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3. To be a medium for the exchange of knowledge and ideas among faculty, researchers and students of Khon Kaen University and other institutions.

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Editor's note

Dear readers,

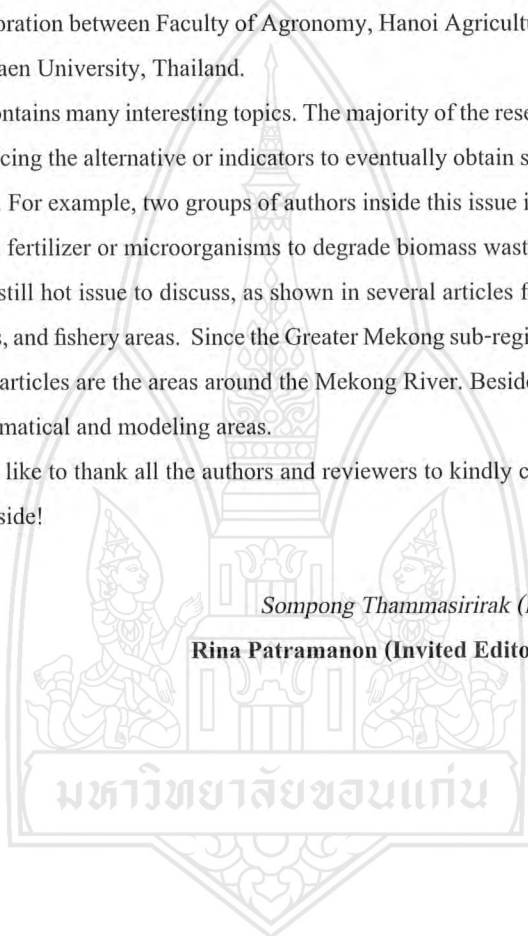
Welcome to the special issue of KKU Science Journal. This copy is a collection of selected articles presented in The Second International Conference on Science and Technology for Sustainable Development of the Greater Mekong sub-region, held on October 2-3, 2008 at Hanoi, Viet Nam. The Conference was a successful outcome of a co-host collaboration between Faculty of Agronomy, Hanoi Agricultural University, Viet Nam and Faculty of Science, Khon Kaen University, Thailand.

Inside this issue contains many interesting topics. The majority of the research articles follow the theme of the Conference in introducing the alternative or indicators to eventually obtain sustainable development in the Greater Mekong sub-region. For example, two groups of authors inside this issue introduce a natural fertilizer to potentially replace chemical fertilizer or microorganisms to degrade biomass waste, respectively. Moreover, the subjects of biodiversity are still hot issue to discuss, as shown in several articles focusing on biodiversity in the forest areas, water reservoirs, and fishery areas. Since the Greater Mekong sub-region is our living place, the sites of research study in several articles are the areas around the Mekong River. Besides those articles, there still are interesting articles in Mathematical and modeling areas.

The editors would like to thank all the authors and reviewers to kindly contribute to this special issue. Please enjoy the contents inside!

Sompong Thammasirirak (Main Editor)

Rina Patramanon (Invited Editor for special issue)



**A preliminary examination of gut contents of three species of
freshwater fishes, *Barbonymus altus* (Günther, 1868), *Notopterus
notopterus* (Pallas, 1769) and *Ompok bimaculatus* (Bloch, 1794)
from Nong Han Kumphawapi, Udon Thani, Thailand**

Chutima Hanjavanit^{*} and Narumon Sangpradub¹

Abstract

Commercially important species, red-tail tinfoil barb, *Barbonymus altus* (Günther, 1868), Grey featherback, *Notopterus notopterus* (Pallas, 1769) and Butter catfish, *Ompok bimaculatus* (Bloch, 1794) were collected from Nong Han Kumphawapi, Udon Thani Province, northeast Thailand in March 2007 in order to examine gut contents. The results showed that the red-tail tinfoil barb, the grey featherback, and the butter catfish fed on mainly sand grains (mean range 29.2-40.5%), detritus (mean range 25.9-31.7%), algae (mean range 9.1-19.0%) and diatoms (mean range 3.8-15.5%). Miscellaneous food items in dietary components were also noted. It was found that Ostracoda, Chironomid larvae, unidentified fragments of aquatic insect larvae, Nematoda and plant materials in mean 10% of gut of the red-tail tinfoil barb. Whereas Cladocera, Ostracoda, Palaemonidae (Decapoda), Coleoptera, Chironomidae (Diptera), Baetidae (Ephemeroptera), Micronectidae (Hemiptera), Libelluliidae (Odonata), scales of fish and plant materials were found in mean 22% of gut of the grey featherback. The butter catfish also fed on similar miscellaneous food items (mean 16%) to those of the grey featherback, including fungi, zooplankton and small unidentified fish. These results indicate that the red-tail tinfoil barb, the grey featherback and the butter catfish are omnivorous.

Keywords : Gut contents, Red-tail tinfoil barb, Grey featherback, Butter catfish, Nong Han Kumphawapi

Introduction

Nong Han Kumphawapi, in Udon Thani Province, is the second largest freshwater wetland in northeast Thailand. The wetland is located in the latitude 17° 06' 14" N, longitude 103° 00' 05" E, altitude 160-170 m above sea level, average depth 1-2 m, width 5 km, length 13 km, and surface area approximately 45 km² (Office of Natural Resources and Environmental Policy and Planning, 1999) (Figure 1). It plays an important role as the major water source in Kumphawapi

District, Udon Thani Province, providing water for irrigation and agriculture for the local villagers. Studies and research have confirmed that the wetland is a site of ecological and conservation significance because it is an important source of biodiversity, providing feeding and breeding sites for numerous plants and animals. The wetland held 18 families and 46 species of fish. Red-tail tinfoil barb, *Barbonymus altus* (Günther, 1868), Grey featherback, *Notopterus notopterus* (Pallas, 1769) and Butter catfish, *Ompok bimaculatus* (Bloch,

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1794), commercial species were also collected from the wetland (Pagdee et al., 2007). The red-tail tinfoil barb, the grey featherback and the butter catfish are valuable sources of protein and delicacy dish. The aim of this study is to preliminary examine gut contents of those three fish species in order to obtain information on food items the fish fed.

Materials and Methods

Fish collection

Fish samples were bought in March 2007 from local fishermen captured daily at Ban Don Klang, Ban Don Nguen and Ban Don Kaew, surrounding the Nong Han Kumphawapi wetland, where there are fishery piers and markets. (Figure 1). Individual fish was measured for total width (cm) (Figure 2) and weigh (g). Fish samples were preserved in 90% ethanol and abdominal cavities were first opened to facilitate penetration of the preservative. The fish samples were transported to the Department of Biology, Faculty of Science, Khon Kaen University, Thailand, for identification. Species identification is based on Rainboth (1996); Vidthayanon (2004).

Preparation and analysis of stomach content

Gut of individual fish was isolated and divided to 3 parts: anterior, middle, and posterior parts. Each part of the gut was preserved in 70% ethanol diluted in glycerin. Three replicates of temporary slides from each part of gut were prepared and examined microscopically. The lowest possible taxonomic level of food items were determined based on Lewmanomont et al. (1995); Morse et al. (1996); Pennak (1989); Sangpradub and Boonsoong (2006). Method of frequency of occurrence (Lagler, 1956) was used for diet analysis. The gut contents were quantified as a percentage of the total stomach content per species of

fish. An average diet composition has been obtained by pooling the data of individual gut of the same species.

Results and Discussion

Sizes of fish samples

The sizes of the red-tail tinfoil barb (n=10) ranged from 10.5-16.5 cm and weighed 24.0-56.0 g, the grey featherback (n=10) were 15.1-18.3 cm, 25.3-30.3 g and the butter catfish (n=7) were 12.4-15.6 cm, and 12.8-18.2 g, respectively.

Gut contents

The results of gut content analyses of the red-tail tinfoil barb, the grey featherback, and the butter catfish are presented in Figure 3. It showed that stomach contents of the red-tail tinfoil barb consisted mainly of sand grains (29%), detritus (26%), algae (19%), diatoms (16%) and miscellaneous items (10%). The major food items of the grey featherback were detritus (31%), followed by sand grains (30%), miscellaneous food items (22%), algae (11%), and diatoms (6%). Whereas, the butter catfish fed mainly on sand grains (40%) and followed by detritus (31%), miscellaneous food items (16%), algae (9%), and diatoms (4%), respectively.

Table 1 shows the different components of miscellaneous food items. The miscellaneous items of the red-tail tinfoil barb (Figure 4) consisted of plant materials, ostracod (O.Ostracoda), chironomid larvae (O.Diptera), unidentified fragments of aquatic insect larvae, and nematode worms. The grey featherback consumed miscellaneous items (Figure 5), which mainly composed of ostracod, prawns (Palaemonidae, O.Decapoda), chironomid larvae, baetid nymphs (O.Ephemeroptera) and micronectid larvae (O.Hemiptera), plant materials, coleopteran (O.Coleoptera), libelluliid nymphs (O.Odonata), cladoceran (O.Cladocera), and fish scales. Whereas the

butter catfish fed on miscellaneous food items similar those of the grey featherback, including ostracod, prawns, small unidentified fish, zooplanktons, baetid nymphs, chironomid larvae, fungal hyphae, plant materials, micronectid larvae and libelluliid nymphs, respectively (Figure 6). Food items of the three fish species in the present study slightly differed from those reported by Rainboth (1996), that diet of the red-tail tinfoil barb in Cambodian Mekong consisted of a wide variety of animals, plant matters and organic debris, the grey featherback is an insect and fish predator, whereas the butter catfish feed on crustaceans, fish and occasionally on mollusks. In addition, Vidthayanon (2004) reported food items of the red-tail tinfoil barb in Thailand consisted of plants and aquatic insects, the grey featherback feed on fish, prawns, aquatic insects and the butter catfish consume on aquatic insects, prawns, crabs and small fish. The differences of food items are probably related to the way fish feed and the prevalence of various food items in the water body at the time of the study. An interesting observation in this study is that the presence of considerable quantities of ingested sand grains, which were found 29%, 30% and 40% in stomach contents of the red-tail tinfoil barb, the grey featherback and the butter catfish, respectively. It appears that no sand particles and diatoms have been reported in the guts of the three fish species (Rainboth, 1996; Vidthayanon, 2004). Amisah and Agbo (2008) have suggested that ingested sand particles presumably assist the maceration of food materials in the

pyloric stomach. Another finding in this study was the presence of diatoms in the guts of the three fish species. Diatoms have outer shells made from silica. According to Amisah and Agbo (2008), the prevalence of sand in guts of fish may facilitate digestive breakdown of the food items. From the present study, both plant and animal materials were analyzed for the gut contents of the three species. It indicated that the three species are omnivorous. Due to the small numbers of fish in this study, it is therefore not possible to determine whether the fish are planktivorous, insectivorous and piscivorous. In general, several tens or hundreds of samples are used in the gut content analysis (Yamagishi et al., 2005). Berg (1979 cited in Hyslop, 1980); Pillay (1952 cited in Hyslop, 1980) reported that the analysis of stomach contents of fish could provide information about the niche of particular fish in its ecosystem and this has become a standard practice in fish ecology works (Hyslop, 1980). The knowledge of the food items of the fish is very important and essential for the stock enhancement through the seedlings release and it will become a guideline for determining the environmental capacity which is useful for the prevention of fish starvation and improvement of their growth after the release (Yamagishi et al., 2005). Therefore, it is necessary to examine gut contents of a large numbers of fish seasonally, and study aquatic biota included benthic macroinvertebrate fauna from the study area before any conclusions of these factors indicating the feeding habits in the three fish species can be deduced.

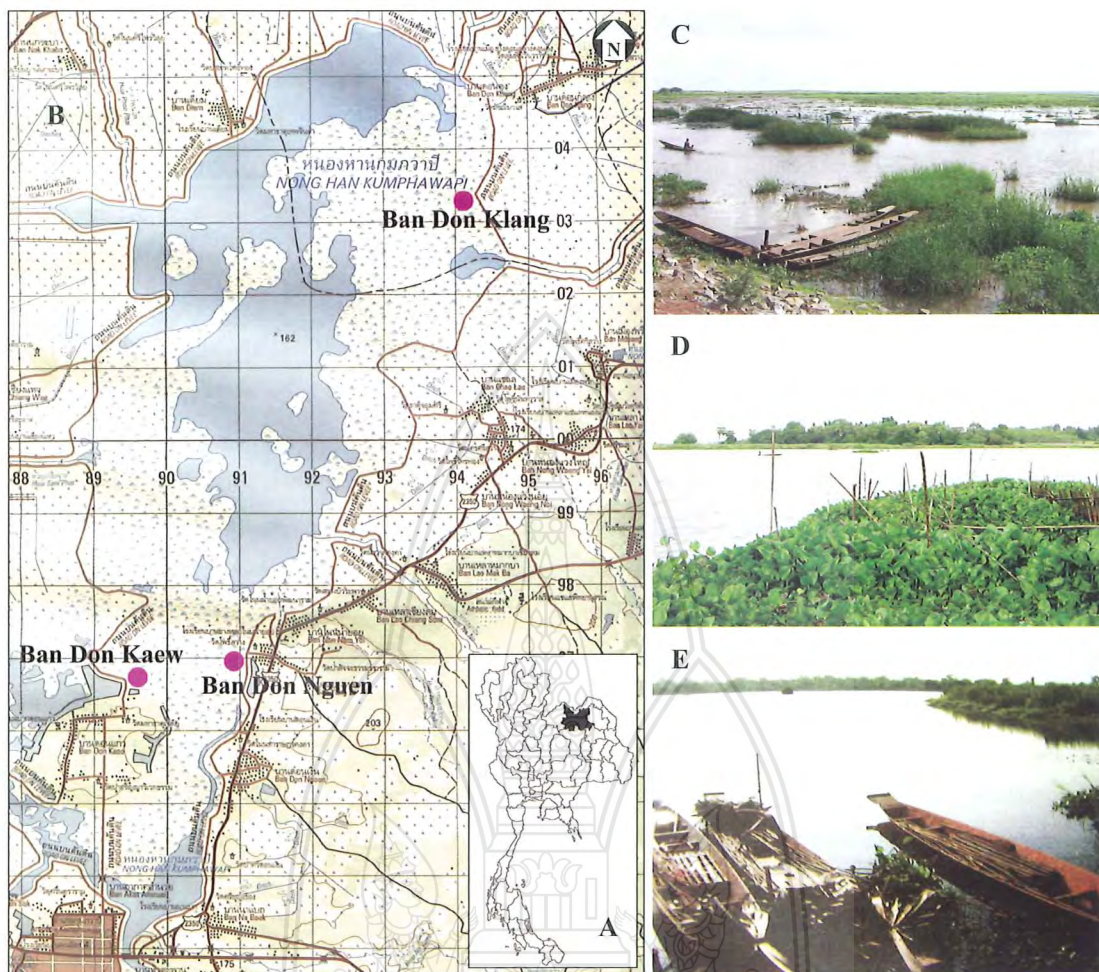


Figure 1. A. Map showing location of Udon Thani Province, Thailand
 B. Map of Nong Han Kumphawapi wetland located three sampling sites
 C. Ban Don Klang (latitude $17^{\circ} 12' 22.02''$ N, longitude $103^{\circ} 3' 52.26''$ E, Tambon Kumphawapi, Kumphawapi District, Udon Thani Province)
 D. Ban Don Nguen (latitude $17^{\circ} 8' 55.38''$ N, longitude $103^{\circ} 2' 6.42''$ E, Tambon Chaelae, Kumphawapi District, Udon Thani Province)
 E. Ban Don Kaew ($17^{\circ} 8' 43.02''$ N, longitude $103^{\circ} 1' 15.52''$ E, Tambon Kumphawapi, Kumphawapi District, Udon Thani Province)

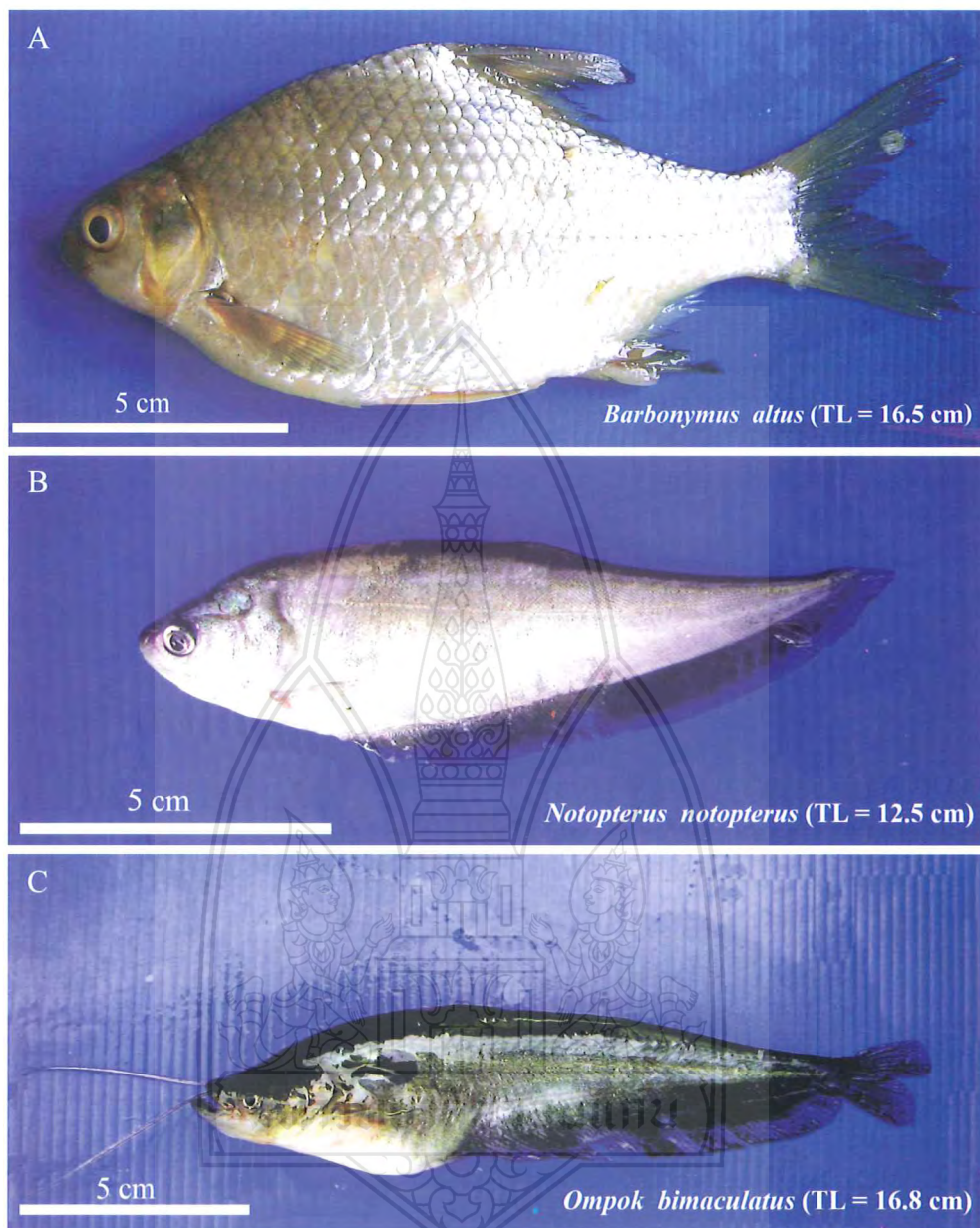


Figure 2. Photographs of three fish species

- A. Red-tail tinfoil barb, *Barbonymus altus* (Günther, 1868),
(16.5 cm total length, Ban Don Kaew, March 2007)
- B. Grey featherback, *Notopterus notopterus* (Pallas, 1769)
(12.5 cm total length, Ban Don Klang, March 2007)
- C. Butter catfish, *Ompok bimaculatus* (Bloch, 1794),
(16.8 cm total length, Ban Don Nguen, March 2007)

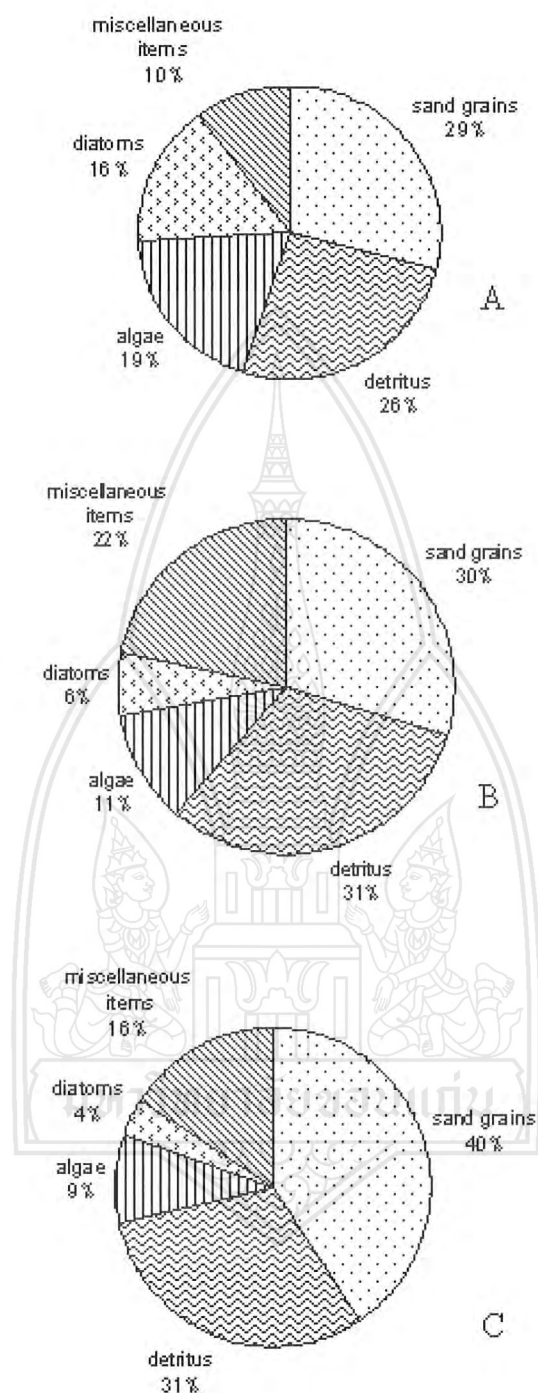


Figure3. Percentage of dietary component

- A. Red-tail tinfoil barb, *Barbonymus altus* (Günther, 1868)
- B. Grey featherback, *Notopterus notopterus* (Pallas, 1769)
- C. Butter catfish, *Ompok bimaculatus* (Bloch, 1794)

