

Every supplier is solved to obtain the effective patterns. If $V_{k^*} = \max \{V_k | k=1, \dots, K\} > 0$ then the new column $a_{mk^*p^*} = (a_{1k^*p^*}, \dots, a_{mk^*p^*})^T$ is introduced into model (30)-(33) as a new basis column. If $V_{k^*} \leq 0$, the current column is proven to be globally optimal. For a profound description of the column-generation procedure for cutting stock problems, see Lasdon (1970). To illustrate the CG procedure, a flow chart is shown in Figure 1.

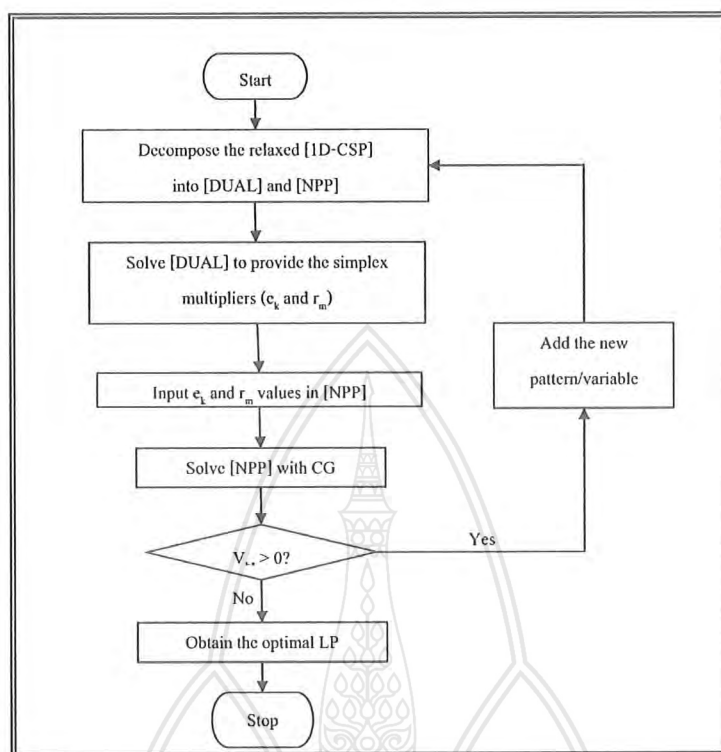


Figure 1. Flow chart of Column-Generation procedure.

4. THE PROPOSED HEURISTIC METHOD FOR SOLVING ILP

This heuristic method is proposed (Wongprakornkul, 2004; 2005) for solving the resulting obtained ILP after solving the relaxed 1D-CSP. The principle of round down method is developed. After solving the problem as an LP, all decision variables are obtained. Consider and there are three categories of the values of such as in the interval; a) greater than or equal to 1, b) between 0 and 1, and c) equal to zero. Hence, three groups of constraints are added under three cases as below:

Case1: If $X_{kp} \geq 1$ then the constraints, $X_{kp} \geq \lfloor x_{kp} \rfloor$, are added.

Case2: If $0 < X_{kp} < 1$ then the constraints, $X_{kp} \leq 1$, are added.

Case3: If $= 0$ then add it in set C, and the constraint, $\sum_{x \in C} x_{kp} \leq 1$, is added.

For solving the ILP with the proposed heuristic method, the constraints (46)-(48) are added. Let $\dot{x}_{kp}$, $\ddot{x}_{kp}$ and $\ddot{x}_{kp}$ be X_{kp} in case1, case2, and case3, respectively. The mathematical model can be written as follows:

$$\text{Minimize } \sum_{k=1}^K \sum_{p=1}^{P_k} g_k x_{kp} \quad (43)$$

Subject to

$$-\sum_{p=1}^{P_k} x_{kp} \geq -R_k, \forall k, \tag{44}$$

$$\sum_{k=1}^K \sum_{p=1}^{P_k} a_{mkp} x_{kp} \geq D_m, \forall m, \tag{45}$$

$$\dot{x}_{kp} \geq \left\lfloor \dot{x}_{kp} \right\rfloor \forall k, p, \tag{46}$$

$$\ddot{x}_{kp} \leq 1 \forall k, p, \tag{47}$$

$$\sum_{x \in C} \ddot{x}_{kp} \leq 1 \forall k, p, \tag{48}$$

$$x_{kp} \geq 0 \text{ and Integer}, \forall k, p. \tag{49}$$

5. RESULTS

In order to prove the effectiveness of the proposed heuristic method, two approaches for solving ILP were raised, A0 and A1. A0 is the direct method, where as A1 is the heuristic method with round down techniques, as shown in Figure 2. And an integrated one-dimensional cutting stock problem and transportation problem, [1D-CSP&TP] (see (Wongprakornkul, 2004)), was created as samples of ILP for testing. Then 192 instances were generated. Two performances were investigated for the effectiveness: the CPU Time and the relative-different Percentage.

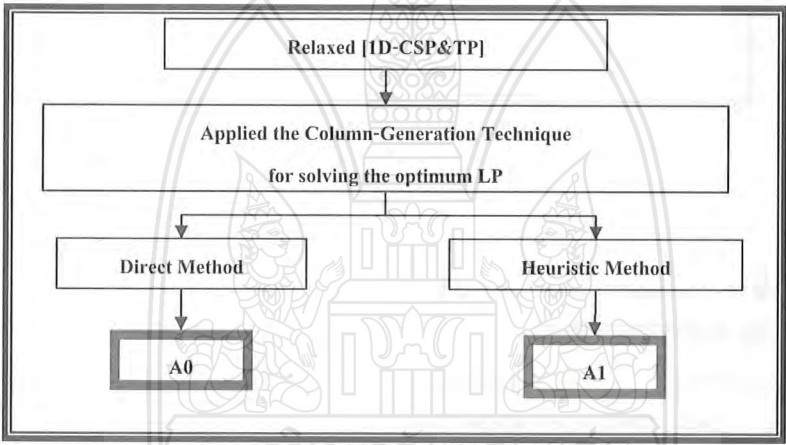


Figure 2. Two approaches for solving ILP.

The relative-different Percentage could compute as the formula below:

$$R_s \% = \left(\frac{ILP_s - LP}{LP} \right) * 100\%; s = 0, 1.$$

In the experiments, the limited computation time is 6 hours. Therefore, any instance, which could not solve within 6 hours, was ignored. The results are shown in Table 2.

Table 2. Results of two performances by solving with A0 and A1.

Approach	Number of Instances	CPU. Time (seconds)		Relative Difference (%)	
		Min.	Max.	Min.	Max.
A0	141*	0.7	19,231.1	0	0.1897
A1	192	0.8	575.3	0	0.1897

Note: *Excluding the instances that took the computation time more than 6 hours.

6. CONCLUSIONS

A1 could solve 192 instances within 10 minutes or within the reasonable computation time and the maximum relative-different percentages of both A0 and A1 were the same. Hence, the heuristic method (A1) with round down techniques is more effective than the direct method for solving ILP.

These results are applied for doing the production planning. If some industries, who can form this particular mathematical model, need to plan quite often, such as every month, every week or even everyday, then an effective heuristic method is an alternative approach for solving the problem.

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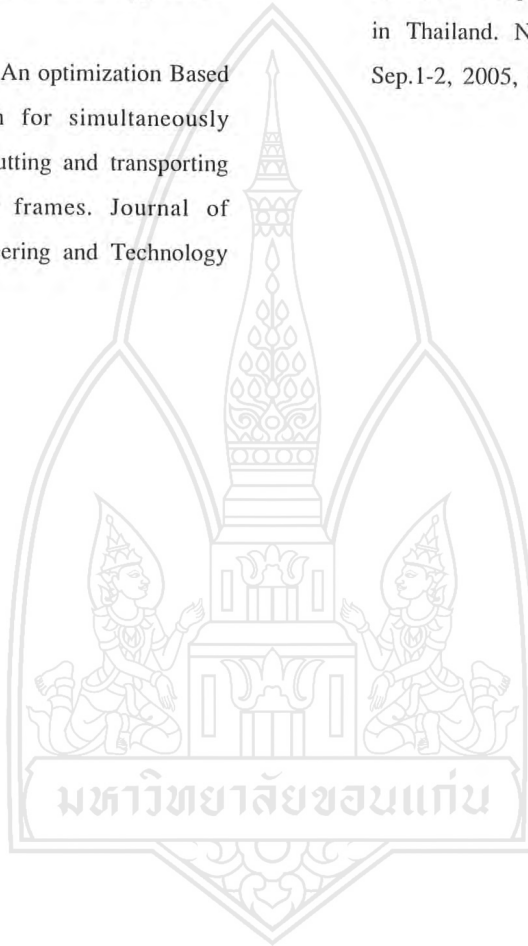
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The Local Livelihood and Natural Resource Management Survey and Its Implication on the Integrated Conservation and Development Projects: A Case Study in Attapeu, Lao PDR

การสำรวจการจัดการทรัพยากรธรรมชาติและความเป็นอยู่ใน
ท้องถิ่นกับการนำไปใช้ในโครงการอนุรักษ์และพัฒนา
แบบผสมผสาน: กรณีศึกษาในจังหวัดอัตตะปือ ประเทศสาธารณรัฐ
ประชาธิปไตยประชาชนลาว

Pongthep Suwanwaree^{1} and Phaivanh Phiapalath²*

Abstract

In recent years, natural resources became ever more threatened due to high population growth and related demands from the increased number of inhabitants on one hand and lack of proper natural resource management on the other. This study looked at the needs for livelihood development and natural resource management of local people in Attapeu province, Laos and presents some recommendations for effective Integrated Conservation and Development projects. Semi-structure interview (SSI) and participatory rural appraisal (PRA) were used in February 2005 with key informants from 12 pilot villages in the Mekong Wetland Biodiversity Programme demonstration site. The study proved that the people in the region preferred buffalo raising and trading for making their personal income. Their second choice for earning money was growth and sale of vegetables and handcraft, followed by fishing, the operation of sugarcane plantations and the ecotourism business. Thus improving quality of agricultural products through proper irrigation schemes and increasing ware value on the marketplace was considered by most farmers as valuable strategy for heightening their profits. As for threats, residents listed encroachment as the main threat to their resources, but also mentioned lack of education and awareness and over harvesting as permanent risks. They identified potential solutions for conserving their environment as enforcement of more effective regulations from the local and national government, establishment of fish conservation zones, and education of people in the value of nature preservation. Setting the right balance between progressive civilization development

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and environment conservation is however a very complex task as it has social, ecological, economic and political dimensions. To ensure success of potential projects, all stakeholders need to be well informed at all levels and should actively participate on every step of initiative from its design all the way through to execution, project orientation and other information schemes are thus highly recommended.