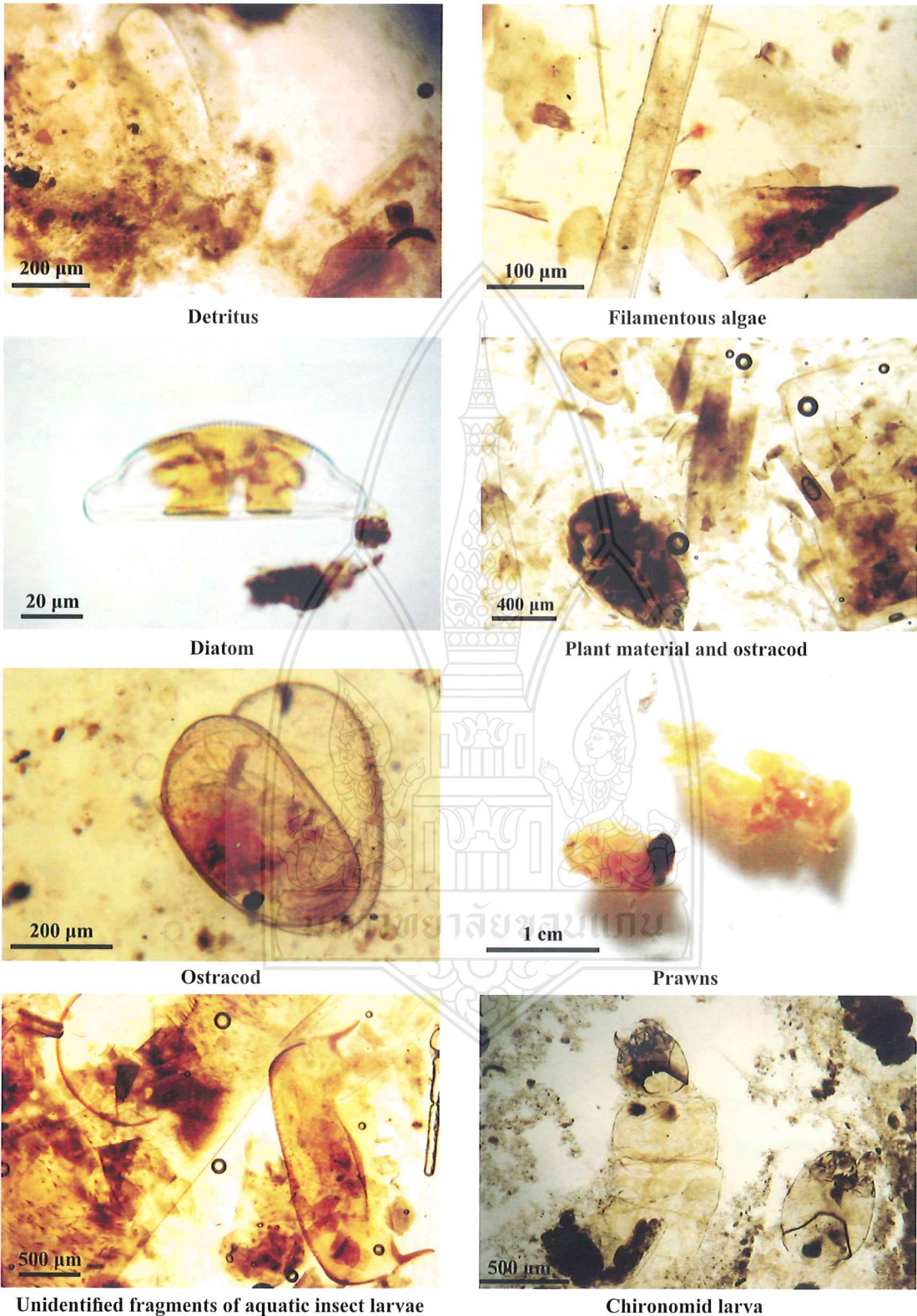


Figure 4. Miscellaneous food items in guts of the red-tail tinfoil barb, *Barbonymus altus* (Günther, 1868)

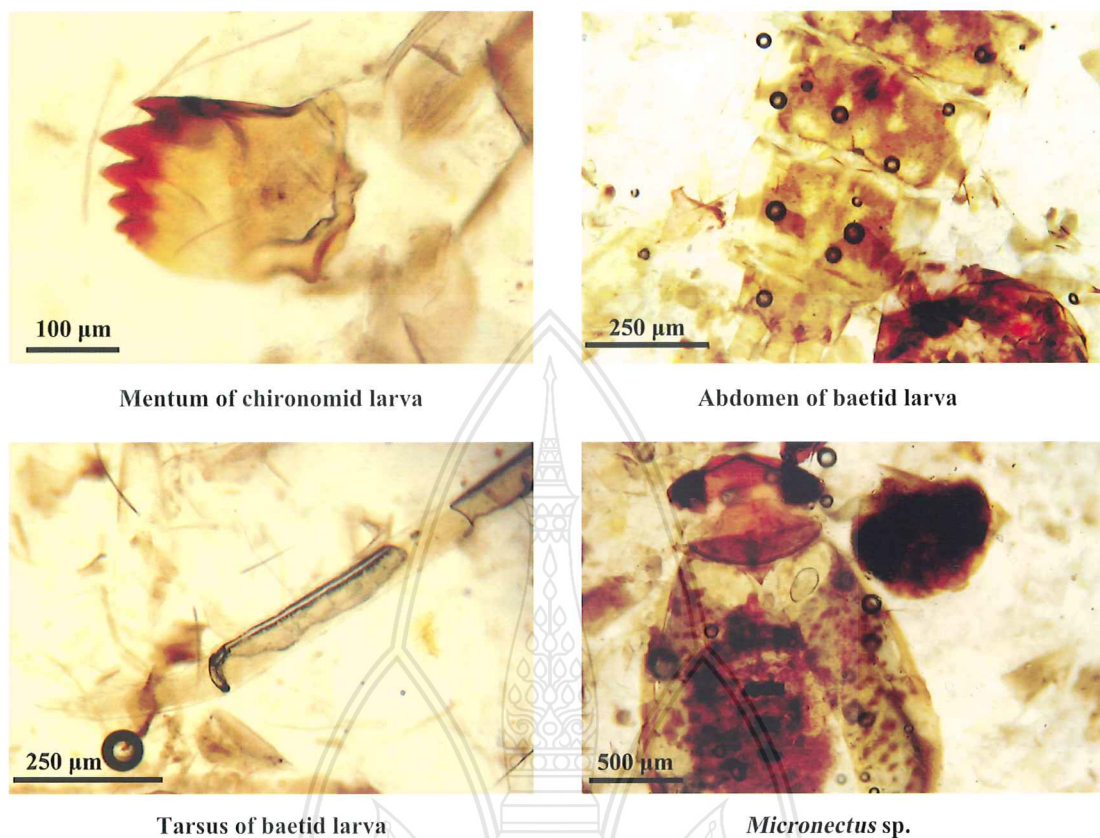


Figure 5. Miscellaneous food items in guts of the grey featherback, *Notopterus notopterus* (Pallas, 1769)

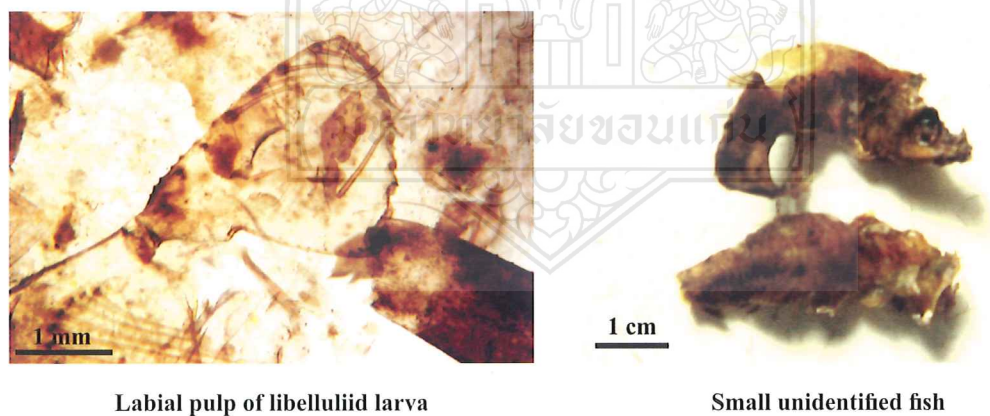


Figure 6. Miscellaneous food items in guts of the butter catfish, *Ompok bimaculatus* (Bloch, 1794)

Table 1. Food items of the red-tail tinfoil barb, the grey featherback and the butter catfish collected from the Nong Han Kumphawapi wetland, Kumphawapi District, Udon Thani Province, Thailand, in March 2007.

Food category	Red-tail tinfoil barb (N=10)	Grey featherback (N=10)	Butter catfish (N=7)
Sand grains	+++++	+++++	+++++
Detritus	+++++	+++++	++++
Algae	+++++	+++++	++++
Diatoms	+++++	+++	++++
Miscellaneous items			
Plant materials	+++++	++++	+
Nematoda	+	-	-
Zooplankton	-	-	+++
Ostracoda	+++++	+++++	++++
Cladocera	-	+	-
Decapoda			
Palaemonidae	-	+++++	++++
Unidentified fragments of aquatic insect larvae	+++	-	-
Coleoptera .	-	+++	-
Diptera			
Chironomidae	+++++	+++++	++
Ephemeroptera			
Baetidae	-	+++++	+++
Hemiptera			
Micronectidae	-	++++	+
Odonata			
Libelluliidae	-	++	+
Fish scale	-	+	-
Small unidentified fish	-	-	++++
Fungal hyphae	-	-	++

-

= absent

++

= present in 3-4 fish

++++

= present in 7-8 fish

+

= present in 1-2 fish

+++

= present in 5-6 fish

+++++

= present in 9-10 fish

Acknowledgements

We would like to thank the Head of the Department of Biology, Faculty of Science, Khon Kaen University, Thailand to permit us to use facilities for this research and Mr. Prayutt Udonphimai for specimen preparation.

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Characterization of Digestive Enzymes from Adult Freshwater Pearl Mussel *Chamberlainia hainesiana* (Lea, 1856)

Wilailuk Khruenat^{1,2}, Uthaiwan Kovitvadh^{1*}, Arunee Engkagul³,
Satit Kovitvadh⁴ and Krisna Rungruangsak-Torrissen⁵

Abstract

Freshwater mussel *Chamberlainia hainesiana* is a vulnerable species in Thailand. The species has a lustrous nacreous shell that can be utilized for various purposes, and has a potential industrial use for producing freshwater pearl. Characterization of digestive enzymes would provide critical information prerequisite for studying the development of digestive enzymes during on-growing as well as for *in vitro* digestibility study important for the development of suitable artificial feed formulations for the commercial culture. The aim of this preliminary study was to determine optimum pH and temperature for three digestive enzymes: amylase, protease and lipase, from digestive gland including stomach of adult *C. hainesiana* under various pH's (2–11) and temperatures (25–80°C). The results revealed at least three isoforms of amylase and protease, and at least 4–5 isoforms of lipase. No alkaline protease activity was detected. For future enzyme expression study, the suitable optimal conditions are 40°C pH 6 for amylase activity, 50°C pH 5 for protease activity, and 50°C pH 8 for lipase activity. At natural habitat of around 30°C, these enzymes are most active at pH 6, 6, and 8 (or 10), respectively, which are suitable conditions for future study on *in vitro* digestibility.

Keywords : Freshwater mussel, Amylase, Protease, Lipase

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Introduction

Freshwater pearl mussel *Chamberlainia hainesiana* (Lea, 1856) is a vulnerable species in Thailand. It is multi-utility aquatic animal with economic values (Kovitvadhii et al., 2002). This mussel has a lustrous nacreous shell that can be utilized for various purposes. The species also has a potential industrial use for producing artificial freshwater pearl (Yeemin, 1997). Furthermore, it is food for the native people as well as for domestic animals (Kovitvadhii et al., 2002). Mussels are filter feeders and their filtering activities contribute to maintaining a clean environment and reducing pollution (Dan et al., 2001). The population of freshwater mussels has decreased as a result of low water quality. To increase the population of freshwater mussels, culture techniques have been used for mass production and conservation (Isom and Hudson, 1982, 1984; Hudson and Isom, 1984; Keller and Zam, 1990; Kovitvadhii et al., 2002; Kovitvadhii et al., 2003). Recently, Kovitvadhii et al. (2006) have successfully cultured *Hyriopsis (Limnoscapha) myersiana* to adult phase, and it should also be possible for *C. hainesiana*.

At present, the hatchery rearing of juveniles and adults bivalves relies on the mass production of microalgae. Coutteau and Sorgeloos (1992) identified the mass production of live algae as a major bottleneck in bivalve hatcheries and nurseries. Algae production comprises up to 30% of hatcheries' operation costs. Furthermore, algae cultures often vary in nutritional value and are subject to seasonal growth patterns and contamination. The use of artificial feeds with optimized nutritional quality is the need in aquaculture. Utilization of nutrients in aquatic animals depends on digestive enzymes (Rungruangsak-Torrissen et al., 2006; Areekijseree et al., 2006; Rungruangsak-Torrissen, 2007; Rungruangsak-Torrissen and Fosseidengen,

2007; Supannapong et al., 2008). Recent studies on feeding of aquatic animals have reported the correlation among digestive protease activity (trypsin specific activity and activity ratio of trypsin to chymotrypsin), protein digestibility, feed conversion efficiency (FCE), fish weight and specific growth rate (SGR), as well as the quality of muscle and oocyte in Atlantic salmon (Sunde et al., 2001; Sunde et al., 2004; Rungruangsak-Torrissen et al., 2002; Rungruangsak-Torrissen, 2007), Atlantic mackerel (Rungruangsak-Torrissen and Fosseidengen, 2007), and among trypsin specific activity, FCE and SGR in Atlantic cod (Lemieux et al., 1999). Study on amylase activity has helped in the development of more rapid and accurate *in vitro* carbohydrate digestibility assay (Areekijseree et al., 2006; Supannapong et al., 2008) and used as indicator for carnivorous feeding habit (Hofer and Schiemer, 1981). *In vitro* digestibility studies of dietary protein (Rungruangsak-Torrissen et al., 2002; Areekijseree et al., 2006; Rungruangsak-Torrissen, 2007; Supannapong et al., 2008) and carbohydrate (Areekijseree et al., 2006; Supannapong et al., 2008) using bivalves crude enzyme extract and based on the activities of protease and amylase, respectively, could provide informations on nutritional quality of feeds and feed raw materials.

This work is the first part of the project on biochemical evaluation of dietary quality in relation to growth performance quality of *C. hainesiana*. The aim of the work was to determine optimum pH and temperature for the main digestive enzymes (amylase, protease and lipase) collected from digestive gland including stomach of the adult *C. hainesiana*, under various conditions. Data obtained from this study are prerequisite for future studies of digestive enzyme expression during the life cycle, and for future development of artificial feed formulation for mussel culture.

Materials and Methods

Animal and rearing

Adult *C. hainesiana* of 14 months old were transferred from Kanchanaburi Inland Fisheries Research and Development Center to the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, where they were sampled for enzyme analyses. They were cultured mussels from glochidia stage until adult, according to Kovitvadhi et al. (unpublished). The mussels from late juvenile (150 days) to adult were fed freely with natural plankton in the pond.

Preparation of enzymes extracts

Eight mussels were cleaned with tap water to remove adhering detritus. The shells were opened by cutting off anterior and posterior adductor muscles, and the digestive glands and stomachs were dissected. The organs were weighed and the digestive gland including stomach from four mussels were pooled and used as one replication. Enzyme extraction was modified from Areekijseer et al. (2004) by adding 1:0.5 (w/v) of 200 mM phosphate buffer, pH 7 during homogenization on ice. After centrifuged at 13,000 g for 15 min at 4°C, the supernatant was collected and kept in aliquots at -80°C for later determination of amylase, protease and lipase specific activities. The protein concentration of the enzyme extracts was determined using the method described by Lowry et al. (1951).

Determination of amylase specific activity

Amylase activity was determined by 3,5-dinitrosalicylic acid (DNS) method according to Areekijseer et al. (2004) based on Benfeld (1951). The reaction mixture contained 125 µl 1% soluble starch (in 100 mM specific buffer containing 6 mM NaCl)

and 125 µl enzyme extract of suitable dilution. For pH profile study, the reaction mixture was incubated in buffers pH 2–11 for 15 min at 25°C. Then the reaction was stopped by adding 250 µl 1% DNS (dissolved in 2 M NaOH and 0.6% sodium potassium tartrate), heated in boiling water bath for 5 min. After cooling down, 2.5 ml distilled water was added before measuring the absorbance at 540 nm. The buffers used were glycine-HCl buffer for pH 2, citrate phosphate buffer for the pH range of 3–5, phosphate buffer for the pH range of 6–8, and NaHCO₃-Na₂CO₃ buffer for the pH range of 9–11 (Areekijseer et al., 2004). For temperature profile study, the reaction mixtures were performed at various temperatures (25–80°C), at different pH. Amylase specific activity was expressed as U mg protein⁻¹. The unit (U) of amylase activity was defined as µmol maltose produced per hour (under the specified reaction conditions).

Determination of protease specific activity

Protease activity was determined by measuring an increase in cleavage of short-chain polypeptide using azocasein as substrate, modified from Areekijseer et al. (2004) based on Garcia-Carreno (1992). The reaction mixture contained 250 µl 1% azocasein (dissolved in 0.1 M NaOH and 100 mM specific buffer) and 10 µl enzyme extract of suitable dilution. The protease activity was too low to be detected at 25°C. Therefore the pH profile study was performed at 30°C in the pH range of 2–11 using the buffers as described above. After 15 min incubation, the reaction was stopped by adding 1.2 ml 10% TCA and centrifuged at 8,000 g for 15 min. The supernatant was collected and 1.4 ml 1 M NaOH was added before measuring the absorbance at 440 nm. For temperature profile study, the reactions were carried out at 25–80°C, at different pH. Total protease

specific activity was expressed as U mg protein⁻¹. The unit (U) of protease activity was defined as an increase in absorbance at 440 nm per hour (under the specified reaction conditions).

Determination of lipase specific activity

Lipase activity was determined using the method modified from Gupta et al. (2002) based on Winkler and Stuckmann (1979). The reaction mixture contained 200 µl 0.01 M *p*-nitrophenyl palmitate (dissolved in isopropanol), 800 µl 0.2 M buffer and 10 µl crude enzyme extract of suitable dilution. For pH profile study, the reaction mixtures were performed at 25°C in various buffers pH 2–11, as described above, for 15 min. The reaction was then stopped by adding 250 µl 0.1 M Na₂CO₃. After centrifuging at 13,000 g for 15 min, the absorbance of the supernatant was measured at 410 nm. For temperature profile study, the reaction mixtures were performed at various temperatures (25–80°C), at different pH's. Lipase specific activity was expressed as U mg protein⁻¹. The unit (U) of lipase activity was defined as the increase in absorbance at 410 nm per hour (under the specified reaction conditions).

Statistical analysis

Mean and standard error of the mean were calculated for each enzyme specific activity.

Results

Amylase activity had pH optimum at pH 6 (Fig. 1A), and at least three isoforms of amylase were observed at pH 5, 6 and 8 (Fig. 1A). Specific activity of amylase was very low at pH ≤ 4 and at pH ≥ 9 (Fig. 1A), showing less than 400 U/mg protein. Study of temperature profiles at pH 5, 6 and 8 indicated different amylase activities with optimal temperatures at 25°C, 40°C and

40°C, respectively (Fig. 1B). Two isoforms had very low activities at ≥ 50°C, while the other isoform had very low activity at ≥ 60°C (Fig. 1B). Amylase showed highest specific activity at 40°C pH 6.

For protease, it had optimum pH 6 (Fig. 2A), and at least three isoforms were observed at pH 3, 5 and 6 (Fig. 2). Protease specific activity was too low to be detected at 25°C. However, the pH profile study at 30°C revealed the specific activity at pH 6 > pH 5 > pH 3 (Fig. 2A). No protease activity was detected at pH ≥ 7 (Fig. 2A). Study of temperature profiles at pH 3, 5 and 6 indicated different proteases with optimal temperatures at 40–60°C, 50°C and 30°C, respectively (Fig. 2B). The optimal condition at 50°C pH 5 revealed the highest protease specific activity following with the condition at 30°C pH 6.

The pH profile of lipase showed a relatively high specific activity in a broad pH range of 2–11. (Fig. 3A). At least 4–5 isoforms of lipase were observed at pH's 2, 6, 8, 10, and probably 11 (Fig. 3A). By selecting not too extreme pH, the pH 6, 8, and 10 were chosen for temperature profile study, showing lipase specific activities with optimal temperatures at 40°C, 50°C 80°C, and 30°C, respectively (Fig. 3B). Lipase showed highest specific activity at 50°C pH 8.

Discussion

In this study, we observed different isoforms of the digestive enzymes with different optimal conditions of pH and temperature for their activities. At least three isoforms were detected for amylase and protease, and at least 4–5 isoforms for lipase. The temperature range of 30–50°C and pH range of 5–8 for amylase activity in the digestive gland including stomach of *C. hainesiana* are similar to those observed in marine mussel *Mytilus chilensis* (Fernandez-Reiriz et al., 2001) and freshwater

pearl mussel *Hyriopsis bialatus* (Areekijseree et al., 2002; Supannapong et al., 2008). A relatively high amylase activity at 30°C, although not optimum, indicated an importance of *C. hainesiana* amylase for carbohydrate digestion in natural

habitat where the temperature range is 28–30°C. However, the general optimum condition suitable for studying amylase expression in *C. hainesiana* is 40°C and at pH 6.

There are many types of protease enzymes with

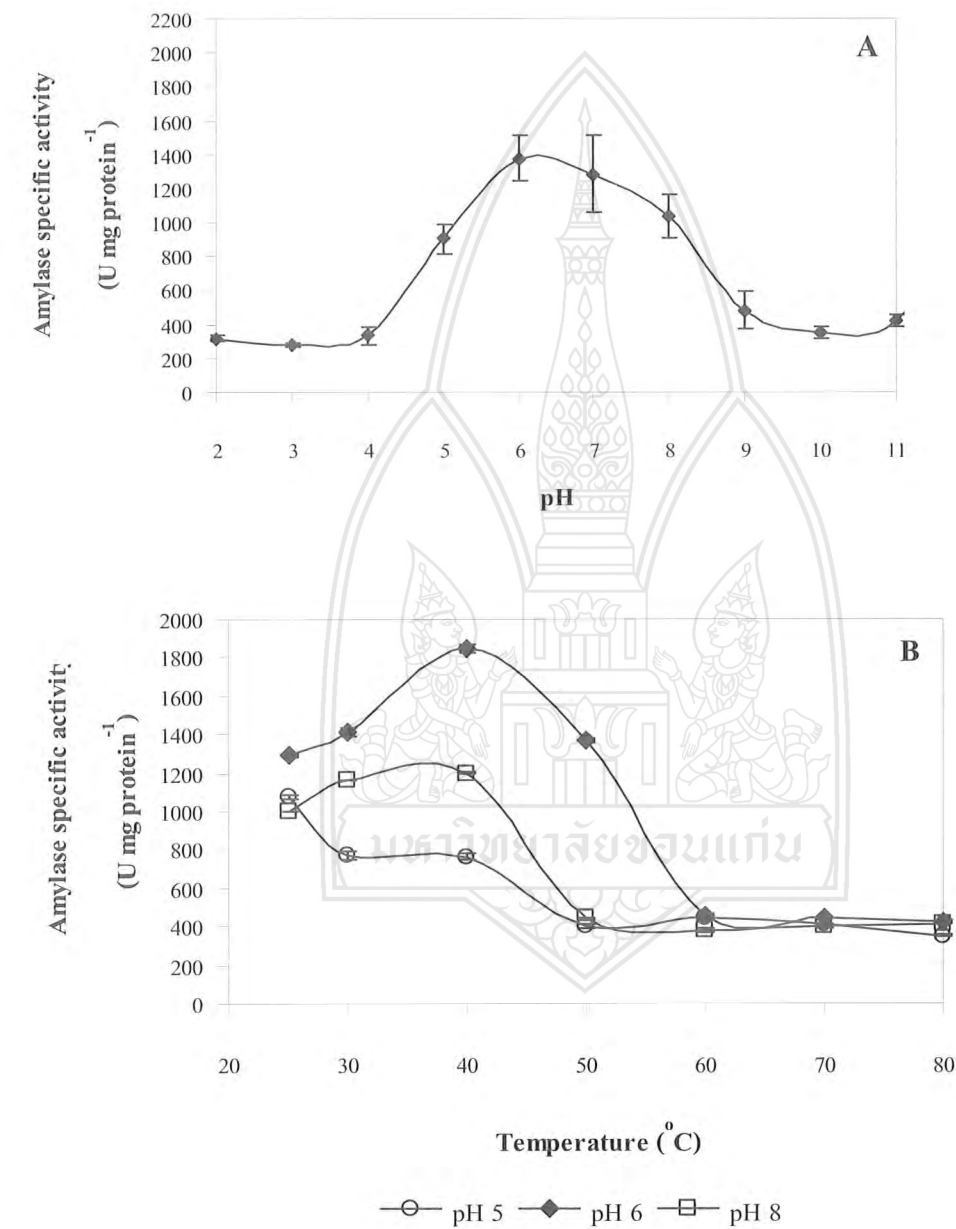


Figure 1. Amylase specific activity (U mg protein⁻¹) showing pH profile at 25°C (A), and temperature profiles at pH's 5, 6 and 8, indicating general optimal condition at 40°C pH 6 (B).

specific activity at different pH (Areekijserree et al., 2004; Supannapong et al., 2008; present work) and temperature (Rungruangsak Torrisen and Male, 2000; present work).

Surprisingly, no alkaline protease activity was detected in the digestive gland including stomach of adult *C. hainesiana*. Among the three isoforms of protease detected, two main

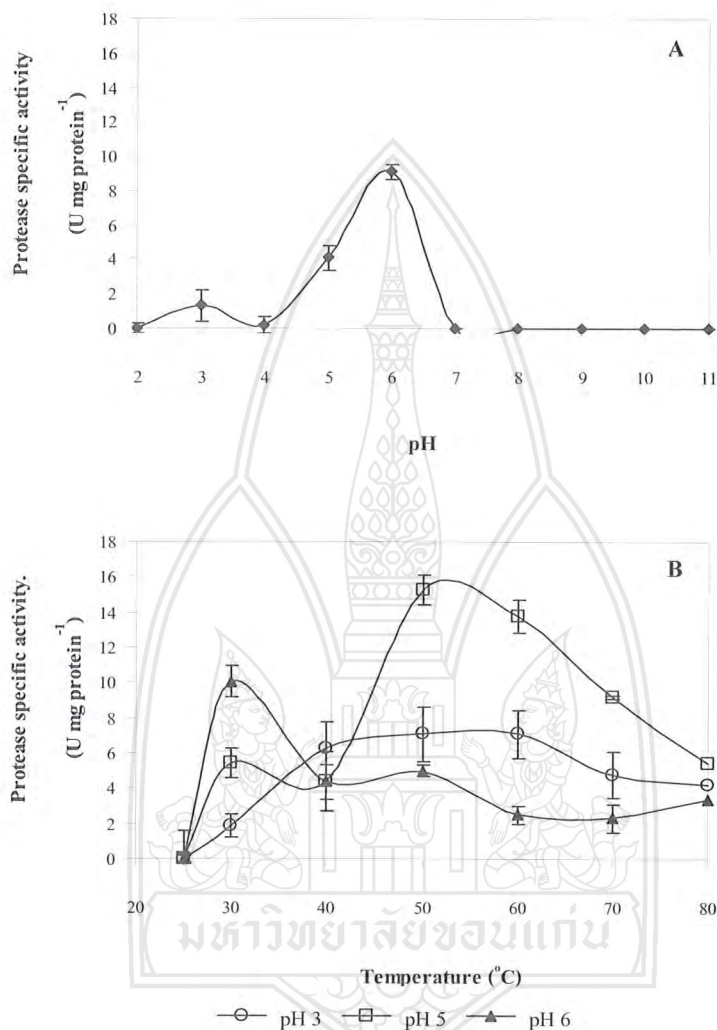


Figure 2. Protease specific activity (U mg protein⁻¹) showing pH profile at 30°C (A), and temperature profiles at pH's 3, 5 and 6, indicating general optimal condition at 50°C pH 5 (B)

protease activities were observed with their optimum conditions at 50°C, pH 5 and 30°C, pH 6. These two main proteases probably represent one from stomach and the other from digestive gland but when extracting was difficult to separate these two organs. pH of the samples extracted from digestive gland and stomach

it was neutral. This indicated that, at natural habitat temperature (28–30°C), neutral protease is important for protein digestion in adult *C. hainesiana*. However, the general optimum condition suitable for studying protease expression in *C. hainesiana* is 50°C, pH 5.

Lipase seemed to consist of a large group

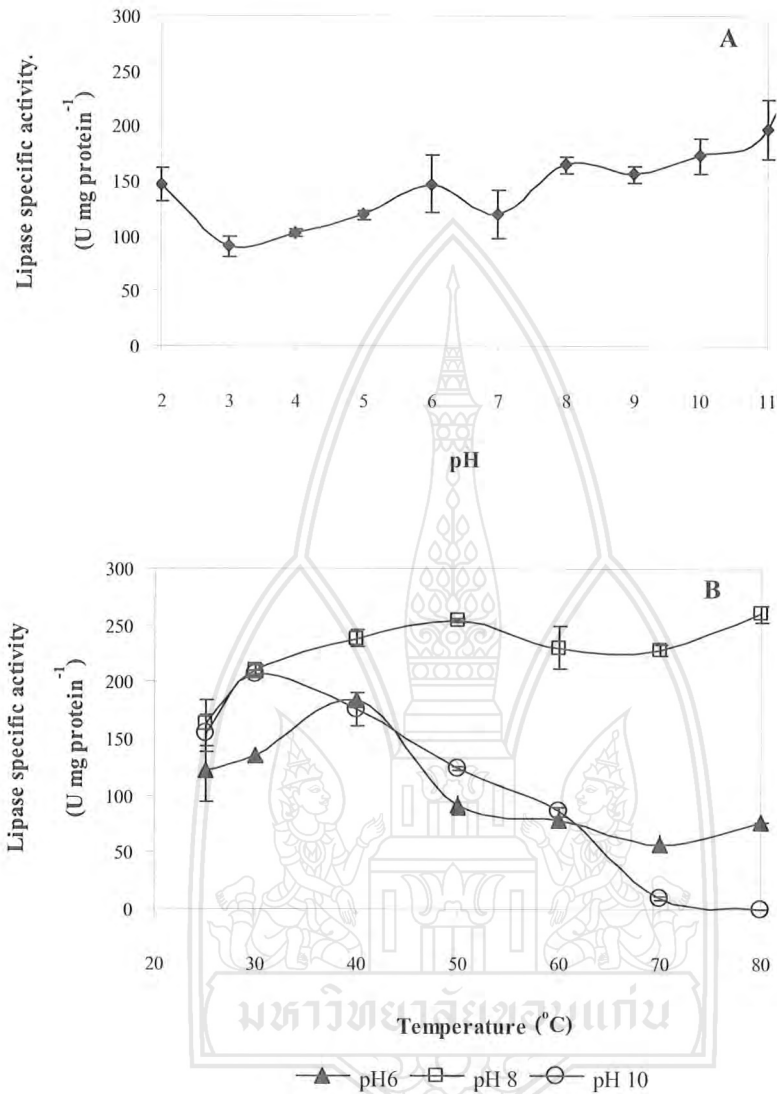


Figure 3. Lipase specific activity (U mg protein⁻¹) showing pH profile at 25°C (A), and temperature profiles at pH's 6, 8 and 10, indicating general optimal condition at 50°C pH 8 (B)

of enzyme types with the activities covering a broad temperature and pH range. Among the different isoforms of lipase observed, and at natural habitat temperature of around 30°C, two alkaline lipases actively at pH 8 and

pH 10 are important for lipid digestion of this mussel species. However, the optimum condition suitable for studying lipase expression in *C. hainesiana* is 50°C pH 8.

Temperature and pH, playing important

roles on enzyme activities, can cause changes in enzyme structure as well as its catalytic performance. The temperature and pH for optimizing digestive enzyme activity are important factors for future study of any impact on expression of each digestive enzyme of interest during the animal life cycle, whereas the condition of pH with high temperature optimum is suitable for such comparison study (Rungruangsak-Torrissen et al., 2006, Rungruangsak-Torrissen, 2007). The enzyme characteristics study could also be useful as a prerequisite knowledge for developing artificial feed formulations through nutritional evaluation of diets by *in vitro* digestibility study (Rungruangsak-Torrissen et al., 2002; Areekijseree et al., 2006; Rungruangsak-Torrissen, 2007; Supannapong et al., 2008), whereas the condition of pH with optimal temperature close to natural habitat is suitable. In addition, it could be useful for formulating artificial diets that are suitable for the digestion under different rearing conditions (Rungruangsak-Torrissen et al., 2002; Rungruangsak-Torrissen, 2007). The ability of digestion is species specific (Rungruangsak-Torrissen et al., 2002). Therefore it is important that temperature and pH for optimizing the activities of the main digestive enzymes are studied in each species of interest. By this way, the optimal temperature and pH could be selected for amylase, protease and lipase expressions, for *in vitro* digestion of carbohydrate, protein and lipid, respectively, in different food raw materials using fish crude enzyme extracts. Among different main nutrient digestibility, protein digestion apparently is the key factor determining food quality even in herbivores like mussels (Areekijseree et al., 2006; Supannapong et al., 2008). Protease activity in acid condition (peptic activity) is not related to feed

utilization quality and fish growth, unlike protease activity in alkaline condition (tryptic activity) that is related to feed conversion efficiency and fish growth (see Rungruangsak-Torrissen et al., 2006). *In vitro* protein digestibility of diets using fish crude enzyme extracts could indicate the qualities of the diets in fish growth trials (Rungruangsak-Torrissen et al., 2002; Rungruangsak-Torrissen, 2007). Unfortunately, in *C. hainesiana*, no protease activity was observed in alkaline pH (Fig. 2A). Therefore, we could not detect tryptic activity, which is active in alkaline pH. However, for *C. hainesiana*, future studies on *in vitro* protein digestibility of food raw materials in neutral condition of pH 6 should provide reasonably good information for quality of the food raw materials for diet formulations that could be utilized optimally by the mussel. For adult *C. hainesiana*, the *in vitro* digestibility condition for future assessing the qualities of food raw materials for diet formulation should be at 30°C pH 6 for protein and carbohydrate digestion. If lipid digestion would be studied, the *in vitro* digestion condition would be performed separately at 30°C pH 8. These temperature and pH conditions could be used for the *in vitro* digestibility study of food raw materials as well as the formulated diets.

Acknowledgements

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Diversity of Anuran and Habitat Type of Their Tadpoles in Kui Buri National Park, Thailand

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Abstract

A study on anuran diversity in Kui Buri National Park, Prachuapkhirikhan Province, Thailand, was carried out during the period from August 2005 to July 2006. Surveys were conducted monthly for approximately 3-4 days. This study found frogs and/or tadpoles of 43 species, belonging to 26 genera, 6 families (Megophryidae, Bufonidae, Dicroglossidae, Ranidae, Rhacophoridae and Microhylidae) and 1 order. *Ansonia malayana*, *Phrynoidis aspera*, *Ingerophrynus parvus* and *Limnonectes blythii*, which are protected animals in Thailand, were found. *Glyphoglossus molossus* and *Limnonectes blythii*, which have near threatened animal status according to Thailand Red Data: Mammals, Reptiles and Amphibians, were also found in this study. This study found 37 tadpole species in 3 different habitat types: 12 species in lotic water type, 22 species in permanent lentic water type, and 20 species in temporary water bodies. Of the 37 tadpole species, 17 species can reside both in permanent and temporary water bodies; they are *Duttaphrynus melanostictus*, *Hoplobatrachus rugulosus*, *Fejervarya limnocharis*, *Polypedates leucomytax*, *Glyphoglossus molossus*, *Micryletta inornata*, for examples. Five species, (*Chiromantis vittatus*, *Rhacophorus maximus*, *Rhacophorus reinwardtii*, *Kaloula* sp. and *Microhyla* sp.,) were restricted to permanent lentic water types, and three species, (*Pelophylax lateralis*, *Microhyla pulchra* and *Kurixalus verrucosus*,) were restricted to temporary water bodies.

Keywords : Anuran Diversity, Tadpole Diversity, Habitat Type

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Introduction

Amphibian diversity is very rich in the tropical rain forest zone. For example, South America has 1,400 species whereas 700 species occur in the Indo-Australian region (Frost, 2002). Thailand is located in the Indo-Malayan Region with the north and the south of Thailand are the Indo-Chinese subregion and the Sundaic subregion, respectively. These subregions are separated at latitude 11°40' N., north of Chumphon Province. Kui Buri National Park, Prachuapkhirikhan Province, covers part of the 2 subregions. This area is important for animal distributions between the Indo-Chinese subregion and the Sundaic subregion (Lekagul and Round, 2005), so determination of amphibian diversity in this area will provide important information. Amphibians have declined dramatically in many areas in present time. They have revealed numerous potential causes from loss of habitat, environmental contaminants, emerging diseases, invasion of alien species, direct exploitation, climate changes and other synergistic effects. It is possible that the physiology and morphology of amphibians has contributed to these population declines. This study to explore amphibian diversity may provide important data for conservation and management in the future.

Materials and Methods

The diversity of anurans and the habitat types of their tadpoles were studied in Kui Buri National Park, Prachuapkhirikhan Province, Thailand, for 1 year period ranging from August 2005 - July 2006. This study included survey and collection of samples in the national park area for 3-4 days in every month. Some tadpoles were collected and reared until they reached adulthood and then their species were classified in the laboratory. Tadpole species were classified based

on morphology: mouth part structures, gill position, intestinal pattern, and other data. For adult anurans, they were classified by using Kampen (1923); Taylor (1962); Berry (1966); Inger (1966); Taylor (1966); Matsui et al. (1996); Leong and Chou (1999); Wut (2003) and Frost (2007).

Results

The study found 43 adult anuran and/or tadpole species belonging to 26 genera, 6 families, including Megophryidae, Bufonidae, Ranidae, Rhacophoridae and Microhylidae and 1 order (Table 1 and Figure 1). These 43 species (Figure 2) consist of:

Twelve species residing in lotic water type; *Amolops panhai*, *Amolops marmolatus*, *Ansonia malayana*, *Brachytarsophrys carinensis*, *Phrynoidis aspera*, *Leptobrachium smithi*, *Limnonectes blythii*, *Odorrana nasica*, *Clinotarsus alticola*, *Hylarana eschatia*, *Hylarana nigrovittata* and *Xenophrys parva*

Seventeen species residing in both permanent and temporary water body, such as *Duttaphrynus melanostictus*, *Hoplobatrachus rugulosus*, *Fejervarya limnocharis*, *Polypedates leucomystax*, *Glyphoglossus molossus* and *Micryletta inornata*.

Five species residing in permanent lentic water type; *Chiromantis vittatus*, *Kaloula* sp., *Microhyla* sp., *Rhacophorus maximus* and *Rhacophorus reinwardtii*

Three species residing in temporary water body *Microhyla pulchra*, *Pelophylax lateralis* and *Kurixalus verrucosus*

Discussion

The study found four adult frog and/or tadpole species which are new distribution in this area; they are *Glyphoglossus molossus*, *Pelophylax lateralis*,

Chiromantis vittatus and *Rhacophorus maximus*. For *G. molossus*, this species was found inhabiting in the ecotone area between the agricultural area and the forest area, and its tadpoles inhabit in lentic water in the dry evergreen forest area. Taylor (1962) and Inthara et al. (2005) reported that this species can be found in northern part of Thailand, Chon Buri, Chanthaburi, Rat Buri, Phra Nakhon Si Ayutthaya and Kanchanaburi. Chan-ard. (2003) stated that it has wide distribution throughout Thailand except southern Thailand and Kanchanaburi locates at the lowest latitude in the Western Thailand. Moreover, *Ansonia malayana*, *Phrynoidis aspera*, *Ingerophrynus parvus* and *Limnonectes blythii*, which are protected animals in Thailand, were found. *Glyphoglossus molossus* and *Limnonectes blythii*, which have near threatened animal status according to Thailand Red Data: Mammals, Reptiles and Amphibians (Nabhitabhata and Chan-ard, 2005), were also found in this study.

Adults and tadpoles of *Pelophylax lateralis* live in the ecotone area between the agricultural area and the forest area but Taylor (1962) and Chan-ard (2003) reported that it has wide distribution in Thailand except the southern part of Thailand and it normally lives in deciduous dipterocarp forest area.

Chan-ard (2003) reported that *Chiromantis vittatus* can be found in the northern part of Thailand but this study found *C. vittatus* strictly lives in dry evergreen forest area at altitude about 100-600 meters (msl.). The study found that population at about 600 meters (msl.) has the same morphological characteristics as those of *Chirixalus punctatus* with many dark brown spots on head, dorsum, trunk, and legs (Wilkinson et

al., 2003). However the color is changed later on and is very similar to that of *Chiromantis vittatus*, which is violet-brown on dorsum with three rather indistinct violet stripes without any dark brown spots. This color pattern is the same as that in the population at altitude about 100 meters (msl.). Therefore it confers to *C. vittatus*.