บทคัดย่อ

หลายปีมานี้ทรัพยากรธรรมชาติถูกคุกคามเนื่องจากการเพิ่มขึ้นของประชากรและความต้องการ โดยขาดการจัดการที่เหมาะสม การศึกษาครั้งนี้ได้สำรวจการพัฒนาชีวิตและการจัดการทรัพยากรธรรมชาติของประชาชนในจังหวัดอุตรดิตถ์ พร้อมทั้งได้ให้ข้อเสนอแนะ เพื่อให้โครงการอนุรักษ์และพัฒนาแบบผสมผสานในประเทศลาวมีประสิทธิภาพมากยิ่งขึ้น การศึกษาทำโดยใช้การพูดคุยถึงสัมพันธภาพและประเมินสภาพท้องถิ่นอย่างมีส่วนร่วม ในพื้นที่ 12 หมู่บ้านของโครงการความหลากหลายทางชีวภาพในพื้นที่ชุ่มน้ำของแม่น้ำโขง ช่วงเดือนกุมภาพันธ์ พ.ศ. 2547 ผลการศึกษาพบว่าชาวบ้านต้องการเพิ่มรายได้โดยการเลี้ยงกระบือเป็นอันดับแรก รองลงไปคือการจำหน่ายพืชผักสวนครัวและงานฝีมือ ตามด้วยการประมง ปลูกอ้อย การท่องเที่ยวเชิงนิเวศ และส่งเสริมการชลประทาน ตามลำดับ ส่วนภัยที่คุกคามทรัพยากรธรรมชาติอันดับหนึ่งคือการบุกรุกจากคนนอกพื้นที่ ตามด้วยการขาดการศึกษาและจิตสำนึกของชุมชน และการเก็บเกี่ยวผลผลิตมากเกินไป ชุมชนได้เสนอแนวทางแก้ไข โดยให้เพิ่มการปราบปรามจับกุมโดยเจ้าหน้าที่ของรัฐ กำหนดพื้นที่อนุรักษ์พันธุ์ปลา และให้การศึกษาแก่ผู้คนถึงประโยชน์ของการอนุรักษ์ธรรมชาติ การผสมผสานระหว่างการอนุรักษ์และการพัฒนาเป็นสิ่งที่ซับซ้อน เพราะครอบคลุมมิติทางสังคม นิเวศวิทยา และการเมือง เพื่อให้ประสบความสำเร็จ ผู้มีส่วนได้ส่วนเสียทั้งหมดควรได้รับข้อมูลอย่างครบถ้วนและมีส่วนร่วมในโครงการพัฒนาทุกขั้นตอน

Keywords: Integrated Conservation and Development (ICAD), natural resource management

คำสำคัญ: การอนุรักษ์และพัฒนาแบบผสมผสาน, การจัดการทรัพยากรธรรมชาติ

INTRODUCTION

Laos PDR is a small land-locked mountainous country endowed with a rich biodiversity and a rather high forest covered area when compared to neighboring countries, with more than 20 % forest area covering 41.5 % of the country's land surface (MAF, 2005). Also, there are 20 National Protected Areas (NPAs) covering approximately 14 % of the country. The organization of protected area management in Laos is fairly different from other countries in view of the fact that local people can live legally inside the designated zones of protection. All NPAs in Laos can be classified as close to category VI of

the classification that *The International Union for the Conservation of Nature* (IUCN) proposed in its standardized protected area system (managed area). Eighty percent of Laotian people are poor, live in rural areas, and rely only on agriculture and forest products (Morris et al., 2004). To fight poverty but also degradation of the environment, a dozen of projects with both conservation and development objectives were implemented in many protected areas in Laos about a decade ago as part of a programme commonly known as *Integrated Conservation and Development* (ICAD). However, ICAD projects focused on development rather than conservation

and rarely succeeded. Moreover, even though the projects used a lot of valuable budget they failed to inform the concerned stakeholders in the areas, did not really involve them in their procedures and had little activities about education and awareness rising. Unfortunately, ICAD projects often ended in short periods with more damage to natural resources than positive effects to the environment.

It is a common belief that (local) people can get a better life when they have access to higher income. Therefore, the creation of alternative

and/or improvement of existing pathways to income for local people are among the basic topics that have to be addressed in the design of every development project. In view of that, this paper describes the priorities of local people in selected villages for income generation, illustrates their opinions on how to conserve and manage natural resources in a sustainable way, discusses potential impacts of ICAD projects based on past experience, and finally provides some recommendations for the establishment of more effective ICAD projects in the future.

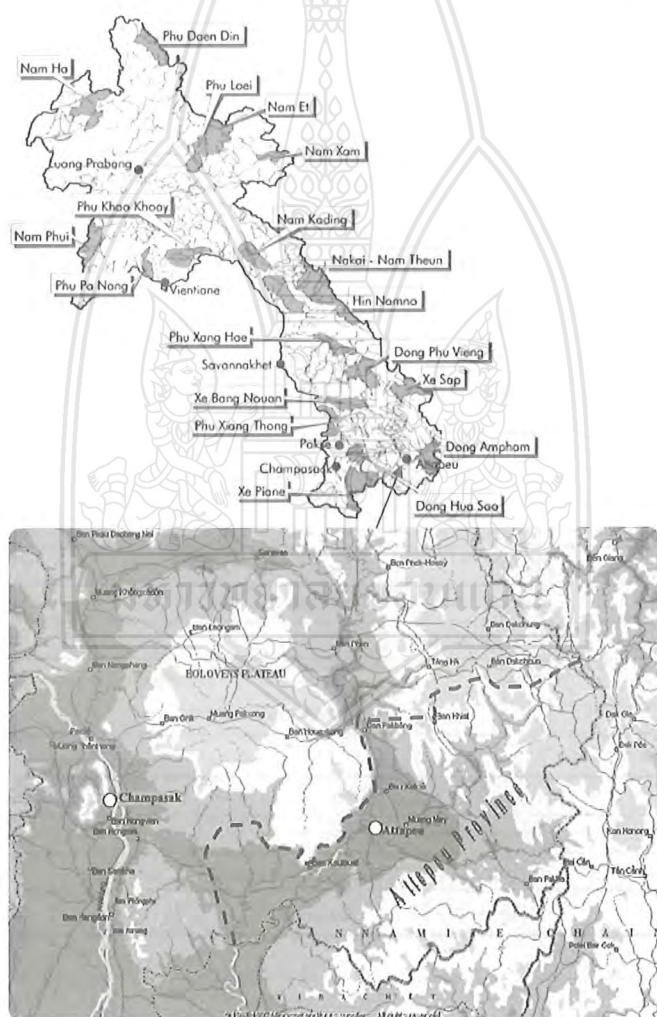


Figure 1. The national protected areas in Laos, PDR and Attapeu province.

THE STUDY AREA

Attapeu province is located in the Southeast of Laos, bordering Vietnam to the east and Cambodia to the south (see the map in Figure 1). The province has approximately 100,000 people with a wide diversity of ethnic groups. Despite the fact that it is one of the seven poorest provinces in the country, the province still has a rich biodiversity and high forest coverage at 61 % (934,489 ha) of the total land area (WWF, 2003). The province has two National Protected Areas (Xe Pian and Dong Ampham) and one proposed National Protected Area (Xe Khampho) all three being home of a great number of wildlife. Importantly, large portions of the province are covered by wetlands (both seasonal and perennial). Local people mainly depend on forest and wetland products for food and as sources of income. The main resource, particularly in the southern lowland areas, is fish, which accounts for 80 - 90 % of animal protein in the diet of lowland

communities (Morris et al., 2004). Forest products and wetland resources do not only provide critical livelihood resources, but also help local people in times of hardship, such as during crop failures or natural disasters. As demands for resources increased, some forest and wetland areas have been converted to agricultural land during the last 15 years.

The Mekong Wetland Biodiversity Programme (MWBP)³ has been working with the officials and local communities in Attapeu province since 2004. The project lies in three lowland districts that cover some part of Xe Pian NPA and Xe Khampho proposed NPA. Project objectives are to assist the local people with the identification of proper strategies for managing their remaining wetlands and wetland resources and enhancing resource quality as well as capacity. At the same time the projects aimed also to be a stimulus for non-profitable livelihood development activities related to the health and agricultural sectors.

Table 1. Population of the studied villages.

District/village	Established	Ethnicity	Population	Household
<i>Sanamxay district</i>				
Ban Sompoy	Before 1975	Lao loun	869	184
Ban Seankeo	1987	Sou	136	29
Ban Hat Oudomxay	1989	Sou	354	69
Ban Phon Sa-at	1985	Lao loun and Sou	495	91
Ban Mai	Before 1960	Lao loun	560	116
Ban Pindong	Before 1975	Sou	232	42
Ban Tamoyot	1983	Sou, Lave, Ouy, Laoloun, Nyaheun, Kacheng,	168	29
<i>Samakhixay district</i>				
Ban Ka Yeur	1996	Ouy	472	81
Ban Halang Yai	Before 1990	Lave	559	98
Ban Kasom	Before 1980	Lao loun	436	96
Ban Haisok	Before 1975	Lao loun	568	110
<i>Xaysetha district</i>				
Saysi	1930	Lao loun	1,030	198

Source: Phiapalath (2005)

³ Mekong Wetlands Biodiversity Programme (MWBP) is a joint funding project of Global Environment Facility (GEF), UNDP, The Mekong River Commissions (MRC) and The World Conservation Union (IUCN)- working in four countries of Lower Mekong Basin (Cambodia, Laos, Thailand and Vietnam).

METHODOLOGY

Attapeu province was selected for this case study because many ICAD projects were going on in this province. Twelve MWBP Pilot villages were selected because they had and used wetlands as important sources for foods and income. The list of villages and their population are described in Table 1.

Key informants (10 men and 10 women) in each village were identified based on their expertise in hunting, fishing and using forest products. The interviews were conducted by using

both semi-structured interview (SSI) and participatory rural appraisal (PRA) to understand the village profile and socio-economic situation. Group discussions of key informants were conducted to identify status of natural resources and wetlands (see Figure 2).

Two teams of investigators held the interviews. The first team (3 staff) questioned four villages during February 15 - 21, 2005 and the second team (4 staff) interviewed eight villages during February 21 - 27, 2005.



Figure 2. Local people and their livelihoods in the studied villages.

RESULTS AND DISCUSSIONS

All 12 visited and examined villages are located in lowland plain areas along Sekong and Xe Pian Rivers, in areas that are mainly dominated by seasonal wetlands. Perennial wetlands are scattered throughout these areas, especially in Sanamxay

district. Sanamxay contains high biodiversity values, and is a part of Xe Pian National Protected Area, the second highest biodiversity of National Protected Area (Ling, 1999) and the most important area for water bird conservation in the country.

The poorest villages⁴ were Ban Seankeo, Ban Hat Oudomxay and Ban Tamoyot, where the poverty rate was 65 %. Noticeable signs for household poverty were rice shortage, lack of animals for plowing, absence of a family leader and labors, lack of paddy lands with regular irrigation supply, natural disasters (floods, drought) and pest infestation. All three villages were located far from the main conservation areas. Since natural resources were not managed properly and human needs were increasing with growing population, fish became scarce and not enough to harvest and sell. This effect became obvious especially in the dry season when only few and small fish were available.

Education: Schools in general were short in teachers and essential teaching material, particularly tables, manuals and student books, were missing. The low numbers of educators led, for instance, in Ban Tamoyot, that in fact, classes had to be cancelled for long times because the instructor became sick. Ban Pindong, as another example of unacceptable educational conditions, had only one small school building with three classrooms, but had five different classes taught by only two teachers. Poor attendance records of sub-ethnic group children as Lave, Sou, and Cheang were found throughout the examined communities. A negative finding was also that teachers had not been paid for five to six months.

Health: Most villages had only few or no toilets. Villagers did not have access to clean tap water. Thus, many villagers had to use water from

ivers, streams or natural ponds as drinking water. Besides, there were insufficient medicinal supplies against the widespread of diarrhea, malaria and stomach-ache.

Agriculture: Most villages had in operation rice paddy field cultivation and vegetable gardening, except at Ban Tamoyot, which still practiced upland rice cultivation. Chemical fertilizers were used for paddy rice cultivation but the rice production was still low with an average of 1.5 tons/hectare. Crop damage from flood, drought, pest and lack of suitable rice variety were also of concern.

Livestock: Villagers mainly raised buffalos, but some villages included cows, pigs and chickens. Buffalo theft and the appearance of livestock diseases were often stated as problems. It appeared in interviews that attempts of disease prevention in pigs and chickens often failed. Villagers reported that they had to inject their livestock themselves with drugs when animals became infected but saw that due to lack of expertise and skills their treatments tend to be improper. Trained staff however were exceptional and thus the quality of livestock disease prevention remained extremely low.

Food: Main food sources were forest and wetland products such as crabs, frogs, snails, shrimps, and a variety of vegetables, mushrooms, and bamboo shoots. According to Meusch (2005), local people performed fishing mainly for their own consumption (70 %) and only little for sale.

Income: Villagers income came mainly from selling livestock (cattle and, though to less

⁴ A village poverty in the study context: more than 50 % of a number of households had insufficient of rice for consumption, lack of suitable paddy land for cultivation, no buffalos, live in temporary houses and lack public facility services such as access, school, health etc.

extent, pigs). Other goods brought to the markets included fish, rice, firewood, saw-wood, local tobacco, vegetables, non-timbered forest products (NTFPs) such as mushrooms, "*Maksengbua*" (*Strychnos nux vomica*) and handicrafts. The average monthly income of a typical local household in the studied regions was about 500 Laotian Kip, which is equivalent to about US\$ 50.

Priority needs for income generation

Highest priority for income generation was given to cattle raising, reaching 43 % of the total of the mentioned income activities. Second choice was gardening (13 %), followed by handcraft (13 %), fish raising, sugarcane plantation and ecotourism (9 % each) and last of all irrigation (4 %). Some villagers said that raising buffaloes was like having an account with a bank, from which they could withdraw money when needed simply by selling one or more of the animals. Accordingly, buffaloes were recognized as most important source of income and saving for a family. However, diseases occasionally affected the buffalo herds and valuable animals were lost by death. Roaming buffaloes, on the other hand, sometimes caused serious damage to forest and farmland, which was costly for the owners.

With respect to handcraft, some villagers raised in the interviews the point of how to add value to forest products (e.g. their handcraft made from rattan). Certainly, the market demand has to be seen as the most influential factor for this type of family business. Many development activities failed because the local marketing system capacity was not enhanced at the same time as the product output. In addition, some handicrafts used raw materials from forests in the back country so evaluation of

the existing forest resources is a prerequisite for better management.

For gardening, local people thought of growth of a variety of crops that suited their needs, among them are banana, tobacco, and chili. The application of chemical fertilizer was seen as a good chance for increasing crop yield but fear of undesired side effects of the possibly toxic chemicals were expressed as a major concern. The introduction of bio-fertilizer as an alternative to conventional fertilizers thus should be thoroughly considered to address the concerns of the local farmers and the protection of their environment against unwanted pollution.

Another local need mentioned again and again was the enterprise of an efficient aquaculture. Villagers wanted to raise fish in a pond or a cage in a river or paddy field during the cultivating season. Native species and simple breeding and fattening techniques should of course be introduced when supporting local people in establishing the desired aquaculture. Additionally, a well-recognized ecotourism could probably be a good way to provide substantial alternative income; however, it requires a capital funding and may have strong impacts on the integrity of the natural resources and local culture. Lastly, irrigation was also mentioned by a good number of participants of the study as strategy they would like to have in well-working place to improve their situation because it could help people gain more cultivated areas and become independent from seasonal changes in humidity as long as the water supply through irrigation schemes is offered at an adequate level. A negative ecological side effect of irrigation, however, may be the impact on fish migration therefore careful technological design is needed from the involved engineers.

Status of natural resources

According to the interviews, local resources had declined gradually for many years. For example, fish caught per person per day reduced from five kilograms in former times to only one kilogram or less nowadays. To establish better resource management, it is of highest importance to support the villagers in many ways. The details of findings are as below:

Wetlands and their products: There were many items that local people harvested for household consumption and sale. For example, eels, snails, frogs, weeds were taken from natural ponds, marshes, and rivers. According to Mather et al., (1999), 150 animal species were available in the back country of Xe Pian region at the time of study, about 40 species of fish could be caught in the local rivers (Poulson and Luanglath, 2005), and frogs, shrimps, snails are present in natural ponds. Seasonal rivers had a similar function as natural ponds with respect to food supply. Within and in the surroundings of wetland and flood plains, a large variety of vegetables and mushrooms grew, especially in the wet season.

NTFPs: According to Poulson and Luanglath (2005), 19 species of NTFPs were harvested in the inspected area. From the interview, the most important NTFPs were Maksengbua (*Strychnos nux vomica*), and Makchong (malva nuts, *Scaphium macropodium*). In fact, Makchong is considered a good natural product for sale associated with high market demand. Interestingly, many Maksengbua and Makchong trees were cut down and fruits were harvested before maturity was reached. The low quality products associated with immature harvest led to low prices, which of course is a disadvantage for the buyer and seller. The observed

misbehavior of locals in this matter most likely is caused by lack of education and ownership (feeling of responsibility) which in turn leads to the waste of these sustainable natural resources.

Wildlife: Many wild animals have been frequently spotted in the surroundings of the Pindong and Phonsa-at villages because both are located inside the Xe Pian NPA. Tracks of some wild animals such as the sambar, barking deer and wild pigs, were even seen close to the villages. Wildlife harvesting was low compared to fish and other forest products since the local villagers' guns had been handed over to the government during the last 10 years.

Fishery and fish management

A majority of villagers fished for their own consumption, only a few for vending. Meusch (2005) reported that 70 % of the caught natural fish were used for consumption. As mentioned already, the amount of fish caught per day per fisherman had declined in recent years from five kg to one kg. People in Ban Mai and Ban Sompoy engaged both fishing and purchasing fish from fishers from other villages to cover personal consumption. Fish price depended on species and season; while in towns, prices often rose up to twice as much as in rural areas even though the locations were close to the settlement. Reason seemed to be the prizing on the urban marketplace, which was performed by middlemen with no regulation from local government.