For *Rhacophorus maximus*, it can be found from 500-2,000 meters (msl.) (IUCN, 2008) but this study found its tadpoles inhabit in permanent lentic water body at altitude about 600 meters (msl.) which is the range of altitude from IUCN. This species can also be found in eastern Nepal, northern Bangladesh, India, northern Burma and southern Xizang and southern Yunnan provinces in China and western Thailand (IUCN, 2008). In Thailand this species was reported in Rat Buri, Kanchanaburi and Phetchaburi provinces. (Chan-ard, 2003; Khonsue et al., 2006) Therefore, the distribution of *R. maximus* in Kui Buri National Park area is the lowest latitude of its distribution.

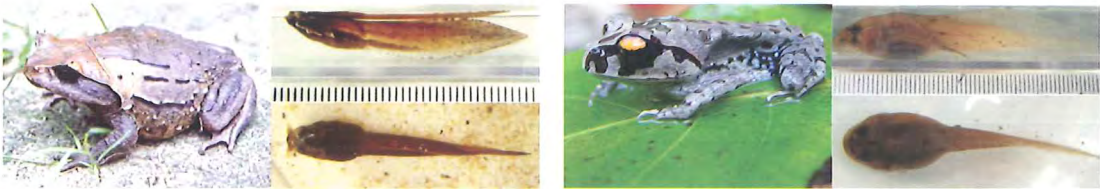
Inthara et al. (2005) divided tadpoles habitat into five types by using microhabitat characteristics including the relationship between mouth part structure and feeding behavior of tadpoles. But tadpole habitat types in Kui Buri National Park area can be divided into three types only. The study also found that some tadpole species have mouth part structure alike but they inhabit in different habitats. Therefore, the mouth part structure and feeding behavior of tadpole species can not be used as an index of tadpole habitat types in this study, but environment factors such as water temperature, dissolve oxygen, may be the important factors.

Table 1. Families and species of frogs and/or tadpoles found in Kui Buri National Park, Prachuapkhirikhan Province, Thailand.

Sceincetific Names*		Sceincetific Names*	
No	Species	No	Species
	<u>Family Megophryidae</u>	21	<i>Hylarana erythraea</i>
1	<i>Brachytarsophrys carinensis</i>	22	<i>Hylarana eschatia</i>
2	<i>Leptobrachium smithi</i>	23	<i>Hylarana nigrovittata</i>
3	<i>Leptolalax melanoleucus</i>	24	<i>Odorrana nasica</i>
4	<i>Xenophrys major</i>	25	<i>Pelophylax lateralis</i>
5	<i>Xenophrys parva</i>		<u>Family Rhacophoridae</u>
	<u>Family Bufonidae</u>	26	<i>Chiromantis nongkhorensis</i>
6	<i>Ansonia malayana</i>	27	<i>Chiromantis vittatus</i>
7	<i>Duttaphrynus melanostictus</i>	28	<i>Kurixalus verrucosus</i>
8	<i>Ingerophrynus parvus</i>	29	<i>Polypedates leucomystax</i>
9	<i>Phrynoidis aspera</i>	30	<i>Rhacophorus bipunctatus</i>
	<u>Family Dicroglossidae</u>	31	<i>Rhacophorus maximus</i>
10	<i>Fejervarya limnocharis</i>	32	<i>Rhacophorus reinwardtii</i>
11	<i>Hoplobatrachus rugulosus</i>		<u>Family Microhylidae</u>
12	<i>Limnonectes blythii</i>	33	<i>Calluella guttulata</i>
13	<i>Limnonectes doriae</i>	34	<i>Glyphoglossus molossus</i>
14	<i>Limnonectes hascheanus</i>	35	<i>Kaloula pulchra</i>
15	<i>Limnonectes kuhlii</i>	36	<i>Kaloula</i> sp.
16	<i>Occidozyga lima</i>	37	<i>Microhyla berdmorei</i>
17	<i>Occidozyga martensii</i>	38	<i>Microhyla butleri</i>
	<u>Family Ranidae</u>	39	<i>Microhyla fissipes</i>
18	<i>Amolops marmoratus</i>	41	<i>Microhyla pulchra</i>
19	<i>Amolops panhai</i>	42	<i>Microhyla</i> sp.
20	<i>Clinotarsus alticola</i>	43	<i>Micryletta inornata</i>

* Sceincetific names followed Frost (2007)

Family Megophryidae



Brachytarsophrys carinensis

Leptobrachium smithi

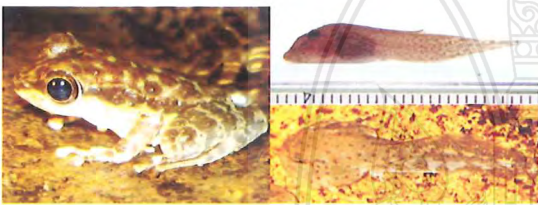
Family Bufonidae



Ansonia malayana

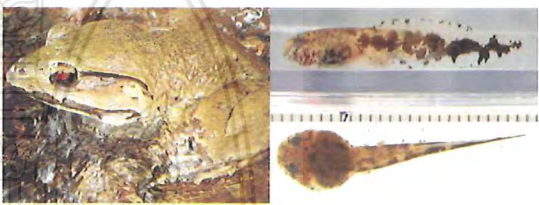
Phrynomantis aspera

Family Ranidae



Amolops panhai

Dicroglossidae



Limnonectes blythii

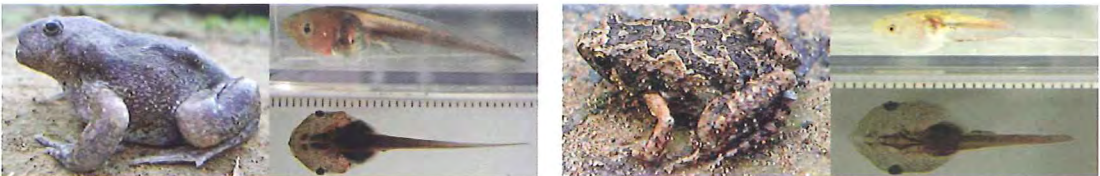
Family Rhacophoridae



Rhacophorus bipunctatus

Chiromantis vittatus

Family Microhylidae



Glyphoglossus molossus

Microhyla butleri

Figure 1. Example anuran adults and tadpoles in Kui Buri National Park, Prachuapkhirikhan Province, Thailand.

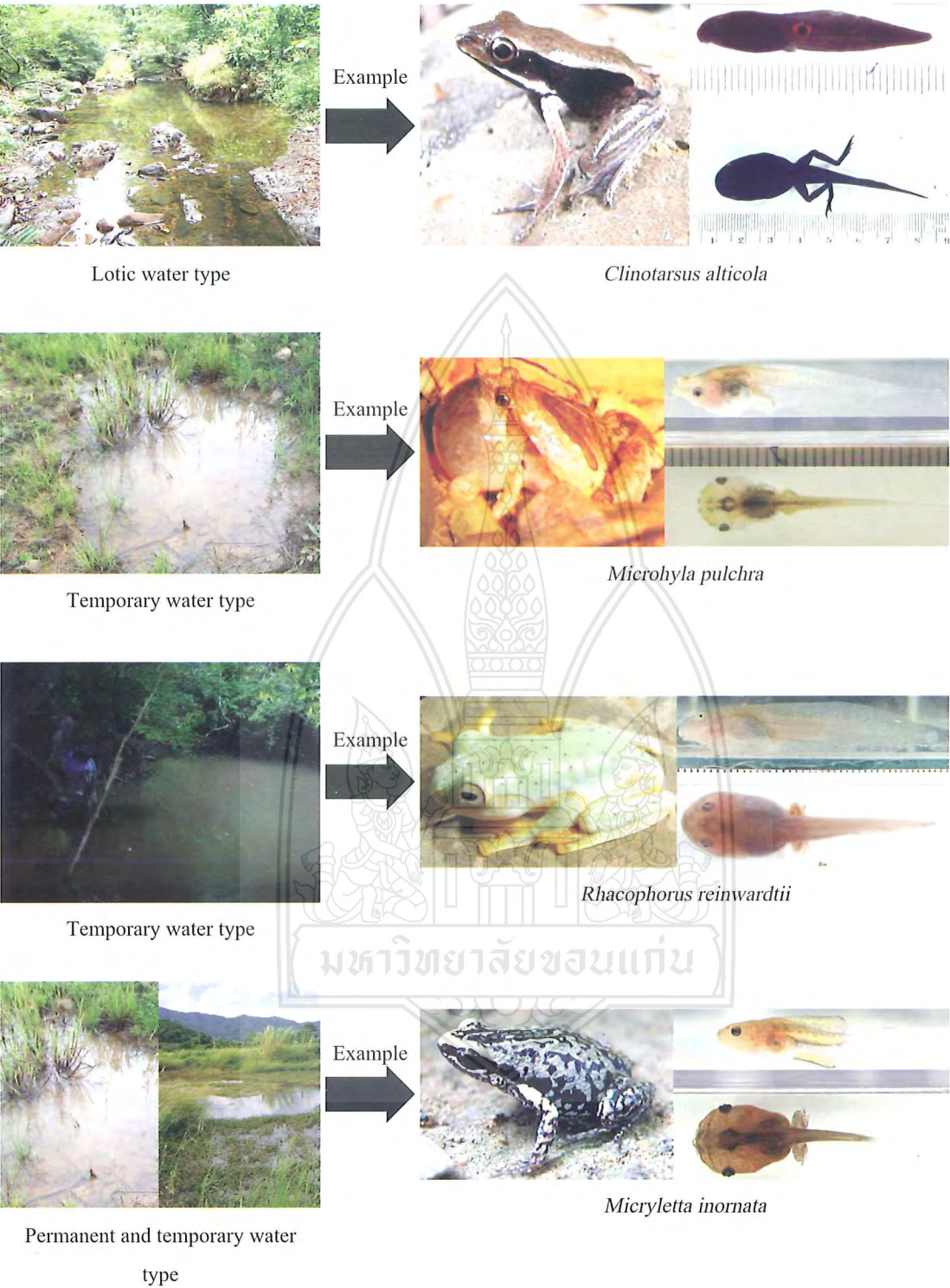


Figure 2. Example of habitat types of anuran adults and of tadpoles in Kui Buri National Park, Prachuapkhirikhan Province, Thailand.

Conclusions

1. In this study, we found 43 species of frogs and/or tadpoles belonging to 26 genera and 6 families (Megophryidae, Bufonidae, Dicroglossidae, Ranidae, Rhacophoridae, and Microhylidae) and 1 order.

2. Three habitat types for amphibians were determined: lotic water type, permanent lentic water type and temporary water type.

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Diversity of Benthic Algae and Water Quality in Tributaries of the Mekong River Passing Thailand and Some Parts of Lao PDR

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Abstract

There are many tributaries which are connected to the Mekong River passing along Thailand. The water quality in the river was affected from these tributaries. Besides, the Mekong River and their tributaries have influenced on life of people surrounded. A study on the diversity of macroalgae and benthic diatoms in the tributaries of Mekong River was carried out from May 2007 to April 2008. Five sampling sites were selected along the Kok River in Chiang Rai Province, the Huerng River in Loei Province, the Songkram River in Nakhon Phanom Province, the Moon River in Ubonratchatani Province and Nam Ngum River in Vientiane, Lao PDR.

In this investigation, twelve species of macroalgae were found, they belong to 3 divisions, viz. Cyanophyta, Chlorophyta and Charophyta. The dominant genera in Division Cyanophyta were *Phormidium* spp. and *Nostoc* spp; *Spirogyra* spp. and *Cladophora* spp. in Division Chlorophyta and *Nitella* spp. were dominant genera of Division Charophyta. One hundred and twenty seven species of benthic diatoms were also found and classified in Division Bacillariophyta. Most of them were pennate diatoms e.g. *Navicula* spp., *Gomphonema* spp., *Nitzschia* spp. and *Achnanthes* spp. The water qualities in these tributaries were moderate and classified by trophic status as mesotrophic.

Keywords : Biodiversity, Macroalgae, Benthic Diatoms, Tributaries of the Mekong River

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Introduction

The Mekong River is the main river in South East Asia. Its estimated length is 4,350 km. From the Tibetan Plateau it runs through China’s Yunnan province, Myanmar, Thailand, Lao PDR, Cambodia and Vietnam. The biodiversity of the Mekong River is one of the richest in the world. More than 1200 species of fish have been identified and there could possibly be as many as 1700 species. The Mekong River has many tributaries with many people live along. They have many daily life activities such as consumption, agriculture, transportation, fishery affected to the water quality in the Mekong River.

Benthic algae are important organisms in running water because They are primary producers in aquatic ecosystem. They can be used to indicate water quality, because we can found them in specific environment in running water. Beside, some macroalgae such as *Cladophora* spp., *Microspora* spp. and *Spirogyra* spp. were used as food by local people in the northern and north-eastern of Thailand. However, there is no survey

of the macroalgae in this area. In this investigation, the water quality based on physical, chemical and diversity of benthic algae were determined. The survey of edible macroalgae in these tributaries of the Mekong River was also done.

Study site

Sampling sites (Figuer 1) were selected along Kok River in Chiang Rai Province, Huerng River in Loei Province, Songkram River in Nakhon Phanom Province, Mool River in Ubonratchatani Province and Nam Ngum River in Vientiane, Lao PDR. Each site was affected by human activities. The Kok River has been affected from transportation by boat travelling. The Huerng River has been affected from land logging and agriculture which resulted in flooding and nutrients washed away from soil and increased velocity of water in river. The Songkram River has high conductivity because of the salt in soil particle, and the Moon River has been affected from fishery and the dam south of sampling site. The Nam Ngum River has been affected from Nam Ngum Reservoir upstream of sampling site.



Figure1. Map of Thailand showing sampling site ,1) Kok River in Chiang Rai Province (site 1), 2) Huerng River in Loei Province (site 2), 3) Nam Ngum River in Lao PDR (site 3), 4) Songkram River in Nakhon Phanom Province (site 4) and 5) Mool River in Ubonratchatani Province (site 5)

Material and Methods

Samples were collected in May 2007 (hot dry season 1), August 2007 (rainy season), January 2008 (cool dry season) and April 2008 (hot dry season 2).

Sampling of benthic algae

Macroalgae were collected by scrapes and scratches from substrates along the river bank of each sampling site such as stone, gravel, stick and were kept at low temperature. A random number table was used to select 10 plot of 1 m² with in sampling area of 100 m x 5 m. The samples were preserved with 2% glutaraldehyde.

Benthic diatoms were collected in same random plot of macroalgae by placing 10 mm² plastic sheath on upper surface of selected substrate and brushing them until cut out area was completely clear. In the laboratory, diatoms were cleaned by centrifugation at 2,500 rpm 15 minutes. Then, layers of diatom cell that appeared between supernatant and solid particle were placed into test tube. Concentrate HNO₃ was added and heated in a boiler at 70°C - 80°C for 15- 30 minutes. The sample was rinsed by distilled water to adjust pH to 6-7. The cleaned cell will be precipitated in the test tube as grey powder. Sample was dropped on microscope slide and slowly dried on hot plate. Naprax is used as mounting agent for making permanent slide. (Rott et al, 1997)

Identification of benthic algae

Benthic algae were identified based on morphology by using relevant books for identification of macroalgae e.g., Desikachary (1959); Entwisle (1989) and Kumano (2002). The identification books for benthic diatoms were Barber and Haworth (1981), Lange-Bertalot (2001) and Kelly and Haworth (2002).

Determination of water quality

Some physical and chemical parameters of water samples were measured at sampling sites by using multimeter, i.e. water temperature, pH, conductivity. Total dissolved solid and turbidity was measured by absorptometric method by using spectrophotometer (HACH model DR/2000). Other parameters were determined in the laboratory with the standard method procedure, i.e. alkalinity, dissolve oxygen (DO), biochemical oxygen demand (BOD) and nutrient. Alkalinity was determined by phenolphthaleine methyl orange indicator method, DO and BOD by azide modification method, and nutrient (Nitrate-nitrogen, Ammonium-nitrogen, orthophosphate) by spectrophotometer, cadmium reduction method, Nessler method and Ascorbic method, respectively.

In the investigation, the values of DO, BOD, conductivity and nutrients were applied to trophic status by using AARL-PC score. This method was modified from those of Wetzel (1983); Lorraine and Vollenweider (1981) and Peerapornpisal et al. (2004).

Result

Twelve species of macroalgae were found. They were classified to 3 divisions, 5 orders and 7 families. Most of them belonged to Division Cyanophyta (50%) and Division Chlorophyta (42%). The dominant species in Division Cyanophyta were *Phormidium* spp., *Nostoc* spp. and *Oscillatoria* princeps Vaucher ex Gomont *Cladophora glomerata* Kützting while *Spirogyra* sp. was a dominant species in Division Chlorophyta and *Nitella* spp. was a dominant species in Division Charophyta. (Table 1).

From the investigation, 127 species of benthic diatom were also found and could be classified to 2 orders, 10 families, and 35 genera. They were benthic

diatoms of Order Pennales and 4 species of Order Centrales. The dominant species were *Achnanthes minutissima* Kützing, *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot, *Gomphonema lagenula* Kützing, *Navicula symmetrica* Patrick, *Nitzschia palea* (Kützing) W. Smith (Table 2 and Figure 3).

In this research, water quality was classified based on AARL-PC score. The water quality of 5 sampling sites was in mesotrophic status and showed moderate water quality (Table 3 and Figure 2).

Table 1. Species list of macroalgae and site distribution in tributaries of the Mekong River during 2007-2008.

Taxon	Site Distribution
Division Cyanophyta	
Order Nostocales	
Family Nostocaceae	
<i>Nostoc</i> sp.	5
Family Oscillatoriaceae	
<i>Lyngbya</i> sp.	5
<i>Microcoleus</i> sp.	2
<i>Oscillatoria princeps</i> Vaucher ex Gomont	5
<i>Phormidium</i> sp.	2
Division Chlorophyta	
Order Tetrasporales	
Family Tetrasporaceae	
<i>Tetraspora</i> sp.	5
Order Siphonocladales	
Family Chladophoraceae	
<i>Cladophora glomerata</i> Kützing	2
Family Zygnemataceae	
<i>Spirogyra</i> sp.1	1, 2, 3, 4
<i>Spirogyra</i> sp.2	4
Order Charophyta	
Order Charales	
Family Characeae	
<i>Nitella</i> sp.	3

Table2. Species list of benthic diatoms and site distribution in tributaries of the Mekong River in 2007-2008.