Fishing problems: The use of effective equipment such as Nakak (diving goggle with spears) was mentioned as a method that inflicted pressure on the existing fish populations, resulting in a decline of fish numbers in rivers. Villagers suggested

that fishing equipment with *Nakak* practice must be curtailed and the government should enforce this regulation effectively. As for other dangerous fishing practices, both the illegal electro fishing and fishing with poisons were reported. From the opinion of the informants, lack of enforcement of laws provided opportunities for outsiders to over harvest the existing resources (Ogawa, 2005). For example, a Vietnamese family lived at the mouth of Xe Pian river for 15 years and extensively caught migrating fish from the Sekong river with various sizes of fishing nets and apparently caused a considerable reduction in the upstream fish content. The family was several times asked to withdraw from the area but refused. Finally, in 2005 they were fined for 4 million kip or USD 400 and then removed against their own will and relocated at the Xe Khong River. Obviously, actions against critical abuse of natural resources such as the fish content should of course not be delayed for as long as in the above example if positive effects are target.

Fishery zoning: Many villages had fish conservation zones, which were usually established by the government. However, some villagers did not well understand the purpose of the establishments and ignored the areas of fish protection. This made the regulations for sheltering fish ineffective so they failed due to incursion by the local people in the villages and others from town. One exception was Ban Kasom, which had successfully established and managed a fish conservation zone by local villagers for many years. In this special case the villagers shared the ownership of the ponds and it was therefore in their own interest to monitor and guard their fish conservation zones cautiously and regularly.

Factors putting pressure on natural resources

The outsider encroachment with modern tools for harvesting and an application of illegal fishing methodologies was the major concern (61 %), followed by the lack of education and awareness (31 %) and then over harvesting of natural resources (8 %), respectively. Local people typically had only a rudimentary form of education and accordingly no adequate knowledge on the values of natural resources. For example, some local villagers used to harvest forest products by cutting down the whole tree, even though the trees fruits were not yet ripe. They reasoned their bad behavior with the competition with other villagers or town people aiming for the same harvest. Consequently, their forest products had low price because they had low quality.

Local opinions for natural resource management

All interviewed people were aware of the declining fish population and the increasing difficulty of harvesting fish from the rivers. Natural resources have been used inappropriately by the means of illegal hunting and over harvesting by outsiders. About 44 % of the total responses mentioned the need of local regulations and strong enforcement. In addition, the need (30 %) was expressed for establishing a fish conservation zone, both restricted and controlled use zones. Finally, 17 % of the respondents reported the needs for better education and environmental awareness rising.

Establishment of fish conservation zone was first priority, then aquatic resource management and aquaculture (Meusch, 2005). This is similar to a protected area management, which demands clear zoning. Local people and outsiders may use natural

resources in managed zones as long as their harvesting is carried out in a sustainable manner. The effective operation of fish conservation zoning requires the organization of a community larger than just one village. It must involve the active and cooperative participation and agreement of all adjacent villages in order to co-manage existing resources and successfully enforce rules and regulations.

Potential impacts of development activities on the environment

The livelihood development activities of local villagers such as buffalos raising, handcraft, and fish raising almost certainly will have an impact on natural resources. Buffalos were released into the forest for browsing and stayed there for at least 6 months per year. They occupied saltlicks and disturbed inland water pools, the important feeding sites for water birds especially in dry season. For example, Nong Pheu (inland natural pond) in Ban Phonsa-at and Patumaung inland pond in Ban Sivilay, Sanamxay were two of the most important bird feeding sites in the Xe Pian NPA. In the dry season local villagers, buffalos and birds all used the same areas for their purposes. Bird feeding sites are limited and local villagers and buffalos certainly will disturb the bird there. However, the degree of negative impacts remains to be unclear and detailed scientific studies should be undertaken for clarification.

Many livelihood development activities have been funded by ICAD projects such as medium scale irrigation, economic/cash crop or tree plantations, agriculture expansion, fish and livestock raising, and clean water supplies. However, the conser-

vation activities have been omitted. It should be emphasized that the benefits from using irrigation would encourage the recipients to conserve forests. However, this approach to conservation might not continue once the funded project ends (Fisher et al., 2005). Some projects supported villagers in planting economic trees without any detailed preceding assessments, such as soil suitability tests, market analysis and even a screening of how to plant certain crops effectively under the local conditions. Such unplanned activities can increase deforestation and generate a negative impact on biodiversity. Many local people did not support the idea of economic tree plantation anyway because it required capital investment, which they rarely had. Similarly, other projects aimed at introducing some alien plant species for buffalo grazing or introducing exotic fish such as Tilapia and Indian carp into natural rivers and ponds. The latter attempt led, however, in parallel to the decline of some native fish species (Baird, 1995). Alien plant species not only affected the ecosystem but also the livelihood of local people given the decreasing rice yields induced by the introduced new weeds. There were also projects that supported the expansion of agriculture into forests and wetland areas and use of chemical fertilizer to improve the yields and quality of crop products but they went along with the risk of deforestation and water pollution. Well-meant human activities can generate severe negative impacts on ecosystems at an alarming rate.

There are many issues of local communities that need to be solved by possible ICAD projects, such as education, clean water supply, and low productivity of crops to name just a few. However, ICAD projects should not only provide what local people may need to live adequately but also educate

them in the value of nature conservation and how much natural resource loss is justified to improve their livelihood, and finally encourage cooperation between government and villagers.

RECOMMENDATIONS

ICAD projects often fail due to inadequate dissemination and awareness creation among the involved officials at early stages. Other reasons of failure are poor project design, bad influence of technical advisors who often have little knowledge on conservation backgrounds, lack of situation analysis, and suffer from unclear project implementation strategies. Here, we provide some recommendations to improve potential ICAD projects in the future.

- o For sustainability, socio-economic development should go hand in hand with nature conservation.
- o ICAD projects in designated areas should focus on improving local livelihoods through careful forest resource management. For example, value added forest products should be considered as obligatory elements.
- o Government officials need to visit the conservation areas very often for inspection and when illegal harvesting or other prohibited actions are noticed, immediate measures should be taken against the law breakers.
- o A wider community network building is required and thus should be targeted to achieve a common understanding and goals among villagers and government.
- o Even though, buffalo raising can affect the natural forest, it is worth funding if

the related adverse impacts are understood and minimized.

- o The ICAD projects should start at small scales with careful monitoring and then develop over time in financial and spatial dimension.
- o Scientists and social researchers should be part of larger ICAD projects and based on their expertise in providing advice on minimizing the impact of any planned human activity on the nature and local culture.
- o The dissemination of scientific information and ICAD project plan should reach wider communities of local people, practitioners, developers and decision-makers.

CONCLUSION

In order to gain more income, local people in the examined areas in the southeast of Laos preferred buffalo raising. Second choice was gardening and handcraft, followed by fishing, sugarcane plantation, ecotourism, and irrigation respectively. What the residents thought of as the major threat to their local natural resources was the encroachment from outsiders. Lack of education and awareness, and over harvesting were also seen as negative. The locals saw solutions to their problems in effectively enforcing more and better regulations from the government, in establishing thoroughly controlled fish conservation zones, and educating people in the value of nature conservation.

ICAD projects are generally complex and may suffer from conflicts between conservation and development, but certainly both two aspects must be considered and implemented equally into the

project design. There is a clear need to check the performance of ongoing projects to avoid negative subsequent impacts on the targeted environment. Emphasizing just on livelihood aspects may impede the ultimate goal of conservation. Though livelihood of local people may improve temporarily, permanent poverty reduction is impossible to achieve without proper and complete management of all facets of natural resources. Moreover, it is mandatory for projects to work closely with the local communities, enhance their quality of education and raising awareness, and provide effective public enforcement and legal support from the government. A parallel focus on conservation and development activities is necessary for ICAD projects from the planning to the final stage to ultimately achieve the goals of improved living conditions of residents on the one hand and prevention of natural resources and wild life on the other.

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How Playing Violent Computer Games Affects Thai Children

ผลกระทบของการเล่นเกมสโคมพิวเตอร์ของเด็กไทย

Saisunee Jabjone¹

Abstract

This study attempted to explore the influence of playing computer games on Thai children. A growing body of research has linked violent video game play to increased time spent playing computer games, poor attitudes and behavior. The goal was to examine the influence of playing computer games on Thai children's behavior such as aggression, adaptive skills, and emotional status. Independent variables include the type of computer game (violent and non-violent). Two hundred Thai students aged between 8 to 11 years from Nakhon Ratchasima Primary School participated in the study. The students were randomly selected from the school population and the data were collected by using survey questionnaire, self-report, parent rating scale and teacher rating scale. The results indicate that children who play violent games spend more time playing computer games, reported higher levels of negative behaviors such as aggression, conduct problems and lower levels of adaptive skills. Furthermore, the findings suggest that playing violent computer games raise risks of increasing maladaptive attitude and behavior.

บทคัดย่อ

ศึกษาหาผลกระทบของการเล่นเกมสโคมพิวเตอร์ในเด็กไทย โดยการวิจัยแสดงการเชื่อมโยงระหว่างพฤติกรรมของเด็กที่ชอบเล่นเกมสโคมประเภทรุนแรงกับการใช้เวลาในการเล่นเกมส์ ผลกระทบต่อทัศนคติและพฤติกรรมของเด็ก เช่น ความก้าวร้าว ความสามารถในการปรับตัว และสถานะทางอารมณ์ เป็นต้น ตัวแปรอิสระ คือ ประเภทของเกมสโคมพิวเตอร์ (เกมสโคมประเภทรุนแรงและเกมสโคมประเภทไม่รุนแรง) กลุ่มตัวอย่างเป็นเด็กอายุระหว่าง 8 ถึง 11 ปี ซึ่งสุ่มเลือกจากโรงเรียนประถมในจังหวัดนครราชสีมา โดยใช้แบบสอบถาม 3 ชุด คือ ทดสอบตัวนักเรียน ถามผู้ปกครองและถามครูผู้สอน ผลของการศึกษาแสดงให้เห็นว่าเด็กที่ชอบเล่นเกมสโคมประเภทรุนแรงมีการใช้เวลาในการเล่นเกมส์มากกว่าและมีพฤติกรรมและทัศนคติในด้านลบมากกว่าเด็กที่เล่นเกมสโคมไม่รุนแรง เช่น มีความก้าวร้าว มักก่อปัญหาในโรงเรียน และไม่สามารถปรับตัวเข้ากับผู้อื่นได้ เป็นต้น ผลสรุปของการวิจัยแสดงให้เห็นว่าการเล่นเกมสโคมรุนแรงเพิ่มความเสี่ยงในด้านลบต่อพฤติกรรมและทัศนคติของเด็ก

Keywords: violent computer games, aggression, maladaptive attitude and behavior

คำสำคัญ: การเล่นเกมสโคมรุนแรง, ความก้าวร้าว, ทัศนคติและพฤติกรรมในการปรับตัวเข้ากับผู้อื่น

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INTRODUCTION

Computer Technology has transformed society in profound ways. It has become integrated into many aspects of daily life from school to work, to banking and shopping, and more. Playing games has long been the most common computer activity for children (Turow, 1999). Video and computer games, therefore, continue to grow in popularity among children and youth. Recent advances in technology have made possible a more realistic, graphic depiction in the themes of electronic games. With the advanced improvement in the presentation of computer games comes an increased use of the technology to present extremely violent, bloody and sexually explicit game scenarios. As the largest consumers of these games are children, some thought needs to be given to the effects the games are having on them.