Taxon	Site Distribution
Order Biddulphiales	
Family Melosiraceae	
<i>Aulacoseira granulata</i> Ehrenberg	1, 2, 3, 4, 5
Family Thalussiosiraceae	
<i>Cyclotella meneghiniana</i> Kützing	3
<i>Cyclotella stelligera</i> Cleve	1, 3, 4, 5
<i>Thalassiosira</i> sp.	1, 3, 4
Order Bacillariales	
Family Acnanthaceae	
<i>Achnanthes crenulata</i> Grunow	5
<i>Achnanthes exiqua</i> var. <i>constricta</i> (Torka) Hustedt	1, 2, 4, 5
<i>Achnanthes helvetica</i> (Hustedt) Lange-Bertalot	1,5
<i>Achnanthes inflata</i> (Kützing) Grunow	2
<i>Achnanthes minutissima</i> Kützing	1, 2, 3, 4, 5
<i>Achnanthes</i> sp.1	2, 3, 5
<i>Cocconeis placentula</i> Ehrenberg	1, 2, 3, 4, 5
<i>Cocconies</i> sp.1	1, 5
<i>Diadesmid contenta</i> (Grunow ex V.Heurck) Mann	1, 3
<i>Planothidium lanceolatum</i> (Breb.) Round & Bukhtiyarova	1, 2, 3, 4, 5
<i>Planothidium rostratum</i> Round & L. Bukhtiyarova	2, 3, 4, 5
Family Naviculaceae	
<i>Amphora montana</i> Krasske	2, 3
<i>Amphora</i> sp.	1, 3, 4
<i>Brachysira neoexillis</i> Lange-Bertalot	3, 4
<i>Bacillaria paradoxa</i> Gmelin	4
<i>Caloneis bacillum</i> (Grunov) Mereschkowsky	1, 5
<i>Diploneis oblongella</i> (Naegeli) Cleve	3, 4
<i>Diploneis</i> sp.	4
<i>Encytomonies costata</i> (hustedt) Reimer	3, 4
<i>Encytomonies</i> sp.	3, 4
<i>Frustulia saxonica</i> Rabenhorst	5

Table 2. (continued)

Taxon	Site Distribution
<i>Frusturia vulgaris</i> (Thwaites) De Toni	3
<i>Frusturia weinholdii</i> Hustedt	3
<i>Frusturia</i> spp.	3
<i>Geissleria boreosiberica</i> Lange-Bertalot	5
<i>Geissleria decussis</i> (Østrup) Lange-Bertalot&Metzeltin	1, 2, 4
<i>Geissleria</i> sp.	5
<i>Gomphonema entolejum</i> Østrup	2, 3, 5
<i>Gomphonema gracile</i> Ehrenberg	1, 3, 4, 5
<i>Gomphonema lagenula</i> Kützing	1, 2, 3, 4, 5
<i>Gomphonema parvulum</i> (Kützing) Grunow	1, 2, 3, 4, 5
<i>Gomphonema pumilum</i> (Grunov) Reichardt & Lange-Bertalot	2, 4
<i>Gomphonema truncatum</i> Ehrenberg	3
<i>Gomphonema</i> sp.1	3, 4, 5
<i>Gomphonema</i> sp.2	3
<i>Gomphonema</i> sp.3	3
<i>Gyrosigma scalproides</i> (Rabenhorst) Cleve	1, 2, 3, 4, 5
<i>Gyrosigma spencerii</i> (Quekett) Griffith&Herfrey	1, 4
<i>Luticola cohnii</i> (Hilse) D.G. Mann	3
<i>Luticola monita</i> (Hustedt) D.G.Mann	3, 4
<i>Luticola mutica</i> (Kützing) D.G. Mann	1
<i>Luticola</i> sp.	3
<i>Navicula catarata-rheni</i> Lange-Bertalot	2
<i>Navicula capitatoradiata</i> Germain	2, 3, 4, 5
<i>Navicula cryptocephala</i> forma terrestris Lund	1, 2, 3, 4
<i>Navicula cryptotenella</i> Lange-Bertalot	1, 2, 3, 4, 5
<i>Navicula germainii</i> J. H. Wallace	1, 2, 3, 4, 5
<i>Navicula rostellata</i> Kützing	1, 2, 3, 4
<i>Navicula viridula</i> (Kützing) Ehrenberg var. <i>viridua</i>	1, 3
<i>Navicula viridula</i> var. <i>germainii</i> (Wallace) Lange-Bertalot	1, 2, 3, 4, 5
<i>Navicula radiosa</i> Kützing	1, 3, 5
<i>Navicula symmetrica</i> Patrick	1, 2, 3, 4, 5

Table 2. (continued)

Taxon	Site Distribution
<i>Navicula</i> sp.1	1, 4, 5
<i>Navicula</i> sp.2	3, 5
<i>Navicula</i> sp.3	4, 5
<i>Navicula</i> sp.4	1, 3, 4, 5
<i>Neidium binodis</i> (Ehrenberg) Hustedt	1, 4
<i>Neidium gracille</i> Hustedt var. <i>aequalis</i> Hustedt	3
<i>Neidium</i> sp.	3
<i>Pinnularia gibba</i> Ehrenberg	3
<i>Pinnularia mesolepta</i> (Ehrenberg) W. Smith	1, 4, 5
<i>Pinnularia subcapitata</i> Gregory	3
<i>Pinnularia</i> sp.1	2, 3, 4, 5
<i>Pinnularia</i> sp.2	3
<i>Rhopalodia gibba</i> Ehrenberg	1, 3, 4
<i>Rhopalodia gibberula</i> (Ehrenberg) O.F. Müller	1, 5
<i>Sellaphora gibbula</i> D. Metzeltin & H. Lange-Bertalot	1, 5
<i>Sellaphora pupula</i> (Kützinger) Mereschkowsky	1, 2, 3, 4, 5
<i>Stauroneis anceps</i> Ehrenberg	5
Family Fragilariaceae	
<i>Fragilaria capucina</i> Desmazières	1, 2, 3, 5
<i>Fragillaria crotonensis</i> Kitton	1, 3, 5
<i>Fragilaria tenera</i> (W. Smith) Lange-Bertalot	2, 3
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	1, 2, 3, 4, 5
<i>Synedra</i> sp.1	1, 3
<i>Synedra</i> sp.2	1
Family Eunotiaceae	
<i>Eunotia bilunaris</i> (Ehrenberg)	3
<i>Eunotia exigua</i> (Breb.) Rabenhorst var. <i>tenella</i> (Grunow) Nörpel et Alles	3, 4
<i>Eunotia</i> sp.1	5
<i>Eunotia</i> sp.2	4

Table 2. .(continued)

Taxon	Site Distribution
Family Bacillariaceae	
<i>Bacillaria paradoxa</i> J.F. Gmelin	4
<i>Hantzchia amphioxys</i> (Ehrenberg) Grunow in cleve et Grunow	1, 3
<i>Hantzschia</i> sp.	1
<i>Nitzschia acicularis</i> (Kützing) W.Smith	1, 4
<i>Nitzschia clausii</i> Hantzsch	3, 4
<i>Nitzschia dissipata</i> (Kützing) Grunow	3
<i>Nitzschia levidensis</i> (W. Smith) Grunow in Van Heurck	1, 2, 3, 4, 5
<i>Nitzschia littoralis</i> Grunow	1, 5
<i>Nitzschia palea</i> (Kützing) W. Smith	1, 2, 3, 4, 5
<i>Nitzschia reversa</i> W. Smith	1
<i>Nitzschia sigma</i> (Kützing) W. Smith	1, 2
<i>Nitzschia sinuata</i> var. <i>tabellaria</i> (Grunow) Grunow	3
<i>Nitzschia subacicularis</i> Hustedt	1, 3, 4, 5
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	1, 3, 4, 5
<i>Nitzschia</i> sp.1	1, 3, 4, 5
<i>Nitzschia</i> sp.2	1, 3, 4
<i>Nitzschia</i> sp.3	1, 3, 4
<i>Nitzschia</i> sp.4	1, 3, 4
<i>Nitzschia</i> sp.5	1, 3, 4, 5
<i>Nitzschia</i> sp.6	2
<i>Nitzschia</i> sp.7	2
Family Cymbellaceae	
<i>Cymbella affinis</i> Kützing	1, 2, 3, 4, 5
<i>Cymbella tumida</i> (Brébisson) Van Heurck	2, 3, 4, 5
<i>Cymbella turgidula</i> Grunow	1, 2, 3, 4, 5
<i>Cymbella helmckeii</i> Krammer	2, 3
<i>Cymbella</i> sp.1	3
<i>Cymbella</i> sp.2	2,3
<i>Cymbella</i> sp.3	3,5
<i>Cymbella</i> sp.4	1,2,5
<i>Encyonema minutum</i> Mann	4

Table 2. (continued)

Taxon	Site Distribution
<i>Encyonema</i> sp.1	1, 3, 4, 5
<i>Encyonema</i> sp.2	1, 2, 3, 4, 5
<i>Encyonema</i> sp.3	2, 3, 4, 5
<i>Encyonema</i> sp.4	2, 3, 5
<i>Enocyonopsis leei</i> var. <i>leei</i> Lange-Bertalet	4, 5
<i>Enocyonopsis</i> sp.	3
Family Epithemiaceae	
<i>Epithemia adnata</i> (Kützing) Brébisson	3
<i>Epithemia</i> sp.1	5
<i>Epithemia</i> sp.2	4
Family Surirellaceae	
<i>Surirella roba</i> Leclercq	5
<i>Surirella</i> sp.	1

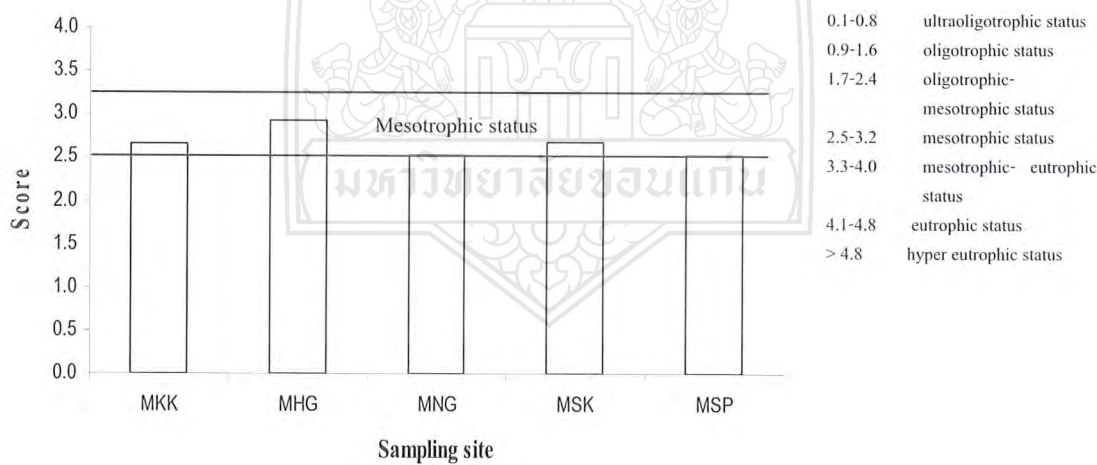


Figure 2 Trophic status of water quality by AARL-PC Score in tributaries of the Mekong River during 2007-2008.

Table 3. Minimum and maximum values of some physical and chemical parameters in tributaries of the Mekong River during 2007-2008.

MKK = Kok River

MHG = Huerng River

MNG = Nam Ngum River

MSK = Songkram River

MSP = Mool river

parameter	Sampling sites				
	MKK	MHG	MNG	MSK	MSP
Water Temp (°C)	22.8-29.7	25.3-31.1	26.2-28.7	31.0-28.2	27.5-31.3
Air T (°C)	27.4-32.5	27.0-34.8	26.9-32.8	27.5-32.4	26.0-31.2
pH	7.0-7.3	5.6-7.6	6.6-7.1	6.8-7.2	6.1-7.5
Conductivity (µs/cm)	108.2-154.1	94.6-362.6	99.1-116.2	1130.0-3120.0	51.9-302.3
TDS (mg/l)	50.6-73.0	44.3-172.3	46.3-55.3	0.7-1566.6	24.6-139.3
Turbidity (mg/l)	32.0-428.6	X > 2.0	3.3-10.6	11.0-23.7	20.2-122.3
Velocity (m/s)	0.2-3.8	0.5-1.5	0.2-7.4	0.1-15.1	0.3-3.5
DO (mg/l)	5.4-6.3	4.9-7.9	3.3-7.6	4.4-6.8	4.5-7.1
BOD (mg/l)	0.3-2.7	0.1-3.4	0.3-1.7	0.1-2.5	0.4-2.5
Alkalinity (mg/l as CaCO ₃)	70.0-104.0	70.0-125.3	60.3-86.0	14.7-69.3	13.0-70.8
Salinity	0.1-0.7	0.0-0.2	0.0-0.1	0.1-1.6	0.0-0.1
NO ₃ -N (mg/l)	0.33-0.77	0.43-15.63	0.10-1.50	0.30-0.97	0.50-1.77
NH ₄ -N (mg/l)	0.33-0.55	0.07-9.43	0.14-0.31	0.20-0.40	0.29-1.11
SRP (mg/l)	0.16-0.47	0.09-0.16	0.06-1.58	0.02-0.58	0.08-0.16
Total coloform bacteria (MPN/100)	280-1100	280-1100	9-2100	1100-2400	1100-4600

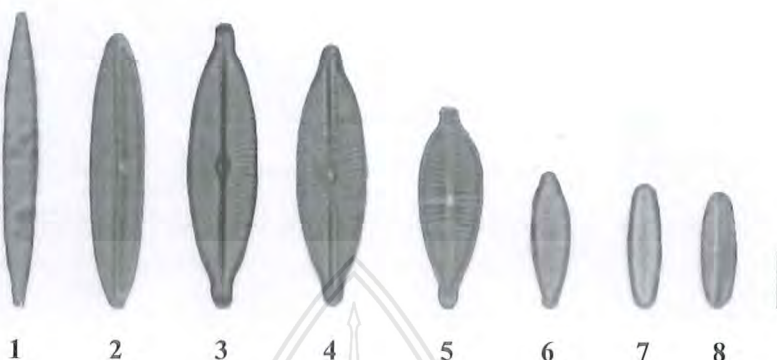


Figure 3. Some species of benthic diatoms in tributaries Mekong River in 2007-2008. (1) *Nitzschia palea* (Kützing) W. Smith, (2) *Navicula symmetrica* Patrick, (3) *Navicula rostellata* Kützing, (4) *Navicula viridula* var. *germainii* (Wallace) Lange-Bertalot, (5) *Gomphonema lagenula* Kützing, (6) *Gomphonema pumilum* (Grunov) Reichardt & Lange-Bertalot, (7-8) *Achnanthes minutissima* Kützing. Scale bar 10 μ m

Discussion

The diversity of benthic algae in each sampling site was different because of different human activity. For example, low amount of macroalgae were found in Huerng River because this sampling site had been affected from land log for agriculture. This impact can increase the velocity in river which was not appropriated for attachment of benthic algae. This finding was in agreement with the study reported by Nozaki (2001) that was quoted by Kunpradid (2005). High amount of macroalgae was found in our sampling sites which had some appropriate parameters for growth and attachment of macroalgae, for example few velocity of water, appropriated substrate, and low turbidity.

The water quality parameters of all sampling sites were classified to mesotrophic status. These relationships were found directly between a number of *Gomphonema pumilum* and turbidity, a number of *Navicula viridula*, *Navicula symmetrica*, *Nitzschia palea* and nutrient. Similarly Kunpradid (2005) found that

G. pumilum was dominant species in high turbid water, whereas *N. viridula*, *N. symmetrica* and *N. palea* presented in high nutrient rivers. *Achnanthes minutissima* was found in mesotrophic water. This finding was similar to report of Suphan (2004) studied diversity of macroalgae and benthic diatom in Area of Golden Jubilee, Thong Pha Phum Project, Kanchanaburi Province, and found *A. minutissima* in the river with mesotrophic status quality. In terms of macroalgae, *Oscillatoria princeps* Vaucher ex Gomont was found directly related with nutrients. Similarly to Palmer (1970) found that *O. princeps* could be grewed in high nutrients concentration environment.

In this study, the species composition of benthic diatoms were clearly changed more than macroalgae because diatoms were more sensitive to water quality than macroalgae. Moreover macroalgae had attached with specific substrates, whereas diatoms could attach on many substrates. Hence, diatoms were suitable for the assessment of water quality in running water.

Acknowledgment

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Diversity of Phytoplankton and Water Quality in the Reservoir of Nam Ngum Dam, Lao PDR

Thanittha Malaiwan^{1*} and Yuwadee Peerapornpisal¹

Abstract

Nam Ngum Dam was built in Vientiane, Lao PDR in 1971 for electricity production, irrigation, fisheries and tourism, with a capacity of $7010 \times 10^6 \text{ m}^3$. In this investigation, the diversity of phytoplankton and the water quality in the reservoir of the Nam Ngum Dam were assessed during June 2007 to April 2008. Water samples were collected every 5 m in vertical depth from the water surface to the bottom of the reservoir. The physical, chemical and some biological parameters, especially the phytoplankton in each depth profile, were studied. Six divisions of phytoplankton were found, i.e. Chlorophyta (green algae), Cyanophyta (blue-green algae), Bacillariophyta (diatoms), Pyrrophyta (dinoflagellates), Chrysophyta (chrysophytes) and Euglenophyta (euglenoids). The dominant species were *Staurastrum tetracerum* Ralfs, *S. freemanii* W. et G.S. West var. *nudiceps* Scott et Prescott, *S. crenulatum* (Nägeli) Delponte, *Cosmarium* sp.1 and *Aulacoseira granulata* (Ehrenberg) Simonsen. The water in the reservoir represents oligo-mesotrophic to mesotrophic status and clean to moderate water quality.

Keywords : Biodiversity, Phytoplankton, Nam Ngum Dam, Lao PDR, *Staurastrum*

Introduction

Nam Ngum Dam is the first hydro dam in Lao PDR. It was financed with assistance from ten countries, under the auspices of the United Nations. It was constructed by a Japanese firm and was completed in 1971. The dam has a generating capacity of 150 megawatts. Nam Ngum Dam mainly provides electricity in Lao, including all the power used in the capital, Vientiane. More importantly, 70 to 80 percent of the electricity are exported to Thailand, accounting for one fourth of Laos' foreign exchange earnings.

The reservoir ("Ang Nam Ngum" in Lao) is a popular day-trip destination, with floating restaurants

moored at the south-western shore (near the dam and the village of Ban Thalat); here there are also boats for hiring to cruise on the lake and visit some of the islands, these activities appear to affect the water quality in the reservoir.

Phytoplankton is an important primary producer. Since it is the basis of the whole food chain in open water. Species composition of the phytoplankton community is an efficient bioindicator for water quality (Peerapornpisal et al., 2004). In this study, the diversity of phytoplankton was studied and the water quality was assessed based on the physical and chemical properties.

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Study Site: Nam Ngum Reservoir is located at $18^{\circ} 32.265'$ N, at $102^{\circ} 34.868'$ E and at an altitude of 197 m above the sea level (Figure 1). The maximum volume of the reservoir is $7010 \times 10^6 \text{ m}^3$. The dam is approximately 90 km

north of Vientiane. Sample was collected from the surface to the deepest point of the reservoir and the physical and chemical properties of the water were determined.

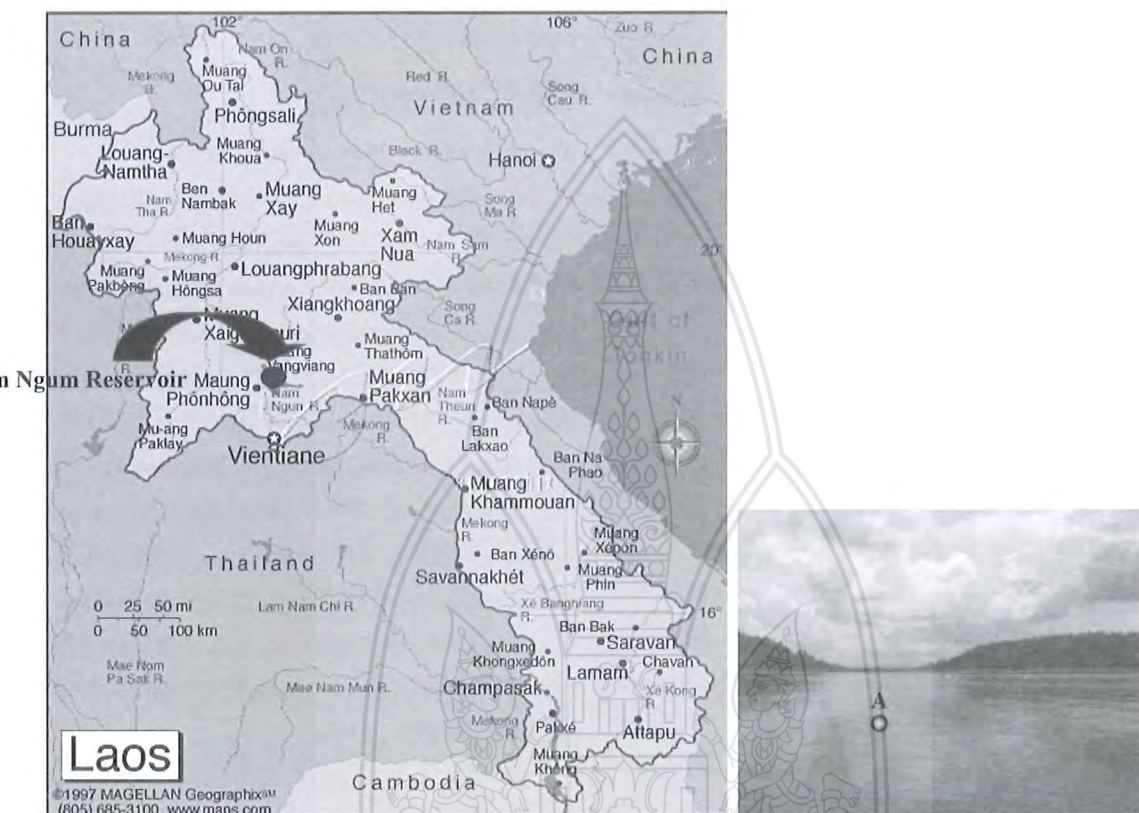


Figure 1. Map of Lao PDR showing the location of Nam Ngum Reservoir and sampling site (A) in the reservoir.

Materials and Methods

Scope of study

The study area located in Nam Ngum of Lao PDR. At the Deepest point of the reservoir, the water samples were collected from the surface to the bottom of the reservoir at every 5 meters. The study was carried out during June 2007 to April 2008.

Physical and Chemical Qualities of Water at the Sampling Sites

Measurement of some physico-chemical

properties of water in the reservoir was done at the sampling sites. The depth of water at which light can penetrate was determined using Secchi disc. The temperature, pH and conductivity was measured by portable meters and dissolved oxygen (DO) was measured by Azide modification method (Greenberg et al., 2005).

Laboratory Investigation of Water Samples

Coliform bacteria was analyzed by the method of Harrigan and Cance (1976). Alkalinity was measured by Phenolphthalein methyl orange indicator

method, BOD was measured using Azide modification method. Nutrients, i.e. soluble reactive phosphorus, nitrate nitrogen, and ammonium nitrogen were measured according to the method described by Greenberg et al. (2005). Chlorophyll a was measured by the method of ISO 10260. Turbidity was measured with a turbidity meter. Water quality was evaluated from the main parameters (DO, BOD, conductivity, nitrate nitrogen, ammonium nitrogen, soluble reactive phosphorus and chlorophyll a) according to Lorrain and Vollenweider (1981), Wetzel (1983) and Peerapornpisal et al. (2004).

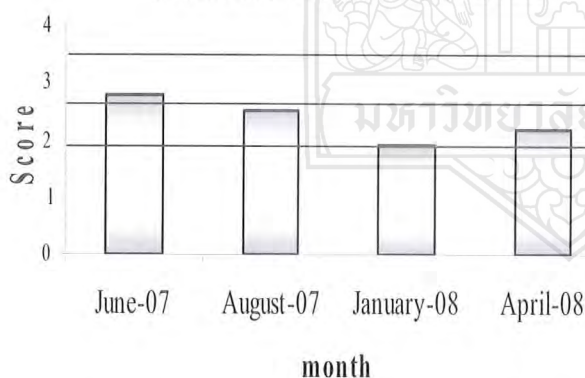
Sampling and Identification of Phytoplankton

1. Sampling of Phytoplankton Samples

Sampling of phytoplankton identification for species and to the determine the density of them, was done at the deepest point of the reservoir using a plankton net (10 μ m pore size). The samples were filtered from 10 litres water samples to give about 100 ml and preserved in 6-7 drops of the Lugol's solution.

2. Identification and Counting

Identification of phytoplankton species



was carried out using the relevant books i.e. Komárek and Anagnostidis (1999); Presscott (1970); Huber-Pestalozzi (1983); Croasdale et al. (1994) and John et al. (2002). The density of each species was done by whole count method.

Results and Discussion

Water Quality of Nam Ngum Reservoir

The water quality of Nam Ngum Reservoir in June 2007 to April 2008 was classified by using the methods of Wetzel (1983), Lorraine and Vollenweider (1981) and Peerapornpisal et al. (2004), these methods have been modified to use in the reservoir of northern Thailand by Applied Algal Research Laboratory, Chiang Mai University (AARL-PC Score) by altering the amounts of DO, BOD, conductivity, chlorophyll a, nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus. The water quality in the reservoir was classified as mesotrophic in June 2007 and as the oligo-mesotrophic status in August 2007, January 2008 and April 2008 (Figure 2).

0.1-0.9	ultraoligotrophic status
1.0-1.8	oligotrophic status
1.9-2.7	oligotrophic – mesotrophic status
2.8-3.6	mesotrophic status
3.7-4.5	mesotrophic –eutrophic

Figure 2. The water quality in trophic status by AARL-PC Score in Nam Ngum Reservoir (June 2007-April 2008).

It was clear that the water quality in Nam Ngum Reservoir was more polluted in the rainy season than the cool-dry season and the summer. According to the standard surface water quality of Thailand, the

water quality in this reservoir was classified in the 2-3 categories, which could be used for consumption after being properly treated.

Biodiversity of Phytoplankton in Nam Ngum Reservoir

The study of biodiversity of the phytoplankton in Nam Ngum Reservoir was investigated during June 2007 to April 2008. Fifty - five species in 6 Divisions were recorded. The highest number of species was in the Division Chlorophyta which include 28 species (54.9%), followed by Bacillariophyta for 6 species (11.76%), Cyanophyta for 6 species (11.76%),

Euglenophyta for 6 species (11.76%), Chrysophyta 5 species (9.8%) and Pyrrhophyta 4 species (7.84%) as shown in Figure 3 and Table 1. The dominant species of the phytoplankton were *Staurostrum tetracerum* Ralfs followed by *S. freemanii* W.et G.S.West var. *nudiceps* Scott et Prescott, *S. crenulatum* (Nägeli) Delponte, *Cosmarium* sp.1 and *Aulacoseira granulata* (Ehrenberg) Simonsen (Figure 3).

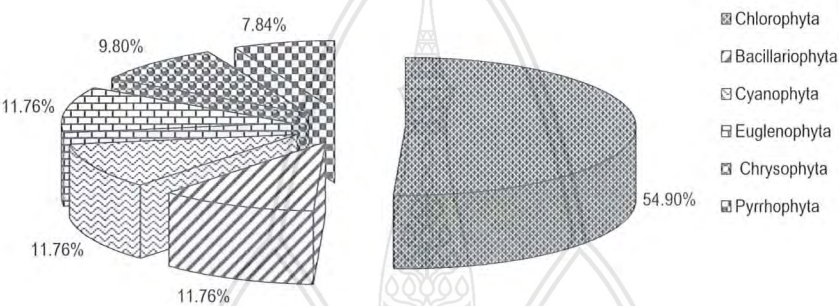


Figure 3. Distribution of the phytoplankton species in each division collected from Nam Ngum Reservoir (June 2007 to April 2008).

Table 1. List of the phytoplankton species in the Nam Ngum Reservoir, Lao PDR (June 2007 - April 2008).

Division Cyanophyta <i>Aphanizomenon aphanizomenoides</i> Forti <i>Cylindrospermopsis raciborskii</i> (Woloszynska) Seenayya & Subba et Raju <i>Oscillatoria</i> sp. <i>Microcystis aeruginosa</i> Kützing <i>Planktolyngbya limnetica</i> Lemmermann <i>Pseudanabaena limnetica</i> Komárek	<i>S. pingue</i> Teiling var. <i>pingue</i> <i>S. sexangulare</i> (Bulnh.) Lundell var. <i>sexangulare</i> <i>S. smithii</i> Teiling <i>S. tetracerum</i> Ralfs <i>S. tohopekaligense</i> Wolle var. <i>tohopekaligense</i> <i>Staurodesmus</i> cf. <i>cuspidatus</i> var. <i>curvatus</i> (W.West) Teil <i>S. pterosporus</i> (P.Lundell) Bourrelly
Division Chlorophyta <i>Cosmarium contractum</i> Kirchner var. <i>ellipsoideum</i> West&West <i>C. cf. capitulum</i> Roy & Bisset <i>Cosmarium moniliforme</i> var. <i>panduriforme</i> (Heimerl) Schmidle	Division Euglenophyta <i>Phacus pleuronectes</i> (Ehrenberg) Dujardin <i>Trachelomonas hispida</i> (Perty) Stein <i>T. hispida</i> var. <i>coronata</i> Lemmermann <i>T. plantonica</i> var. <i>plantonica</i> fo. <i>oblonga</i> (Drezepolski) <i>T. volvocina</i> Ehrenberg <i>Trachelomonas</i> sp.

<i>Cosmarium</i> sp.1	Division Bacillariophyta
<i>Cosmarium</i> sp.2	<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen
<i>Cosmarium</i> sp.3	<i>Cyclotella meneghiniana</i> Kützing
<i>Crucigeniella neglecta</i> (Fott & Ettl) Komárek	<i>Fragilaria</i> sp.
<i>Crucigenia tetrapedia</i> (Kirchner) W.&G.S.West var. <i>tetrapedia</i>	<i>Gomphonema</i> sp.
<i>Dictyosphaerium sphagnale</i> Hind Konien	<i>Meiloseira varian</i> Agardh
<i>Golenkinia radiata</i> Chodat var. <i>radiata</i>	<i>Navicula</i> sp.
<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová	Division Pyrrhophyta
<i>Nephrocytium limneticum</i> (Smith) Smith	<i>Gymnodinium</i> sp.
<i>Pediastrum simplex</i> var. <i>simplex</i> Meyen	<i>Peridiniopsis</i> sp.
<i>Scenedesmus</i> sp.	<i>Peridinium</i> sp.1
<i>Sphaerocystis</i> sp.	<i>Peridinium</i> sp.2
<i>Staurostrum crenulatum</i> (Nägeli) Delponte	Division Chrysophyta
<i>S. excavatum</i> West & West var. <i>excavatum</i>	<i>Centritractus belanophorus</i> Lemmermann
<i>S. freemanii</i> W.et G.S.West var. <i>nudiceps</i> Scott et Prescott	<i>Dinobryon behningii</i> Swirenko
<i>S. limneticum</i> Schmidle var. <i>burnemse</i> West & West	<i>D. divergen</i> Imhof
<i>S. octodontum</i> var. <i>tetradontum</i> Scott et Grönblad	<i>D. sertularia</i> Ehrenberg
<i>S. octoverrucosum</i> var. <i>octoverrucosum</i> Scott & Grönblad	<i>Isthmochloron gracile</i> Chodat

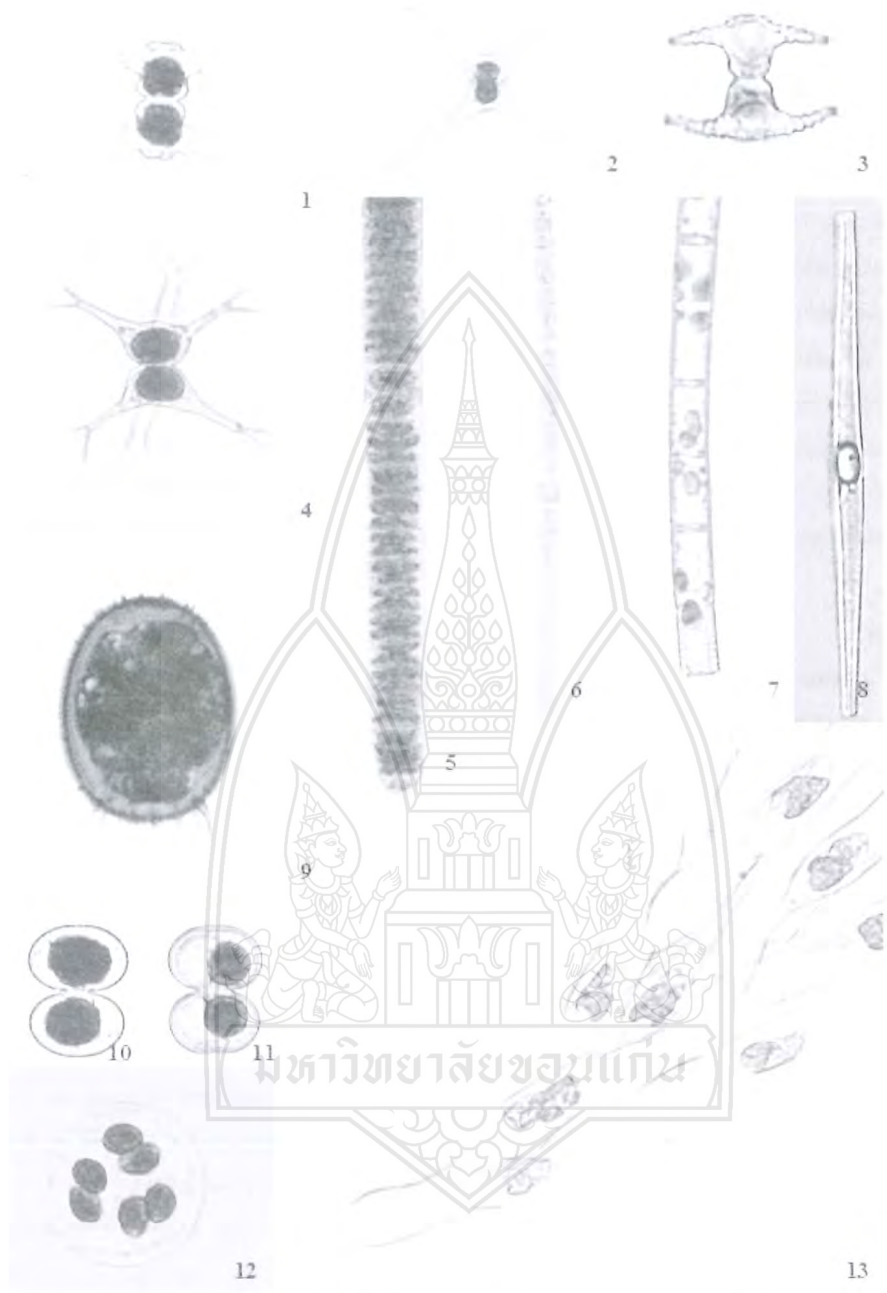


Figure 3. Some species of phytoplankton collected from the Nam Ngum Reservoir (June 2007 - April 2008)
1) *Sraurastrum octoverrucosum* var. *octoverrucosum* Scott & Grönblad, 2) *S. tetracerum* Ralfs, 3) *S. crenulatum* (Nägeli) Delponte, 4) *Staurastrum* sp., 5) *Oscillatoria* sp., 6) *Aphanizomenon aphanizomenoides* Forti, 7) *Aulacoseira granulata* (Ehrenberg) Simonsen, 8) *Fragilaria* sp., 9) *Trachelomonas* sp. 10) *Cosmarium contractum* Kirchner var. *ellipsoideum* West&West, 11) *Cosmarium* sp., 12) *Sphaerocystis* sp., 13) *Dinobryon divergen* Imhof. (Scale bar = 10 µm)

Phytoplankton as Bioindicator for Water Quality in Nam Ngum Reservoir

The species of phytoplankton which indicated oligo-mesotrophic status were *Staurastrum tetracerum* Ralfs and *S. freemanii* W. et G.S. West var. *nudiceps* Scott et Prescott. They were found in high numbers in every season. Moreover, the species that indicated mesoeutrophic status were *Aulacoseira granulata*, and *Peridinium* spp., which were similar to the report of John et al. (2002). Some toxic species of the blue-green algae such as *Pseudanabaena* sp. Was also found in this study. Its filaments were usually found in the sites near the bottom of the reservoir, where nutrient accumulated.

Discussion

The water quality of Nam Ngum Reservoir during the investigation was classified in oligo-mesotrophic to mesotrophic status. The dominant species indicating clean water were *Staurastrum tetracerum* Ralfs, *S. freemanii* W. et G.S. West var. *nudiceps* Scott et Prescott and *S. crenulatum* (Nägeli) Delponte. This finding are in accordance with the reports of Palmer (1970) and Peerapornpisal et al. (2007).

Moreover, this result also had same direction with the reports of Ngearnpat and Peerapornpisal (2007) which found desmid algae such as *Cosmarium capitulum* Roy & Bisset, *Staurastrum gutwinskii* Bernard var. *gutwinskii* and *S. tortum* West & West from oligo-mesotrophic reservoirs. The desmids are generally more common and diverse in oligotrophic lakes and ponds (Gerrath, 1993). Whilst *Peridinium* spp. and *Aulacoseira granulata* (Ehrenberg) Simonsen were indicated of moderate water quality (Palmer, 1970)

It can be concluded that water quality of Nam Ngum Reservoir is safe and clean enough for consumption and water supply production. Although in the rainy seasons, the water quality was decreased due to increase nutrients from irrigation canal. However, because of the large size of water volume, nutrients would be diluted until the water quality was acceptable.

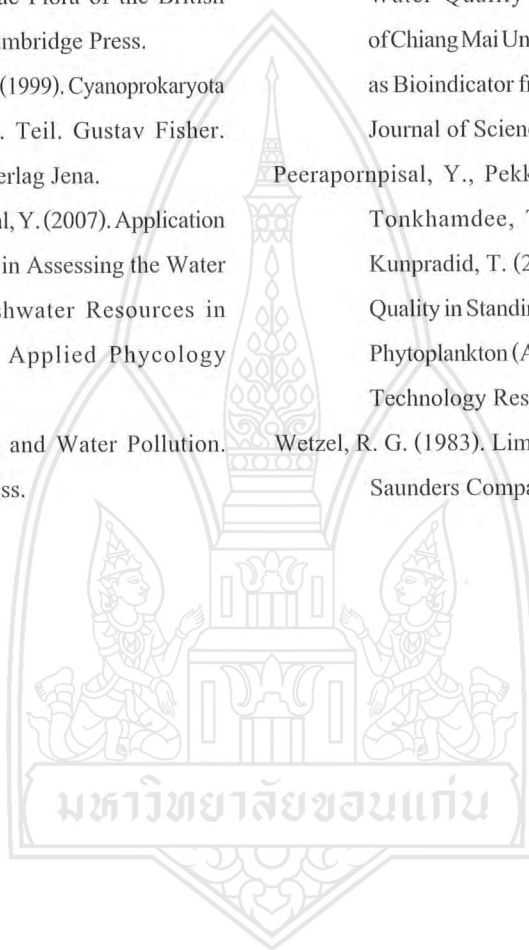
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Efficiency of *Jatropha gossypifolia* L. (Euphorbiaceae) Against *Spodoptera exigua* Hübner (Lepidoptera:Noctuidae): Toxicity and its Detoxifying Enzyme Activities

Nutchaya Khumrungee¹ Vasakorn Bullangpoti^{1*} and Wanchai Pluempanupat²

Abstract

This study explored the efficacy of leaf extract of *Jatropha gossypifolia* against second instar of *Spodoptera exigua* Hübner using dipping method. The leaf extract showed insecticidal activity with an LC_{50} of 35,000 ppm ($r^2 = 0.978$) and 26,500 ppm ($r^2 = 0.99$) after 24 and 48 hours of expose. The detoxification activities of glutathione-s-transferase (GST), and carboxylesterase (CE) in extract-treated insects was also determined to show a induction for CE and inhibited for GST.

Keywords : *Spodoptera exigua* (Hübner), *Jatropha gossypifolia* L., Carboxylesterase, Glutathione-s-transferase

Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous pest that damages many cultivated crops like corn, cotton, beet, tomato, celery, lettuce, cabbage, alfalfa (Wang et al., 2006). This insect is well-known to widely resistant to many synthetic insecticides, therefore a safer method is required to develop an eco-friendly biopesticide.

Botanicals play an important role as biodegradable pesticides and *Jatropha gossypifolia* L. (Euphorbiaceae) was chosen for evaluation. This species contains many secondary metabolites like apigenin (Subramanian et al., 1971); cyclogossin A (Horsten et al., 1996) jatrophatrione, jatropholone (Rahman et al., 1990). The extracts from plant species

have biological properties such as anti-allergic (Adolf et al., 1984), molluscicidal (Adewunmi et al., 1987), and insect repellent activity (Areekul et al., 1987). The main objective of this research was to develop a new botanical insecticide from *Jatropha gossypifolia* extract for controlling *S. exigua*. In addition, detoxifying enzyme activities, carboxylesterase and glutathione-s-transferase (GST), were investigated using enzyme-substrate assays.

Methods and materials

1. Insect rearing method

Larvae of *S. exigua* were collected from onion fields in Ratchaburi province (50 km west from Bangkok), Thailand, and were reared on an artificial diet. Group of 5-10 neonates was put in a plastic box

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(5x5 cm) with the mesh lid, and reared on the diet at 27°C and 70% RH under 16L8Dh photoperiod. Pupae were collected from this culture line and then placed in a cylindrical plastic cage (15 cm high x 5 cm diam.) for adult emergence. New adults were provided with 10% honey solution via a cotton pad and were allowed to lay eggs on a paper sheets in a glass jar.

2. Chemicals

Ethanol 95%, Potassium phosphate (Sigma® Chemical), Polyvinylpyrrolidone (Sigma® Chemical), Glutathione reduce form (Sigma® Chemical), Paranitrophenyl acetate (pNPA) (Sigma® Chemical) and Ethylsene diamine tetra acetic acid (EDTA) (Sigma® Chemical) was used in various experiments.

3. Extraction method

Dried leaf powder of *Jatropha gossypifolia* L. (Euphorbiaceae) (90 g) was used Soxhlet's extractor with 95% ethanol (1800 ml) for 8 hrs. Extract was concentrated by rotary evaporator (BUCHI B-850) under reduced pressure at 60°C. The extract was stored at 4°C until preparation of a stock solution. Stock solution was prepared by weighing a certain amount of the extract and diluting in distilled water to give a series of concentrations.

4. Toxicity bioassay

This research used Completely Randomized Design with 3 replicates of 60 1 day old- 2nd instar larvae for each replicate in each concentration. In this experiment, the 2nd instar larvae were dipped in range of concentrations of crude extracts which diluted with distilled water or were dipped in distilled water as control treatment for 5 second to obtain LC_{50} values. Insects

that did not move and/or did not stay upright were inferred to be dead. The mortality was recorded within 24 and 48 hours after treatment. Lethal concentration (LC50) was calculated using Probit analysis program (StatPlus version 2008).

5. Enzyme assay *in vivo*

GST activity was assayed by the method modified from Cheng et al. (2007). GST activity was determined using CDNB and reduced GSH as substrates. The reaction mixture consisting of CDNB (1.67mM), GSH (1.67mM) and the enzyme preparation (0.1mL) was prepared in a final volume of 3.0 mL with phosphate buffer. The non-enzymatic reaction of CDNB with GSH measured without homogenate served as control. The change in absorbance was measured continuously for 5 min at 340 nm and 25°C in the spectrophotometer.

Carboxylesterase activity was assayed by the method modified from Oakeshott et al. (2005) from 5°C to 50°C by using p-nitrophenyl-acetate (pNPA) as substrate. The assay mixture (1ml final volume) contained 5 mM pNPA in 20 mM sodium Phosphate buffer pH 7.4, and the assay was started by adding the enzyme sample to the mixture. The product, p-nitrophenol was continuously monitored at 400 nm by ultraviolet - visible spectrophotometer.

Results and Discussion

The preliminary screening shows a good potential larvicidal activity. The leaf *Jatropha gossypifolia* extract exhibited strong insecticide activity with a LC_{50} 35,000 ppm ($r^2 = 0.978$) and 26,500 ppm ($r^2 = 0.99$) at 24 and 48 hours after expose (Table 1).

Table1. Toxicity of *Jatropha gossypifolia* crude leaf extracts against second instar larvae of *S.exigua* after 24 and 48 hours post-treatment

Concentration (ppm)	Mortality percentage ⁽²⁾ ± SD	
	24 hours	48 hours
0.00(1)	0.00±0.00a	0.00±0.00a
10,000	15.00±5.77b	22.50±5.00b
20,000	27.50±5.00c	40.00±0.00c
30,000	47.50±5.00d	57.50±5.00d
40,000	52.50±5.00e	72.50±5.00e

⁽¹⁾control treatment: distilled water

⁽²⁾Means ± SD, all experiments followed by different letters within the same row are significantly different at p<0.05, DMRT.

Significant difference was observed between the treatment after 24 and 48 hours (t-test, p< 0.05), suggesting that the exposure time plays important role and long exposure enhances the present activity.