1. Defining violent games

There is much confusion about the definition of 'violent' and terms like 'media violence' and 'violent video\computer games'. Goldstein (1999) defines 'video game violence' as simulated aggression, being different from the real thing in many ways. Violence has numerous roles in games: for instance, violence as a problem-solver, story element, action-movie appeal, mindless entertainment, and effective way of visually conveying physical triumph within a story. Types of violence are realistic, intrinsic, excessive, and glamorized (Thompson and Haninger, 2001). The generic classification of computer and video games is widely employed by the Entertainment Software Rating Board (ESRB), the organization which rates videogames according to their content. The ESRB applies age-specific rating, ranging from "Early Childhood" (three years

and older) to "Adults Only" (18 years and older). Action/adventure games containing smashing, shooting, graphic assault, flying into and rolling over objects and characters were justified as being classified as violent. On the other hand, racing and sports games were characterized as containing minimal violence (Entertainment Software Rating Board, 2005). Defining games by genre aids in the exploration of the motivations behind the use of violence as a narrative or game play element, and Berens and Howard (2001) offer a schema for consideration. These include the genres of: action, driving and racing, first-person shooter, platform, role playing, strategy and simulation, sports and beat-'em ups.

2. History of violence in computer games

In the past 30 years, interactive video games have emerged as one of the most popular forms of entertainment, particularly among youth. The first generation of video games was nothing more than simple geometric shapes, one or more of which could be controlled by the game player (Kookjamas, 2004). In the 1980s, the first of the popular games to be considered violent was Pac Man. This game consisted of a small circle with a mouth that tried to eat pills and destroy ghosts. It was one of the first games to involve destruction of any kind (Kookjamas, 2004). Each generation of games always uses the newest technologies available, leading to more impressive graphics and realism. In the 1990s the face of video games changed dramatically. The consoles such as PlayStation, Nintendo 64 and Saturn brought the ability to generate real-time three-dimensional worlds and video-game designers seized the opportunity to offer yet more realistic and complex graphics (Newman, 2004).

The most popular video game of 1993 was *Mortal Kombat* (Elmer-Dewitt, 1993). This game features realistically rendered humanoid characters engaging in battle. As the name of the game implies, the goal of the player in *Mortal Kombat* is to kill any opponent he/she faces. Along with these new technologies come more realistic violent acts and situations. Another example is the game *Soldier of Fortune*, released in 2000 for the personal computer. In this game each character has 26 kill zones or areas that the character can be hit by a bullet (Gentile et al., 2004). Unfortunately, such violent games now dominate the market. Dietz (1998) sampled 33 popular Sega and Nintendo games and found that nearly 80 % of the games were violent in nature. Interestingly, she also found that 21% of these game portrayed violence towards women. Also, with each new generation of video games or computer games, people are spending more time and money on them.

3. Purpose and goals of the Study

While research on the impact of playing computers is still sketchy and unclear concerning the effects of playing computer games on Thai children, some initial research indications of positive and negative effects are beginning to emerge (Harris, 2001). The goals of this project were therefore to study and explore the effect of computer games in a scientific and controlled manner: It is hoped that the results will provide valuable insights into the influence of both non-violent games and violent games on Thai children.

4. Hypothesis

The study considers the following research question and hypothesis:

H0: There is no significant difference in the Self Report scores, Parent Rating scales, and Teacher Rating scales between children who like to play violent and non violent computer games.

METHOD

The children who participated in this study were recruited from a specific area in Thailand, namely Nakhon Ratchasima province. The school approached to participate in the study was the Nakhon Ratchasima primary school. This school was chosen because it is a prominent primary school having a large group of children from which the sample was randomly selected. The sample was drawn from fourth grade and fifth grade students. Subjects were selected from a population of at least 1,000 male and female children. In general, the age level of children in the fourth and fifth grades was 8 to 11 years. The sample eventually comprised 200 children who were stratified in the study so that equivalent numbers of subjects were male and female, with the final sample consisting of 104 males and 96 females, as shown in Tables 1 and 2. Students participating in the study were required to complete the following procedures: survey questionnaire, Self Report, discussion in separate groups and consent forms signed both by themselves and their parent or legal guardian. To measure the behavior of children in their home and school environments, the parent and teacher were also asked to complete the Parent Rating Scale (PRS) and Teacher Rating Scale (TRS) respectively.

Table 1. The number of children in each group.

Sex	violent games	Non violent games	Total
Male	86	18	104
Female	53	43	96
Total	139	61	200

Table 2. Mean ages and standard deviation of all participant children.

Sex	Mean ages (SD)	
	violent games	Non violent games
Male	10.2 (0.35)	10.1 (0.26)
Female	10.14 (0.44)	10.13 (0.35)

RESULTS

1. Genre preferences

Participants were asked to choose their favorite games in the following eight genres: action, fighting/combat, non violent sports, racing or violent sport, role play/adventure, simulation, puzzle, and educational games. Table 3 shows the percentage and frequency of the games that boys and girls

mostly like to play. The results show that the majority of participants prefer to play fighting/combat games (20 percent). Furthermore, boys and girls report different preferences. Boys prefer games with lots of fighting (25 percent) and action (21.1 percent). In contrast, girls are more likely to enjoy playing educational (33.3 percent) or role play/adventure games (19.8 percent).

Table 3. The percentages of game preference.

Game preference	Percentage (Frequency)		
	Boys	Girls	Total
Action games*	20.1 (21)	11.5 (11)	16.0 (32)
Fighting/combat games*	25.0 (26)	13.5 (13)	20.0 (40)
Non violent sports games	8.7 (9)	3.1 (3)	6.0 (12)
violent sports games*	10.6 (11)	4.2 (4)	7.5 (15)
Role play/adventure *	18.3 (19)	19.8 (19)	18.5 (37)
Simulation games*	8.7 (9)	6.3 (6)	7.5 (15)
Puzzle	4.8 (5)	8.3 (8)	6.5 (13)
Educational	3.8 (4)	33.3 (32)	18 (36)
Total	100.0 (104)	100.0 (96)	100.0 (200)

*Violent games

2. Time spent playing computer games

Table 4 shows that the majority of participants spend on average 4.5 hours playing computer games per week (34 percent), while 40.4 % of

boys spent an average 4.5 hours per week playing computer games which is more than girls do. Most of the girls spend about 1.5 hours per week playing computer games (45.8 percent).

Table 4. Distribution of hours of computer games played each week.

Average of hours per week	Percentage (Frequency)		
	Boys	Girls	Total
0	-	-	-
1.5	12.5 (13)	45.8 (44)	28.5 (57)
4.5	40.4 (42)	27.1 (26)	34 (68)
8.5	19.2 (20)	13.6 (13)	16.5 (33)
12.5	13.51 (14)	12.5 (12)	13 (26)
15	14.4 (15)	1.0 (1)	8 (16)

3. Type of games and children behavior

To test the influence of playing computer games on the children's behavior the following three sources were analyzed: children's self-report, parent rating scale, and teacher rating scale.

The hypothesis was tested by multiple analysis of covariance (MANCOVA) with sex as the covariate. If the MANCOVA was significant, then this was followed by analysis of variance (ANOVA) performed on the thirteen dependent variables (DV) from student's self-reports (attitude to school, attitude to teacher, atypicality, locus of control, social stress, anxiety, depression, sense of inadequacy, relation with parent, interpersonal relations, self-esteem, self-reliance, and emotional symptoms index) and thirteen dependent variables from parent rating scales and teacher rating scales (hyperactivity, aggression, conduct problems, anxiety, depression, somatization, attention problems, atypicality, withdrawal, behavioural symptoms index, adaptability,

social skills, and leadership). The independent variable (IV) was "type of computer game" (violent versus non violent games). The total number of children was 200 (Violent game (vg) = 139 and Non violent game (nvg) = 61).