Toxicity of *Jatropha gossypifolia* leaf extract against *S. exigua* larvae seems to be slightly higher than other botanical insecticides such as *Capsicum frutescens* extracts (LC₅₀ = 48,000 mg/ml) on *Spodoptera* sp. (Saisongkhroh, 2006) but less toxicity than *O. sanctum* extract (LC₅₀ =36.46 ppm) (Kamaraj et al., 2008); although direct comparisons may be impossible because of differences in bioassay methods and related species.

Although no knockdown effect was recorded, symptoms in dead larvae were paralysis, enfolded bodies, darkening of cuticle and abnormal pupation. This suggests that *Jatropha gossypifolia* extract was responsible for biological disturbances in insects.

As the protection of crops from serious pests with chemical pesticides has led to increased resistance to many chemical pesticides along with pest resurgence and secondary pest infestations; there is an increasing interest in the use of botanical pesticides.

Enzyme based experiments revealed that carboxylesterase activity of *S.exigua* was induced after *Jatropha gossypifolia* extract treatment with a correction factor as 0.12 fold (Figure 1). This means *S. exigua* may use carboxylesterase to reduce toxicity which may be causes of resistance in future. Similar observation were made for GST activity of treated *S. exigua*. The inhibition of enzyme activity is not different at 5% DMRT of protein concentration. The correction factor when compared between control and LC₅₀ concentrations suggesting this enzyme inhibited at 1.62 fold (Figure 2). So, the activity of this extract may possibly affect GST.

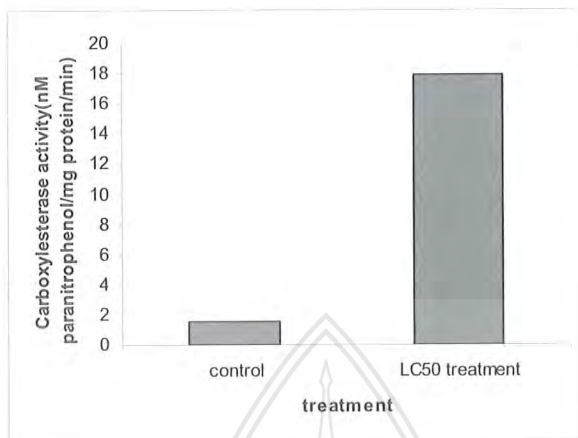


Figure 1. Carboxylesterase activity (nM paranitrophenol/mg protein/min) of 2nd instars *S. exigua* treated larvae after treated with LC₅₀ concentration of senescent leaf *Jatropha gossypifolia* L. crude extract.

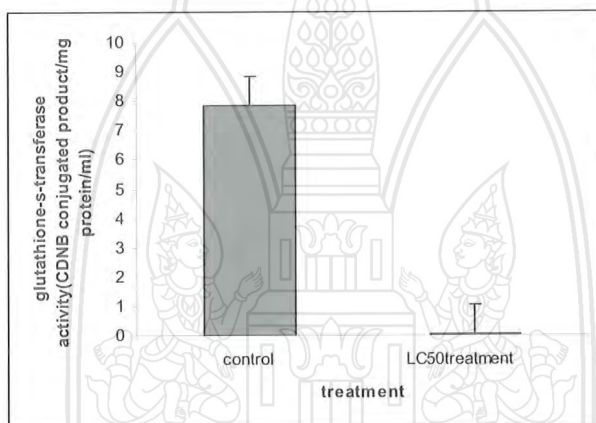


Figure 2. Glutathione-s-transferase activity (CDNB conjugated product/mg protein/ml) of 2nd instars *S. exigua* treated larvae of LC₅₀ concentration of senescent leaf *Jatropha gossypifolia* L. crude extract.

In addition to their insecticidal activity, most of toxicological approaches on botanical insecticides have been focused on inhibition or induction of detoxifying enzymes such as carboxylesterase, glutathione-S-transferase and cytochrom P450. Rachokarn et al. (2008) reported similar results on different enzymes, that is, carboxylesterase activity had a tendency to be induced after treated with *Amaranthus viridis* extracts,

whereas GST activities was inhibited in *S. exigua*. These inducences of detoxifying enzyme suggest a possibility on development of resistance against the extracts as shown in many reports on increased induction in detoxifying enzyme activity and discussed on development of resistance of many insect pests. (Chen and Sun, 1994; Small and Hemingway, 2000, Vontas et al., 2001; Bullangpoti et al., 2007 and Rattanapan, 2007)

Conclusions

This study explored the effects of senescent leaf *Jatropha gossypifolia* extract on second instar *S. exigua* larvae survival by dipping method, exhibiting LC_{50} value as 35,000 ppm ($r^2 = 0.965$). Significant increase in toxicity over time was also recorded. This extract also inhibited glutathione-S-transferase activities but induced the carboxylesterase activity. This is the first report of effective insecticidal activity of the senescent leaf extract of *Jatropha gossypifolia* extract against *S. exigua* which can be used as alternative tool

for the management of this pest.

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Ferns in the Waterfall Areas in Nam Nao National Park, Thailand

Kittima Makgomol¹

Abstract

A survey of ferns in the waterfall areas in Nam Nao National Park was conducted from October 2006 to October 2007. Three locations namely Heaw Sai, Sai Thong and Sum Ma Kao Waterfall areas were surveyed. Ferns were found in 10 families, 18 genera and 29 species. The common family, Polypodiaceae included 5 genera, *Drynaria*, *Goniophlebium*, *Phymatosorus*, *Platynerium* and *Pyrrosia*. Among these, the epiphytic fern *Platynerium holttumii* de Jonch. & Hennipman was abundant. While, among terrestrial species, *Helminthostachys zeylanica* (L.) Hook. was rarely found.

Keywords : Ferns, Nam Nao National Park, Heaw Sai Waterfall, Sai Thong Waterfall, Sum Ma Kao Waterfall

Introduction

Nam Nao National Park covers the area between northeastern and northern Thailand. It is part of the Phetchabun mountainous range that runs through Chaiyaphum and Phetchabun provinces. The national park headquarters is in an unusual and fascinating landscape of deciduous forest dominated by tall pine trees. The vegetation in Nam Nao National Park consists of many forest types, including deciduous, mixed deciduous, hill evergreen, dry evergreen and pine forest. The mountains and forests create a cooler climate in the national park. Nam Nao is one of Thailand's most

beautiful national parks, and includes many attraction sites, such as Phu Pha Jit, Suan Son Dong Bag, Tam Par Hong, Heaw Sai Waterfall and Sai Thong Waterfall.

Fern expeditions to Nam Nao National Park are rather few compared with studies of flowering plant. The previous study reported on fern diversity of some 41 species on the nature trails in Nam Nao National Park (Makgomol and Thangthong, 2008). This research project aimed to explore fern diversity in the waterfall areas in Nam Nao National Park for ecotourism and conservation purposes. A specific aim was to accumulate the information of fern distribution of Thailand.

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Study site

Nam Nao National Park covers in total an area of about 966 square kilometers within Phetchabun and Chaiyaphum provinces. It is approximately marked out by the geographical coordinates of 16° 30' - 16° 57' N and 101° 23' - 101° 45' E and its elevation ranges from 650-1,200 m. The daytime temperatures are very pleasant with an average annual temperature of 25 °C.

Heaw Sai Waterfall is located at Km 67 on Highway 12 (Lom Sak- Khon Kaen) some 1,000 m by forest trail to the waterfall. The water here

comes from Sa Nam Sai Stream. This forms a natural border between Nam Nao district and Korn San district. Sai Thong Waterfall, about 500 m from Heaw Sai Waterfall, has about 30 m width and 4 m high. Sum Ma Kao Waterfall is located at Km 64 on Highway 12 by the 4 m forest trail to the waterfall. It is a small waterfall with 3 m high and the water comes from Sa Num Sai Stream. Heaw Sai and Sai Thong Waterfall are in the deciduous and mixed deciduous forests. Sum Ma Kao Waterfall is situated among mixed deciduous forest (Figure 1).

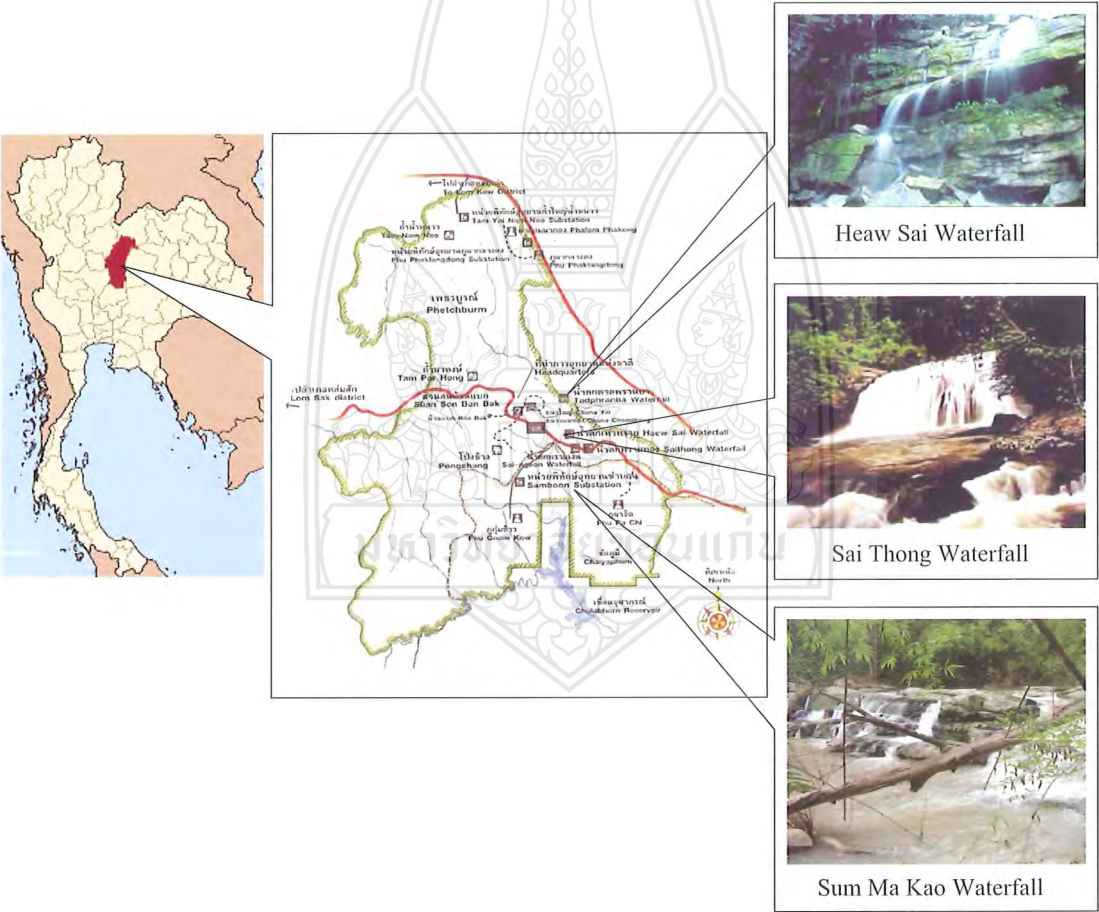


Figure 1. The map showing sampling locations in the waterfall areas in Nam Nao National Park

Materials and Methods

The fern specimens were collected along the main existing forest trails, extending about 10 m from both sides. Three locations: Heaw Sai, Sai Thong and Sum Ma Kao Waterfall areas were surveyed. Field trips were conducted between October 2006 and October 2007. Field notes, habitat and photographs of each species were recorded. The fern identification generally followed the system in Tagawa and Iwatsuki (1979, 1985, 1988, 1989). The voucher specimens were deposited at the Khon Kaen University Herbarium,

Department of Biology, Faculty of Science, Khon Kaen University (KKU).

Results

A total of 10 families, 18 genera and 29 species of ferns were found in the waterfall areas in Nam Nao National Park. The list of 29 species of ferns, together with their locations, habits and abundance of each species is shown in table 1 photographs of some species are shown in Figure 2.

Table 1. Ferns in the waterfall areas in Nam Nao National Park
(Habits: T = Terrestrial, E = Epiphyte, L = Lithophyte; Locations: 1 = Heaw Sai Waterfall, 2 = Sai Thong Waterfall, 3 = Sum Ma Kao Waterfall; Abundance: A = Abundant, C = Common, UC = Uncommon, R = Rarely found)

Family/Species	Habit	Location & Abundance
ATHYRIACEAE		
<i>Anisocampium cumingianum</i> C. Presl	T	3, C
DENNSTAEDTIACEAE		
<i>Microlepia herbacea</i> Ching & C. Chr. ex C. Chr. & Tardieu.	T	3, C
DRYOPTERIDACEAE		
<i>Quercifix zeylanica</i> (Houtt.) Copel.	T/L	1, 3, C
<i>Tectaria impressa</i> (Fée) Holttum	T	1, 2, 3, C
OPHIOGLOSSACEAE		
<i>Helminthostachys zeylanica</i> (Houtt.) Copel.	T	3, R
<i>Ophioglossum costatum</i> R. Br.	T	1, 2, 3, C
<i>Ophioglossum petiolatum</i> Hook.	T	1, 2, C
PARKERIACEAE		
<i>Adiantum caudatum</i> L.	T	1, 2, 3, C
<i>Adiantum erylliae</i> C. Chr. & Tardieu	T	1, 2, C
<i>Adiantum philippense</i> L.	T	1, 2, 3, C
<i>Adiantum soboliferum</i> Wall. ex Hook.	T	1, 2, UC
<i>Doryopteris ludens</i> (Wall. ex Hook.) J. Sm.	T	3, C
<i>Hemionitis arifolia</i> (Burm.f.) T. Moore	T	1, UC

Table 1. (continued)

Family/Species	Habit	Location & Abundance
POLYPODIACEAE		
<i>Drynaria bonii</i> H. Christ	E	1, 2, 3,C
<i>Goniophlebium subauriculatum</i> (Blume) C. Presl	E	2, 3, C
<i>Phymatosorus cuspidatus</i> (D. Don) Pic. Serm.	E/L	2, C
<i>Platynerium holttumii</i> de Jonch. & Hennipman	E	1, 2, 3, A
<i>Pyrrosia adnascens</i> (Sw.) Ching	E	1, 2, 3, C
<i>Pyrrosia heteractis</i> (Mett. ex Kuhn) Ching	E	3, UC
<i>Pyrrosia mollis</i> (Kunze) Ching	E	1, UC
<i>Pyrrosia stigmosa</i> (Sw.) Ching	E/L	1, 2, C
PTERIDACEAE		
<i>Pteris biaurita</i> L.	T	3, C
<i>Pteris ensiformis</i> Burm. f.	T	1, 2, 3, C
<i>Pteris venusta</i> Kunze	T	3, C
SCHIZAEACEAE		
<i>Lygodium flexuosum</i> (L.) Sw.	T	1, 2, 3, C
THELYPTERIDACEAE		
<i>Thelypteris parasitica</i> (L.) Fosberg	T	3, C
<i>Thelypteris terminans</i> (Hook.) Tagawa & K. Iwats.	T	3, C
<i>Thelypteris viscosa</i> (Baker) Ching	T	1, UC
VITTARIACEAE		
<i>Antrophyum parvulum</i> Blume	L	1, UC

Conclusion and Discussion

A total of 10 families, 18 genera and 29 species of ferns were found in the waterfall areas in Nam Nao National Park. The common family, Polypodiaceae, included 5 genera, *Drynaria*, *Goniophlebium*, *Phymatosorus*, *Platynerium* and *Pyrrosia*. The other families contained only 1 or 2 genera. The ferns were found in the study areas thrive in various habitats. Most of them are terrestrials and epiphytes, with only a few species of lithophytes. Among these, the large epiphytic fern *Platynerium holttumii* was abundant. This Holttum’s staghorn fern usually grows on high

branches of trees. The terrestrial ferns such as *Adiantum caudatum*, *Pteris ensiformis* and *Tectaria impressa* were commonly found. While among terrestrial species, *Helminthostachys zeylanica* was rarely found. This eusporangiate fern was found on humus rich soil in shade. The other eusporangiate ferns were more common namely, *Ophioglossum costatum* and *O. petiolatum*. *Antrophyum parvulum*, a lithophytic fern was found on the moist rocks near to the waterfall. However, small plant, *Quercifilix zeylanica* was found on both muddy rocks and soil in light shade.

Although, the Staghorn ferns were abundant in this study, *O. pendulum* was hardly observed with those Staghorn ferns. When compared with the previous study on the nature trails in Nam Nao National Park, it has been reported that *O. pendulum* grows in old nest leaves of the Staghorn fern (see Makgomol and Thangthong, 2008). With regard to the ecotourism and conservation purposes, these waterfall areas were suitable for in-situ of Holttum's

staghorn ferns. However, many ferns found in the areas should be protected from human disturbance, for example the rare *H. zeylanica*, and small plants of *A. parvulum*, *Q. zeylanica*, *O. costatum*, and *O. petiolatum*. A demand for some ferns has been reported from the plant markets. Attractive ferns such as *Doryopteris ludens* and *P. holttumii* might be considered for a further propagation study to support the conservation.



Figure 2. Some ferns which were found in the waterfall areas in Nam Nao National Park:

- a. *Antrophyum parvulum* Blume
- b. *Helminthostachys zeylanica* (Houtt.) Copel.
- c. *Platycerium holttumii* de Jonch. & Hennipman
- d. *Quercifix zeylanica* (Houtt.) Copel.

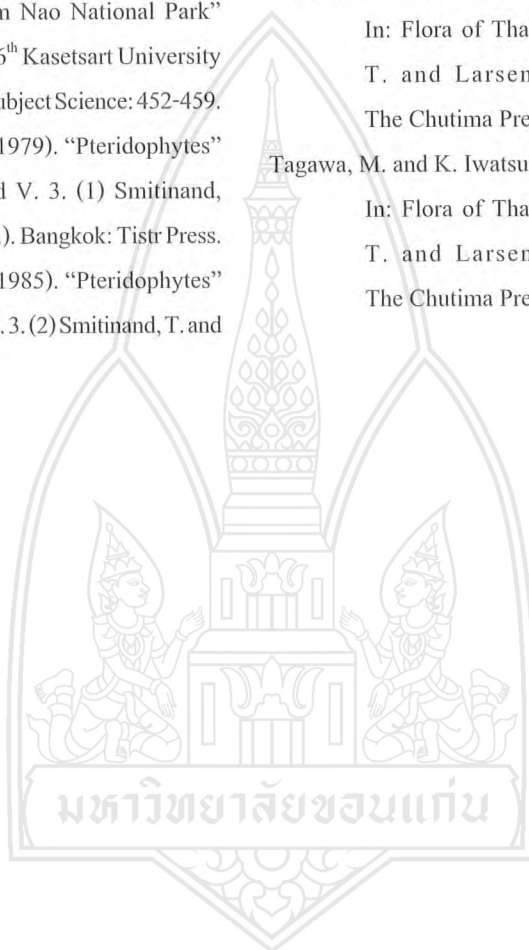
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Fish Parasite Diversity in the Mekong River in the North of Thailand

Watchariya Purivirojkul¹

Abstract

A total of 745 fishes were collected from the Mekong river, Chiang Rai province for parasitic examination. The fish were identified in 5 orders, 10 families, 20 genera and 23 species; Siluriformes (*Pangasianodon gigas*, *Pangasius sanitwongsei*, *Pangasius conchophilus*, *Pangasius bocourti*, *Pangasius micronemus*, *Clupisoma sinensis*, *Belodontichthys dinema*, *Wallago attu*, *Kryptopterus cryptopterus*, *Bagarius bagarius*, *Hemibagrus nemurus*, *Mystus wyckii*), Cypriniformes (*Barbonymus gonionotus*, *Cirrhinus jullieni*, *Labiobarbus siamensis*, *Macrochirichthys macrochirus*, *Mekongina erythrospila*, *Osteochilus hasselti*, *Puntioplites proctozystron*, *Syncrossus helodes*), Osteoglossiformes (*Notopterus notopterus*), Perciformes (*Channa striata*) and Syngnathiformes (*Doryichthys deokhatoides*). The parasite assembly consisted of 2 species of protozoa (*Myxidium* sp., *Thelohanellus* sp.), 21 species of monogenes (*Cornudiscoides malayensis*, *Dactylogyrus* spp., *Thaparocleidus* spp., *Thaparogyrus* sp., *Trianchoratus* sp.), 19 species of digenetic trematodes (*Clinostomum* sp., *Dollfustrema* sp., *Haplorchis* sp., *Haplorchoides* sp., *Metadena* spp., *Opisthorchis* spp., *Paracryptogonimus* spp., *Phyllodistomum* sp., *Proisorhynchoides* spp., *Rhipidocotyle* sp., Unidentified digene), 4 species of cestodes (*Gephyrolina* spp., *Lytocestus* sp., *Senga* sp.), 5 species of acanthocephalans (*Arhythmorhynchus* spp., *Polyacanthorhynchus* sp., *Polymorphus* sp., *Pallisentis* sp.), 7 species of nematodes (*Cucullanus* sp., *Monhysterides* sp., *Proleptus* sp., *Procamallanus* sp., *Spinitectus* sp.) and 4 species of arthropods (*Alitropus typus*, *Ergasilus* spp., *Lamproglana* sp.). The highest diversity of parasites was found in *Bagarius bagarius*, *Hemibagrus nemurus* and *Clupisoma sinensis* with 16, 10 and 9 species of parasites, respectively. All of monogenean parasites found in this study, showed specificity to their hosts compared to many reports from Thailand. Other species of parasite, such as digenea, cestode also showed possibility of specificity to their hosts.