3.1Self report

The results of the MANCOVA (covariate sex) conducted for thirteen dependent variables found significant differences between the two groups of children in self reported attitudes ($p = .009$). Individual ANOVAs were then conducted in order to find where differences existed, as shown in Tables 5 and 6. Significant differences were found for nine dependent measures in the self report (at $p < 0.05$): and these were: attitude to school ($F(1, 198) = 14.68, p = .000$); attitude to teachers ($F(1, 198) = 7.04, p = .009$); atypicality ($F(1, 198) = 7.42, p = .007$); locus of control ($F(1, 198) = 10.42, p = .001$); social stress ($F(1, 198) = 12.77, p = .000$); anxiety ($F(1, 198) = 8.51, p = .004$); depression

($F(1, 198) = 11.21, p = .000$); sense of inadequacy ($F(1, 198) = 6.67, p = .001$); and emotional symptoms index ($F(1, 198) = 8.35, p = .004$). These analyses indicate that the children who favour violent games have higher mean scores in nine characteristics than children who prefer non-violent games. As a result, the children who mostly like to play violent games have more undesirable characteristics in their attitude to school ($M = 3.3$ vs. $M = 2.38$), attitude to the teacher ($M = 4.34$ vs. $M = 3.71$), atypicality ($M = 4.88$ vs. $M = 3.67$), locus of control ($M = 6.46$ vs. $M = 4.9$), social stress ($M = 5.0$ vs. $M = 3.62$), anxiety ($M = 8.36$ vs. $M = 6.75$), depression ($M = 4.47$ vs. $M = 3.46$), and sense of inadequacy ($M = 3.25$ vs. $M = 2.34$) than children

who favor non-violent games. Furthermore, the analyses indicate that the type of computer game played by children significantly affects children's self-reported emotional behavior ($p < .05$). It shows that children who mostly like to play violent games have a higher "Emotional Symptoms Index" than children who like to play non-violent games ($M = 27.5$ vs. $M = 23.2$). However, the analyses revealed no significant effect of the type of computer game on the children's attitude towards their relations to parents ($F(1, 198) = 2.41, p = .122$), interpersonal relations ($F(1, 198) = 2.38, p = .125$), self-esteem ($F(1, 198) = .72, p = .398$), or self-reliance ($F(1, 198) = .00, p = .995$).

Table 5. Mean and Standard Deviation of Self Report by Type of games.

	Violent games (n = 139)		Non violent games (n = 61)	
	M	S.D.	M	S.D.
Attitude to school	3.30	1.64	2.38	1.36
Attitude to teacher	4.34	1.61	3.71	1.42
Atypicality	4.88	2.97	3.67	2.72
Locus of Control	6.46	3.29	4.90	2.77
Social Stress	5.00	2.53	3.62	2.45
Anxiety	8.36	3.63	6.75	3.48
Depression	4.47	2.08	3.46	1.65
Sense of inadequacy	3.25	2.49	2.34	1.72
Relation with parent	5.68	1.71	6.12	2.02
Interpersonal relations	3.81	1.83	4.26	2.05
Self-esteem	2.61	1.07	2.75	1.15
Self-reliance	8.10	2.22	8.10	2.42
Emotional Symptoms Index	27.50	9.99	23.20	8.00

Table 6. Analysis of Variance of Self Report Scores and Type of computer games.

Dependent Variable	F	Sig.
Attitude to school	14.68	.000**
Attitude to teacher	7.04	.009*
Atypicality	7.42	.007*
Locus of Control	10.42	.001*
Social Stress	12.77	.000**
Anxiety	8.51	.004*
Depression	11.21	.000**
Sense of inadequacy	6.67	.001*
Relation with parent	2.41	.122
Interpersonal relations	2.38	.125
Self-esteem	.72	.398
Self-reliance	.00	.995
Emotional Symptoms Index	8.35	.004*

* p < 0.05
** p < 0.001

3.2 Parent Rating Scale

The results of MANCOVA (covariate sex) conducted for thirteen dependent variables found significant differences between the two groups of children in parent attitudes ($p = .000$). Individual ANOVAs were then conducted in order to find where differences existed, as shown in Tables 7 and 8. Significant differences were found for eleven dependent measures in parent rating scale ($p < .05$): These were: hyperactivity ($F(1,198) = 4.82, p = .029$); aggression ($F(1,198) = 6.30, p = .013$); conduct problems ($F(1,198) = 10.71, p = .001$); depression ($F(1,198) = 9.50, p = .002$); somatization ($F(1,198) = 7.67, p = .006$); withdrawal ($F(1,198) = 14.47, p = .000$); attention problems ($F(1,198) = 6.39, p = .012$); behavioral symptoms index ($F(1,198) = 11.24, p = .001$); adaptability ($F(1,198) = 64.5, p = .000$); social skills ($F(1,198) = 51.95, p = .000$); and leadership ($F(1,198) = 100.67, p = .000$). These analyses indicated that the vg children had higher levels of hyperactivity ($M = 9.55$ vs. $M = 8.62$), aggression ($M = 10.73$ vs. $M = 9.25$), conduct problems ($M = 6.41$ vs. $M = 4.82$), depression ($M = 6.25$ vs. $M = 4.77$), somatization ($M = 10.68$ vs. $M = 9.11$), withdrawal ($M = 8.41$ vs. $M = 6.93$), and attention problems ($M = 10.02$ vs. $M = 9.07$) than nvg children. On the other hand, the nvg children had significantly higher adaptability ($M = 14.34$ vs. $M = 11.51$), social skills ($M = 27.26$ vs. $M = 21.37$), leadership ($M = 22.54$ vs. $M = 15.69$), and adaptive skills ($M = 64.64$ vs. $M = 48.58$) than vg children. The type of computer games significantly affects children's behavior ($p < .05$). The data show that children who mostly like to play violent games

have a significantly higher behavioral symptoms index effect than children who like to play non-violent games ($\underline{M}$ = 55.91 vs. $\underline{M}$ = 48.98). However, the analyses revealed no significant effect of type of computer games for anxiety ($F(1,198) = 2.99, p = .085$), and atypicality ($F(1,198) = 3.63, p = .058$), though a non significant trend was found for the vg children to be more anxious and be more atypical.

Table 7. Mean and Standard Deviation of Parent Rating Scale by Type of game.

	Violent games (n = 139)		Non violent games (n = 61)	
	<u>M</u>	<u>S.D.</u>	<u>M</u>	<u>S.D.</u>
Hyperactivity	9.55	2.88	8.62	2.39
Aggression	10.73	4.08	9.25	3.23
Conduct problems	6.41	3.37	4.82	2.62
Anxiety	10.62	4.45	9.49	3.73
Depression	6.25	3.31	4.77	2.67
Somatization	10.68	3.99	9.11	2.81
Atypicality	8.75	3.41	7.79	2.98
Withdrawal	8.41	2.59	6.93	2.35
Attention problems	10.02	2.52	9.07	2.33
Behavioral Symptoms Index	55.91	14.52	48.98	10.64
Adaptability	11.51	2.68	14.34	2.74
Social Skills	21.37	5.39	27.26	5.14
Leadership	15.69	4.37	22.54	4.62

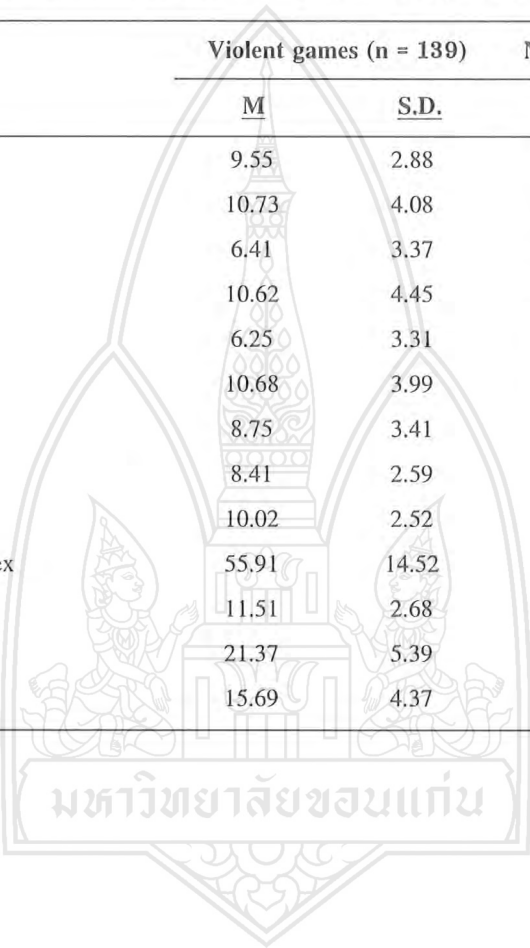


Table 8. Analysis of Variance of Parent Rating Scale and Type of computer game.

Dependent Variable	F	Sig.
Hyperactivity	4.82	.029*
Aggression	6.30	.013*
Conduct problems	10.71	.001*
Anxiety	2.99	.085
Depression	9.50	.002*
Somatization	7.67	.006*
Atypicality	3.63	.058
Withdrawal	14.47	.000**
Attention problems	6.39	.012*
Behavioral Symptoms Index	11.24	.001*
Adaptability	64.5	.000**
Social Skills	51.95	.000**
Leadership	100.67	.000**

* $p < 0.05$
** $p < 0.001$

3.3 Teacher Rating Scale

The results of the MANCOVA (covariate sex) conducted for sixteen dependent variables found significant differences between the two groups of children in teacher attitudes ($p = .000$). Individual ANOVAs were then conducted in order to find where differences existed, as shown in Tables 9 and 10. Significant differences were found for two dependent measures in the teacher rating scale ($p < .05$); and these were aggression ($F(1,198) = 12.93$, $p = .000$), and conduct problems ($F(1,198) = 4.98$,

$p = .027$). The analyses revealed no significant ($p > .05$) effect on the type of computer games to hyperactivity, anxiety, depression, somatization, atypicality, withdrawal, attention problems, behavioral symptoms index, adaptability, social skills, and leadership. According to the teachers, the results showed that children who mostly like to play violent games have significantly higher levels of aggression ($M = 10.51$ vs. $M = 8.66$), and conduct problems ($M = 4.42$ vs. $M = 3.54$) than children who mostly like to play non-violent games.

Table 9. Mean and Standard Deviation of Teacher Rating Scale by Type of game.

	Violent games (n = 139)		Non violent games (n = 61)	
	<u>M</u>	<u>S.D.</u>	<u>M</u>	<u>S.D.</u>
Hyperactivity	12.35	3.83	11.34	3.84
Aggression	10.51	3.44	8.66	3.16
Conduct problems	4.42	2.74	3.54	2.07
Anxiety	5.53	2.77	5.49	3.18
Depression	6.62	2.81	6.74	3.0
Somatization	8.18	2.69	7.74	2.73
Atypicality	8.71	3.59	9.07	3.11
Withdrawal	6.23	2.79	6.59	2.67
Attention problems	7.17	2.59	6.87	2.74
Behavioral Symptoms Index	50.89	11.97	48.16	12.53
Adaptability	6.67	2.18	6.17	2.4
Social Skills	16.23	4.53	15.25	4.63
Leadership	11.73	3.91	10.87	4.19

Table 10. Analysis of Variance of Teacher Rating Scale and Type of computer game.

<u>Dependent Variable</u>	<u>F</u>	<u>Sig.</u>
Hyperactivity	2.89	.091
Aggression	12.93	.000**
Conduct problems	4.98	.027*
Anxiety	.01	.928
Depression	.07	.787
Somatization	1.14	.288
Atypicality	.56	.454
Withdrawal	.44	.506
Attention problems	.72	.396
Behavioral Symptoms Index	2.14	.145
Adaptability	2.15	.144
Social Skills	1.97	.162
Leadership	1.99	.160

* p < 0.05

** p < 0.001

DISCUSSION

1. Preferences for violent computer games

The demographic data show that the majority of Thai children aged between eight and eleven years play computer games for around 3 to 6 hours per week, with a mean of 4.5 hours a week. Studies reported in the literature (e.g. Comber et al., 1997; Dorman, 1997; Funk and Buchman, 1996; Miller et al., 1996; Mumtaz, 2001) consistently show that boys play more electronic games than girls, although girls perceive themselves to have peer approval for a moderate amount of game playing. A clear and consistent difference exists between boys and girls in their reported preferences. The favourite games for boys are fighting/combat type games. The favourite games for girls, on the other hand, are educational type games. This finding supports the male and female preferences for video games found in international studies (Durkin, 1995). Buchman and Funk (1996) also noted that gender differences characterize electronic game playing in childhood: boys play more than girls and prefer more realistic violent games. As the different types of games represent different content these findings probably reflect well-known gender differences with regard to relevant interests. These gender differences also are connected to the fact that most of the games do not present active female characters (Frommer, 2003) nor deal with topics that girls usually show interest in - such as matching, memory and verbal skills (Schorb, 1993; Jungwirth, 1993; Kafai, 1996). In addition, nearly 80 % of boys prefer to play violent games. The percentage of girls who mostly like to play violent games was different from girls who prefer non-violent games. This sug-

gests that a percentage of boys (larger proportion) and girls (smaller proportion) enjoy violent electronic games.

2. Time spent playing computer games

The increasing amount of time that Thai children are spending playing computers games has raised questions about how this technology may make a difference in their lives. The results show that children who mostly like to play violent games spend more hours per week than children who mostly like to play non-violent games. Playing violent games is believed to fulfill certain requirements; it must have a moral story, in which good triumphs over evil, and it must carry cues in its music, sound effects, fantasy story-line, and cartoon-like characters (Deitz, 1998). Therefore, many video game players may feel that they have wasted their efforts if they do not reach the next goal in a game, which may lead to additional time spent playing the game that the person otherwise would have spent in a more constructive task (McCauley, 1998; Zillmann, 1998). Furthermore, the results show that boys spend more hours per week playing computer games than girls do. Boys reported spending approximately double the time of girls. However, the results also show that boys who mostly play either violent or non-violent games spend more time playing computer game than girls do. Girls' and boys' game-playing habits have been addressed by many studies, which have consistently found that boys play video games more than girls (Buchman and Funk, 1996; Davies et al., 2000). This suggests that boys and girls have different objectives for playing computer games. Boys are more interested in completing or winning a game, while girls take a more exploratory approach (Klawe et al., 2002).

3. Influence of violent games on children's behavior

The hypothesis investigated the influence of violent computer games on children's behavior and psychology. This was achieved by measuring the child's self-report, parent rating scale, and teacher rating scale. The findings showed that the type of games (vg vs. nvg) was significantly associated with the children's behavior and psychology using the self-reported, parent and teacher assessments. Significant differences were found for nine dependent measures in the self-report, eleven dependent measures in the parent rating scale, and two dependent measures in the teacher rating scale. Children in the violent games group were found to have more negative outcomes than those in the non-violent games group.

3.1 Playing violent video games increases poorer attitudes towards school.

Children who play violent games have more negative attitude to school and their teachers than children in the non-violent games group. Most of the violent games consist of fighting and killing. Furthermore, children who play more violent computer games are more likely to have been involved in physical fights and imitated their favorite characters. They may also have higher risks of getting into arguments with teachers more frequently. Children who play violent games might spend more time on playing computer games and this may also contribute to their regular neglect of their homework. These factors may be associated with their negative attitudes to school and teacher.

3.2 Playing violent video games increases Emotional Symptoms.

Playing violent video games may affect

both cognitive and emotional functioning in vulnerable children. Children with healthy emotion regulation skills are able to experience a range of positive and negative emotions, to control the intensity and duration of each emotional experience, and to express emotion in socially appropriate ways (Grossman, 1999). Children with deficits in emotion regulation may also seek immersion in game-playing to avoid negative effects such as anxiety and depression. Immersion in violent games may give them a sense of control or power they lack in real life (Funk et al., 1999). Therefore, children who constantly seek stimulation may have problems with emotion regulation. Funk et al. (2004) found that children with a stronger preference for violent games had more clinically significant elevations on a standardized measure of emotional and behavior problems than those with a low preference for violent games. Although some players may seek control over arousal, it is clear that prolonged play can also be over-arousing and frustrating (Grodal, 2000). Negative emotional states such as frustration undermine regulatory capacities, already impaired in the stimulation-seeking child (Bierman and Pope, 1999).

3.3 Playing violent video games increases Behavioral Symptoms and decreases Adaptive Skill.

Children who playing violent games had higher scores in hyperactivity, depression, somatization, withdrawal, and attention problems as well as lower scores in adaptability, social skills, and leadership than the non-violent group. Studies measuring response to playing violent video games have shown that pro-social behaviors are decreased compared to playing nonviolent video games (Chambers and Ascione, 1987; Ballard and Lineberger, 1999).

Children who named violent games as their favorite games to play were rated by their peers as exhibiting fewer pro-social behaviors and more aggressive behaviors in the classroom (Wiegman and van Schie, 2000).

3.4 Playing violent video games increase Aggression.

Both the parent and teacher found that children who mostly like to play violent games had increased levels of aggression and conduct problems. Overall, the majority of researchers have supported the relationship between playing violent video games and increases in aggression or aggression-related outcomes (Anderson and Ford, 1986; Anderson and Morrow, 1995; Ballard and Weist, 1996). Playing video games may increase aggressive behavior because violent acts are continually repeated throughout the video game (Gentile and Anderson, 2003). Violent video games may also encourage players to identify with the character role in their favorite games. Therefore, the players are able to make decisions affecting the actions of the character they are imitating (Anderson and Dill, 2000). Furthermore, players who have prior experience playing violent video games responded with an increased level of aggression when they encountered confrontation (Bushman and Anderson, 2002).

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ข้อแนะนำในการเขียนบทความลงวารสารวิทยาศาสตร์ มข.

ประเภทของเรื่องที่จะตีพิมพ์

1. รายงานผลการวิจัยและค้นคว้าหรือการสำรวจที่ยังไม่เคยตีพิมพ์ในวารสารหรือหนังสืออื่นมาก่อน
2. บทความปริทัศน์ ได้แก่ งานเขียนที่รวบรวมหรือเรียบเรียงจากเอกสารหรือหนังสือต่างๆ เพื่อเผยแพร่และฟื้นฟูงานด้านวิชาการระดับต่างๆ
3. บทความแสดงข้อคิดเห็นหรือข้อเสนอแนะที่เป็นประโยชน์ในด้านวิชาการ เรื่องแปล ข่าววิชาการย่อความจากงานวิจัยหรือหนังสือใหม่ที่น่าสนใจ

รูปแบบของการเขียนและเตรียมต้นฉบับ

1. ต้นฉบับ ต้องเป็นตัวพิมพ์ Angsana New
 - 1.1 ชื่อเรื่อง ขนาดตัวอักษร 20 ตัวเข้ม ตำแหน่งตรงกลาง มีทั้งชื่อภาษาไทยและภาษาอังกฤษ
 - 1.2 ชื่อผู้เขียน ขนาดตัวอักษร 16 เอน ตำแหน่งขวาสุด และใส่หมายเลขไว้ท้ายนามสกุล เพื่อการระบุที่อยู่ด้านล่าง
 - 1.3 หัวข้อใหญ่ในแต่ละส่วน ขนาดตัวอักษร 16 ตัวเข้ม ตำแหน่งซ้ายสุด ยกเว้น บทคัดย่อ และ Abstract อยู่ตรงกลาง หัวข้อย่อยตามความเหมาะสม
 - 1.4 เนื้อความ ขนาดตัวอักษร 14
 - 1.5 คำว่า รูปที่ x และตารางที่ x ขนาดตัวอักษร 14 ตัวเข้ม ส่วนข้อความบรรยายค่อยๆ ตัวปกติ
 - 1.6 ที่อยู่ ขนาดอักษร 12
 - 1.7 ช่องว่างระหว่างบรรทัดตามมาตรฐาน
 - 1.8 พิมพ์ลงในกระดาษขาว A4 (โรเนียวสัน) โดยใช้กระดาษหน้าเดียว ความยาวไม่เกิน 10 หน้า ส่งต้นฉบับ 2 ชุด (ไม่ต้องส่งแผ่นดิสก์ หรือแผ่นซีดีในขั้นตอนนี้ เมื่อได้รับแจ้งว่าได้ลงพิมพ์แล้ว ผู้เขียนส่งฉบับแก้ไข 1 ชุด พร้อมแผ่นดิสก์ หรือแผ่นซีดี)
2. ต้นฉบับ จะถูกพิจารณาโดยกองบรรณาธิการ และจะตอบรับการได้รับบทความโดยไม่ส่งต้นฉบับคืน ผู้เขียนควรจะอดสำเนาเก็บไว้เป็นหลักฐานของตัวเอง 1 ฉบับ
3. ต้นฉบับ จะต้องมีความถูกต้องทั้งในด้านเนื้อหา และการใช้ภาษา รวมทั้งมีความสมบูรณ์ในรูปแบบพร้อมที่จะนำลงตีพิมพ์ได้
4. ชื่อเรื่อง ให้ใช้ภาษาไทยหรือภาษาอังกฤษ ไม่ยาวเกินไป และหลีกเลี่ยงการใช้คำย่อโดยไม่จำเป็น
5. ชื่อผู้เขียน เขียนชื่อภาษาไทยหรือภาษาอังกฤษพร้อมทั้งบอกสถานที่ทำงานให้ชัดเจน
6. เนื้อเรื่อง ใช้ได้ทั้งภาษาไทยล้วนและภาษาอังกฤษล้วน ถ้าใช้ภาษาไทย ภาษาอังกฤษที่ใช้ปนกับภาษาไทยนั้นให้พยายามแปลเป็นไทยเท่าที่จะทำได้และให้เขียนคำเดิมกำกับในวงเล็บ การทับศัพท์ภาษาอังกฤษตลอดทั้งการเขียนตัวสะกด การันต์ ในภาษาไทยให้ใช้ตามแบบราชบัณฑิตยสถาน

ข้อแนะนำในการเขียนบทความลงวารสารวิทยาศาสตร์ มข.

7. ในกรณีของบทความวิจัย ต้องมีบทคัดย่อทั้งภาษาไทยและภาษาอังกฤษ และแบ่งเนื้อหาของบทความเป็นบทนำ วิธีดำเนินงาน ผลการวิเคราะห์ บทสรุปและวิจารณ์

8. เชิงอรรถ (footnote) ใช้เฉพาะที่จำเป็นเพื่อขยายหรือให้รายละเอียดเพิ่มเติมแก่ใจความเฉพาะตอนในบทความ

9. ตารางและภาพประกอบ คำบรรยายประกอบตารางหรือภาพประกอบควรจะสั้นและชัดเจน ถ้าเป็นภาพถ่ายให้ใช้ภาพขาว-ดำ ขนาดโปสการ์ด ภาพเขียนลายเส้นควรเขียนด้วยหมึกดำ ภาพที่เขียนต้องชัดเจนและมีขนาดที่เหมาะสม

10. เอกสารอ้างอิง หรือบรรณานุกรม แนะนำให้เขียนตามแบบที่ทางวารสารกำหนด

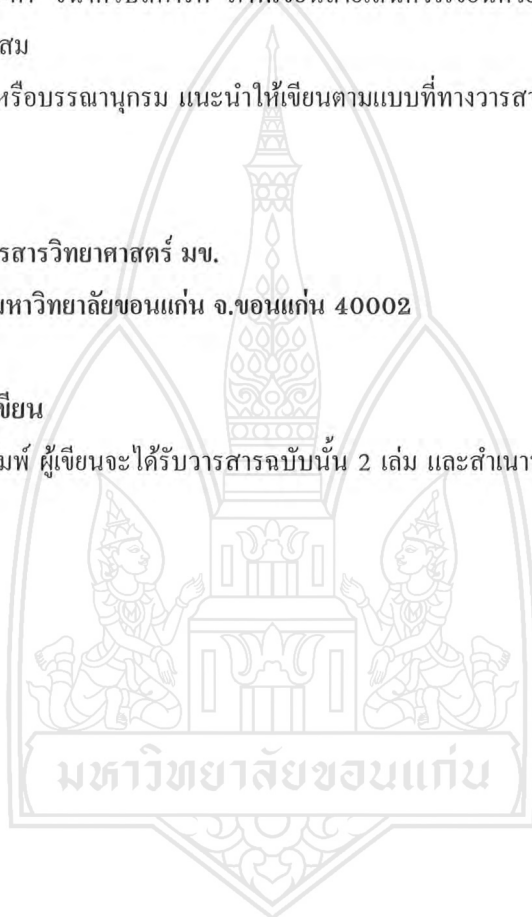
ส่งต้นฉบับมาที่

กองบรรณาธิการวารสารวิทยาศาสตร์ มข.

คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จ.ขอนแก่น 40002

สิทธิันทนาการสำหรับผู้เขียน

เรื่องที่ได้รับการตีพิมพ์ ผู้เขียนจะได้รับวารสารฉบับนั้น 2 เล่ม และสำเนาพิมพ์อีก 15 ชุด



ตัวอย่าง

การวิเคราะห์ปริมาณน้ำมันและกรดไขมันของจิ้งหรีดทองลาย (*Gryllus* sp.) ที่เลี้ยงเพื่อการค้า

บรรจง เชื้อเมืองพาน¹ วิรัช ว่องพัฒนานกุล² และ ปวีณา พงษ์ดนตรี¹

บทคัดย่อ

XX
XX

บทนำ

XX
XX
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วิธีการ

XX
XX
XX

รูปที่ 1 XXXXXXXXXXXXXXXXXXXXXXX

ตารางที่ 1 XXXXXXXXXXXXXXXXXXXXXXX

¹ ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จ.ขอนแก่น 40002

² ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จ.ขอนแก่น 40002

* correspondening author, E-mail

การเขียนเอกสารอ้างอิงในวารสารวิทยาศาสตร์ มข.

เอกสารอ้างอิง (Reference) หมายถึงเอกสารหรือส่วนหนึ่งของเอกสารที่อ้างอิงถึงหรือแปลมาโดยตรง การเขียนเอกสารอ้างอิงในเนื้อหานั้น ให้ใช้ระบบชื่อและปี เช่น Williams (2001) รายงานว่า... หรือ... (Williams, 2001) การเขียนเอกสารอ้างอิงในตอนท้ายบทความนั้น ให้ใส่เฉพาะเล่มที่มีการอ้างอิงไว้แล้วในเนื้อหาเท่านั้น หากอ้างอิงจากเล่มภาษาไทย ต้องเขียนทั้งชื่อและนามสกุล

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การเขียนเอกสารอ้างอิง หรือบรรณานุกรม มีแนวทางการเขียนดังนี้

1. หนังสือ

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2. เอกสารราชการ

ชื่อหน่วยราชการ. (เลขปี พ.ศ. หรือ ค.ศ.) ชื่อเรื่อง. ชื่อหนังสือ. เมืองที่พิมพ์: โรงพิมพ์ หรือสำนักพิมพ์.

3. วารสาร

ก. ภาษาไทย

ชื่อตัว นามสกุลของผู้แต่ง. (เลขปี พ.ศ.). ชื่อบทความ. ชื่อวารสาร. เล่มที่ (ฉบับที่): เลขหน้าเริ่มต้น-เลขหน้าสุดท้าย.

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หมายเหตุ

- กรณีที่เป็นการอ้างอิงจากแหล่งอื่นๆ นอกเหนือจากที่กำหนดข้างต้น เช่น จุลสาร หนังสือพิมพ์ ฯลฯ ให้ใช้คดหลักการเขียนตามแบบข้างต้นโดยอนุโลม
- กรณีที่ผู้เขียน 2 คน ให้เขียนตามแบบของผู้เขียนคนแรก แต่ให้เติมคำว่า “และ” หรือ “and” หน้าชื่อสุดท้ายในกรณีของภาษาไทย หรือภาษาอังกฤษตามลำดับ
- กรณีที่ผู้เขียนมากกว่า 2 คนขึ้นไป ให้ใส่เฉพาะผู้แต่งชื่อแรกและใส่คำว่า “และคณะ” หรือ “et al.” ในกรณีของภาษาไทย หรือภาษาอังกฤษตามลำดับ (เฉพาะในเนื้อหาเท่านั้น)
- ถ้าเอกสารที่ถูกอ้างอิงอยู่ในระหว่างตีพิมพ์ ให้บอกรายละเอียดมากที่สุดและใช้คำว่า “ระหว่างตีพิมพ์” หรือ “in press” ในกรณีของภาษาไทย หรือภาษาอังกฤษตามลำดับ โดยวงเล็บต่อท้ายเอกสารอ้างอิงนั้น



หนังสือในโครงการผลิตตำรา คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น



สถิติพื้นฐาน

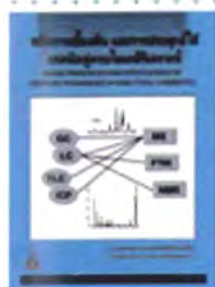
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MINITAB SPSS และ SAS

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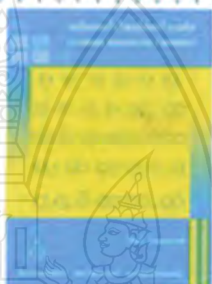
สมการเชิงอนุพันธ์สามัญ Ordinary Differential Equations

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ราคา 200 บาท



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รองศาสตราจารย์ ดร. นิรันดร์ สัตยพันธ์
2551 • 164 หน้า
ISBN 978-974-10-2673-9
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นางสาวนันทวัน เชื้อวงศ์กุล
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ราคา 160 บาท

ท่านที่สนใจหนังสือในโครงการผลิตตำรา ติดต่อสั่งซื้อได้ที่... รองศาสตราจารย์จรรยา จีระวัฒน์
คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จ.ขอนแก่น 40002 โทรศัพท์โทรสาร 04320-2375 E-mail: charachar@kku.ac.th