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Introduction

The Mekong River is the 11th longest river in the world, 7th longest river in Asia and the longest river in the South-East Asia. The Mekong River starts in the southeastern Himalayan mountains and flows estimated 4,350 kms. to the bank at the South China Sea (Kazama et al., 2007). From the Himalayan mountains it runs through the People's Republic of China, the Union of Myanmar, Thailand, the Lao People's Democratic Republic, the Kingdom of Cambodia and the Socialist Republic of Vietnam. The Mekong River provides rich water resources, forest resources and aquatic resources. More than 1,200 species of fish have been identified and there could be possibly as many as 1,700. Fishing is a very important part of the economic activities in the surrounding area and is a vital source of protein for the local diet. It is indicate that some 120 fish species are commercially traded but most of the fishery is based on 10-20 species (Mekong River Commission, 2003).

Many species of parasites were reported in wild fishes (Hymen, 1951; Yamaguti, 1958; Bykhovskaya-Pavlovskaya et al., 1964; Kabata, 1985; Lerssutthichawal, 1999). Parasitic organisms play an important role directly or indirectly causing mortality in fish (Lerssutthichawal, 1999). The monogeneans can cause severe gill damage, results in loss of gill surface area for respiration. Some arthropods affect wild fish of commercial significance as they can affect host survival or cause unsightly changes in the flesh (Lester and Hayward, 2006). The parasites also feed on host tissue and blood leading to secondary microbial infection.

The rich diversity of the Mekong River is striking, not only for fish but also for other groups of aquatic organisms such as mollusks (Rainboth, 1996). Digeneans undergo part or all of their larval development

in mollusks (Paperna and Dzikowski, 2006). Metacercarial infection in fish is the main reason of disease, causing subsequent economic loss. Some metacercaria in fisheries and aquaculture products are sources of infections in humans and domestic animals (Ito, 1964; Deardorff and Overstreet, 1991; Paperna and Dzikowski, 2006).

In this study, parasitic diversity was reported from all species of fishes collected from the Mekong River. The result from this study can be used for public health information involved the infestation of metacercaria encysting in muscle, internal organs and peritoneum of freshwater fishes. Furthermore, parasites have been used widely as biological tags to provide information for fishery managers on the movements and population structure of their hosts such as monogeneans, digeneans, acanthocephalans and nematodes (Brickle and MacKenzie, 2007; Timi, 2007).

Materials and methods

Fish samples were collected from the Mekong River at Chiang Rai Province between 2003-2006. The species of fish were identified according to Nelson (2006) and Rainboth (1996). Examination for ectoparasites (eyes, mouth, nostril and fins) were made using the magnifier. The mucus was also examined under the microscope. Parts of fins, gill and skin of the fishes were removed and examined under stereomicroscope. The visceral organs including liver, intestine, stomach and also in the muscle of the fishes were examined under compound microscope. Collection of the parasites was done separately for further identification. Digeneans, cestodes, acanthocephalans, nematodes and arthropods were kept in the 70% ethanol while monogeneans were fixed and preserved in ammonium-picrate glycerine.

Identification of the parasitic species was performed according to the methods of Hymen (1951); Yamaguti (1958); Bykhovskaya-Pavlovskaya et al. (1964); Jahn and Jahn (1979); Kabata (1985); Lim and Lerssutthichawal (1996); Lerssutthichawal (1999); Petrochenko (1971); Purivirojkul and Sirikanchana (2006). Prevalence and mean intensity of each parasitic species were calculated according to Margolis et al. (1982).

Results and discussion

Twenty - three species from five orders of fishes, namely *Pangasianodon gigas*, *Pangasius sanitwongsei*, *Pangasius conchophilus*, *Pangasius bocourti*, *Pangasius micronemus*, *Clupisoma sinensis*, *Belodontichthys dinema*, *Wallago attu*, *Kryptopterus cryptopterus*, *Bagarius bagarius*, *Hemibagrus nemurus*, *Mystus wyckii* (Siluriformes), *Barbonymus gonionotus*, *Cirrhinus jullieni*, *Labiobarbus siamensis*, *Macrochirichthys macrochirus*, *Mekongina erythrospila*, *Osteochilus hasselti*, *Puntioplites proctozystron*, *Syncrossus helodes* (Cypriniformes), *Notopterus notopterus* (Osteoglossiformes), *Channa striata* (Perciformes) and *Doryichthys deokhatoides* (Syngnathiformes), were examined for parasites. Number of fish samples per species were not consistent because many species of fishes such as *Pangasianodon gigas*, *Pangasius sanitwongsei* and *Mystus wyckii* could be collected only on particular season.

Twenty - six species of the external parasites were found. There were one species of protozoa, *Thelohanellus* sp.; 21 species of monogenes, *Cornudiscoides malayensis*, *Dactylogyrus* spp., *Thaparocleidus* spp., *Thapargyrus* sp., *Trianchoratus* sp. and four species of arthropods, *Alitropus typus*, *Ergasilus* spp. and *Lamproglena* sp. Cyst of *Thelohanellus* sp.,

Monogeneans, copepods *Lamproglena* and *Ergasilus* sp. occurred on the gill filaments while isopod, *Alitropus typus* occurred on the gill cavity and skin. Although they were ectoparasites but only one species of parasite in one species of fish was found.

Thirty - six species of the internal parasites were found in this study. The endoparasites consisted of one species of protozoa, *Myxidium* sp.; 19 species of digenes, *Clinostomum* sp., *Dollfustrema* sp., *Haplorchis* sp., *Haplorchoides* sp., *Metadena* spp., *Opisthorchis* spp., *Paracryptogonimus* spp., *Phyllodistomum* sp., *Proisorhynchoides* spp., *Rhipidocotyle* sp. and Unidentified digene; four species of cestodes, *Gephyrolina* spp., *Lytocestus* sp. and *Senga* sp.; five species of acanthocephalans, *Arhythmorhynchus* spp., *Polyacanthorhynchus* sp., *Polymorphus* sp. and *Pallisentis* sp.; and seven species of nematodes, namely, *Cucullanus* sp., *Monhysterides* sp., *Procamallanus* sp., *Proleptus* sp. and *Spinitectus* sp. (Table 1). *Myxidium* sp. and *Opisthorchis* spp. were found in the gall bladder of fish. Other endoparasites were found in the intestine and the body cavity.

Pangasianodon gigas, *Pangasius sanitwongsei*, *Pangasius conchophilus*, *Pangasius micronemus* and *Doryichthys deokhatoides* had highest percentage parasitic infestation (100%), followed by *Bagarius bagarius* and *Hemibagrus nemurus* which had 96.76% and 96% parasitic infestation, respectively. Three species of fishes, *Labiobarbus siamensis*, *Macrochirichthys macrochirus* and *Mekongina erythrospila* did not infected with any parasite (Figure 1). The highest diversity of parasites occurred in *Bagarius bagarius* (16 species), *Hemibagrus nemurus* (10 species) and *Clupisoma sinensis* (9 species) (Figure 2).

Table 1. Prevalence and mean intensity of parasites from each fishes species in Mekong River in the North of Thailand;
Ac: acanthocephalan, Ar: arthropod, C: cestode, D: digenean, M: monogenean, N: nematode, P: protozoa.

Fish			Parasite	prevalence	mean intensity
order	Family	species (samples)			
Cypriniformes	Cobitidae	<i>Syncrossus helodes</i> (2)	<i>Arthymorhynchus</i> sp. (Ac)	50.00	1.00
	Cyprinidae	<i>Barbonymus gonionotus</i> (15)	<i>Thelohanelus</i> sp. (P)	40.00	-
			<i>Dactylogyrus</i> sp.1 (M)	20.00	1.67
		<i>Cirrhinus jullieni</i> (20)	<i>Thaparogyrus jullieni</i> (M)	55.00	2.50
			<i>Dactylogyrus</i> sp.2 (M)	5.00	1.00
		<i>Labiobarbus siamensis</i> (5)	-		
		<i>Macrochirichthys macrochirus</i> (1)	-		
		<i>Mekongina erythrospila</i> (2)	-		
		<i>Osteochilus hasselti</i> (2)	<i>Dactylogyrus torquegenitalis</i> (M)	50.00	1.00
		<i>Puntioplites proctozystron</i> (6)	<i>Dactylogyrus hamatus</i> (M)	16.67	1.00
Osteoglossi- formes	Notopteridae	<i>Notopterus notopterus</i> (9)	<i>Myxidium</i> sp. (P)	11.11	-
			<i>Malayanodiscoides bihamuli</i> (M)	66.67	3.33
Perciformes	Channidae	<i>Channa striata</i> (10)	<i>Trianchoratus</i> sp. (M)	80.00	3.75
Siluriformes	Bagridae	<i>Hemibagrus nemurus</i> (200)	<i>Cornudiscoides malayensis</i> (M)	66.00	41.27
			<i>Thaparocleidus</i> sp.1 (M)	47.00	39.42
			<i>Thaparocleidus</i> sp.2 (M)	34.00	6.61
			<i>Thaparocleidus</i> sp.3 (M)	81.50	52.24
			<i>Haplorchoides</i> sp. (D)	91.00	40.63
			<i>Haplorchis</i> sp. (D)	73.50	32.12
			<i>Lytocestus</i> sp. (C)	1.00	1.00
			<i>Spinitectus</i> sp. (N)	59.00	1.84
			<i>Polymorphus striatus</i> (Ac)	14.50	6.96
			<i>Pallisentis</i> sp. (Ac)	1.00	2.50
		<i>Myustus wyckii</i> (12)	<i>Paracryptogonimus</i> sp.1 (D)	41.67	1000
	Pangasiidae	<i>Pangasius bocourti</i> (12)	<i>Thaparocleidus</i> sp.4 (M)	83.33	32.40
			<i>Thaparocleidus</i> sp.5 (M)	83.33	56.40
		<i>Pangasius conchophilus</i> (21)	<i>Thaparocleidus</i> sp.6 (M)	100.00	100.00
			<i>Thaparocleidus</i> sp.7 (M)	100.00	91.00
			<i>Monhysterides</i> sp. (N)	100.00	38.00
		<i>Pangasianodon gigas</i> (6)	<i>Prosorhynchoides siamensis</i> (D)	100.00	70.00
			<i>Prosorhynchoides</i>	33.33	8.00
			<i>chiangkongensis</i> (D)		
		<i>Pangasianodon gigas</i> (6)	<i>Alitropus typus</i> (Ar)	100.00	20.00

Table 1. Prevalence and mean intensity of parasites from each fishes species in Mekong River in the North of Thailand;
Ac: acanthocephalan, Ar: arthropod, C: cestode, D: digenean, M: monogenean, N: nematode, P: protozoa. (continue)

Fish			Parasite	prevalence	mean intensity
order	Family	species (samples)			
Siluriformes	Pangasiidae	<i>Pangasius micronemus</i> (20)	<i>Thaparocleidus siamensis</i> (M)	100.00	13.09
		<i>Pangasius sanitwongsei</i> (1)	<i>Cucullanus</i> sp. (N)	100.00	12.00
	Schilbeidae	<i>Clupisoma sinensis</i> (200)	<i>Thaparocleidus</i> sp.8 (M)	45.50	4.63
			<i>Prosorhynchoides</i> sp.1 (D)	47.50	3.86
			<i>Prosorhynchoides</i> sp.2 (D)	45.00	2.89
			<i>Metadena</i> sp. (D)	21.50	1.11
			<i>Gephyrolina</i> sp. (C)	6.00	1.42
			<i>Proleptus</i> sp. (N)	22.50	1.50
			<i>Procamallanus</i> sp.1 (N)	36.50	1.10
			<i>Ergasilus</i> sp.1 (Ar)	29.00	1.19
			<i>Lamproglena</i> sp. (Ar)	25.50	1.24
	Sisoridae	<i>Bagarius bagarius</i> (130)	<i>Thaparocleidus</i> sp.9 (M)	79.23	51.00
			<i>Thaparocleidus</i> sp.10 (M)	3.85	2.60
			<i>Opisthorchis</i> sp.1 (D)	0.77	2.00
			<i>Opisthorchis</i> sp.2 (D)	15.38	2.33
			<i>Dollfusotrema</i> sp. (D)	16.15	6.50
			<i>Rhipidocotyle</i> sp. (D)	10.00	1.75
			<i>Prosorhynchoides phillipinorum</i> (D)	26.92	30.86
			<i>Prosorhynchoides</i> sp. (D)	13.08	26.35
			<i>Clinostomum</i> sp. (D)	3.85	1.40
			<i>Phyllodistomum</i> sp. (D)	6.92	8.89
			<i>Metadena bagarii</i> (D)	4.62	6.83
			<i>Gephyrolina</i> sp. (C)	30.00	4.05
			<i>Procamallanus</i> sp.2 (N)	20.00	2.12
			<i>Arhythmorhynchus</i> sp. (Ac)	2.31	2.00
			<i>Polyacanthorhynchus</i> sp. (Ac)	16.92	2.40
			<i>Ergasilus</i> sp.2 (Ar)	3.08	1.00
	Siluridae	<i>Belodontichtys dinema</i> (22)	<i>Thaparocleidus</i> sp.11 (M)	72.73	7.88
		<i>Kryptopterus cryptopterus</i> (24)	<i>Procamallanus</i> sp.3 (N)	33.33	2.00
		<i>Wallago attu</i> (24)	<i>Thaparocleidus kao</i> (M)	91.67	21.18
			Unidentified digene (D)	8.33	1.00
Syngnathi- formes	Syngnathidae	<i>Doryichthys deokhatoides</i> (1)	<i>Paracryptogonimus</i> sp.2 (D)	100.00	1.00
			<i>Senga</i> sp. (C)	100.00	1.00

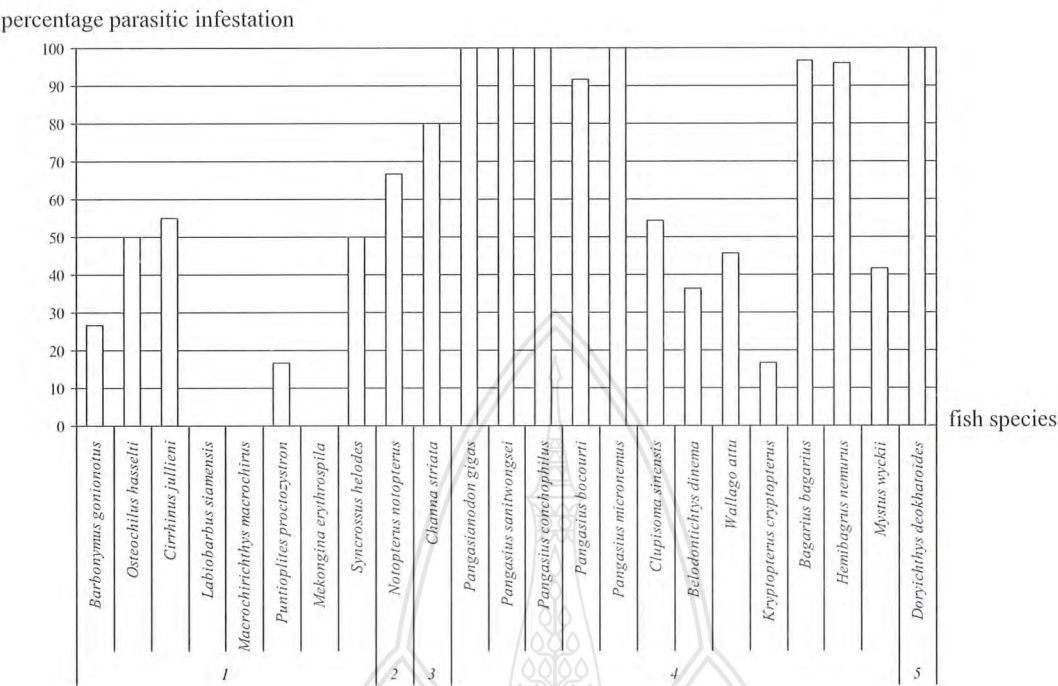


Figure 1. Percentage parasitic infestation of each fish species.
(1 = Cypriniformes, 2 = Osteoglossiformes, 3 = Perciformes, 4 = Siluriformes, 5 = Syngnathiformes)

Some aspects of parasitic protozoa observations found in this study, *Thelohanellus* sp. Was often found as cyst in Cypriniformes fish such as *Puntiplites proctozysron* (Theanhom, 1997), *Cirrhinus jullieni* (Purivirojkul, 1999), *Morulus chrysophekadion* (Suramane, 2000) and *Barbodes gonionotus* (Lerssutthichawal and Supamattaya, 2005). *Myxidium* sp. was often found in gall bladder of fish such as *Hampala macrolepidota* and *Osteochilus hasselti* (Lukphet, 1997).

In most cases of host specificity, the results also showed that parasites could be found in particular host species (in generic and specific levels). The monogeneans, were particularly limited to one host species that was also reported by Lim and Lerssutthichawal (1996), Lerssutthichawal (1999), Purivirojkul (1999), Lerssutthichawal and Supamattaya (2005). For example,

Thaparogyrus jullieni was only found on *Cirrhinus jullieni*, *Thaparocleidus kao* was only found on *Wallago attu*, and *Cornudiscoides malayensis* only on *Hemibagrus nemurus*. The results obtained from this study suggested that the parasites of each fish species might possibly be specific to their host. However, it might be under some conditions such as their intermediate hosts.

Selection of certain host species by monogenean may involve mainly with factors on the host surface. Chemical stimuli emitted from the host have been suggested to attract the parasites (Buchmann and Lindenstrom, 2002). Chemical, mechanical and behavioral mechanisms have been suggested to explain this host specificity (Whittington et al., 2000; Buchmann and Lindenstrom, 2002).

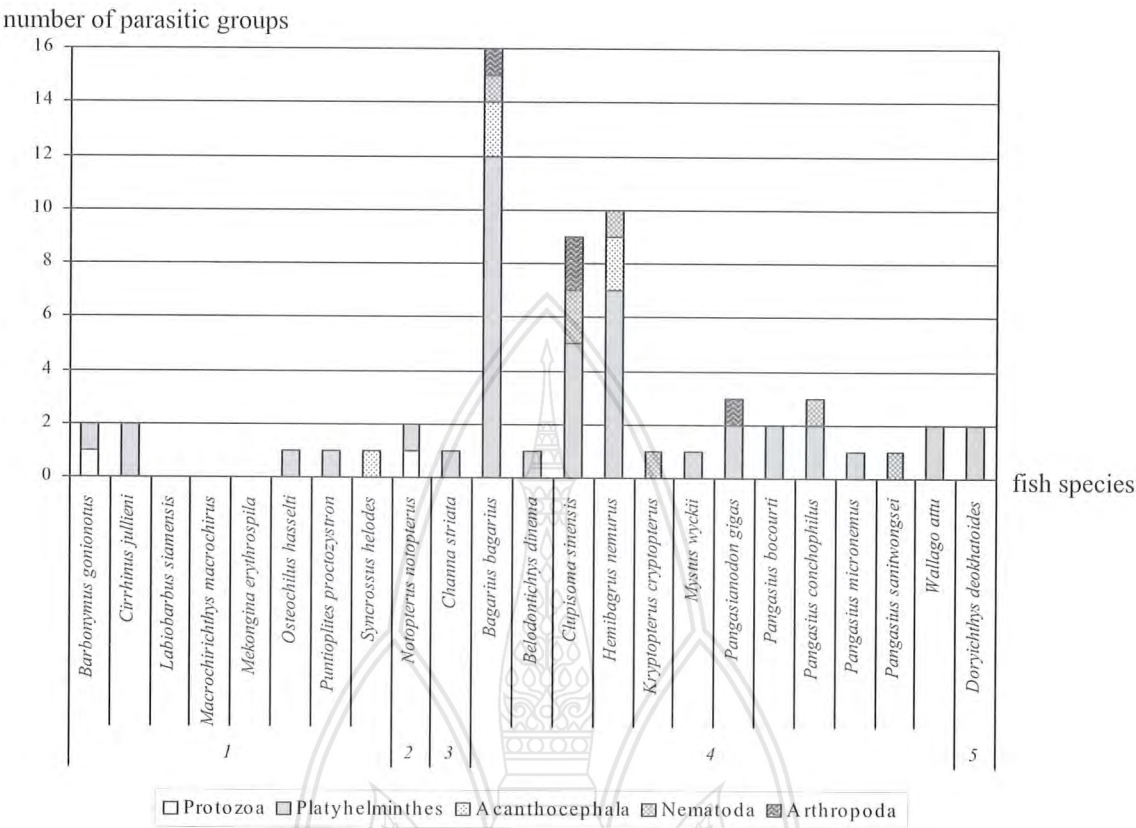


Figure 2. Number of parasitic groups observed from each fish host.
(1 = Cypriniformes, 2 = Osteoglossiformes, 3 = Perciformes, 4 = Siluriformes, 5 = Syngnathiformes)

Conclusions

All monogenean species found in this study are specific to their hosts comparing to severed reports from Thailand (Lim and Lerssutthichawal, 1996; Lerssutthichawal, 1999; Purivirojkul, 1999; Lerssutthichawal and Supamattaya, 2005). Other groups, such as digenean, and cestode also seem to have host specificity. However, this might associate with environmental factors (mollusk or other animals) because in their life cycles need at least one intermediate host.

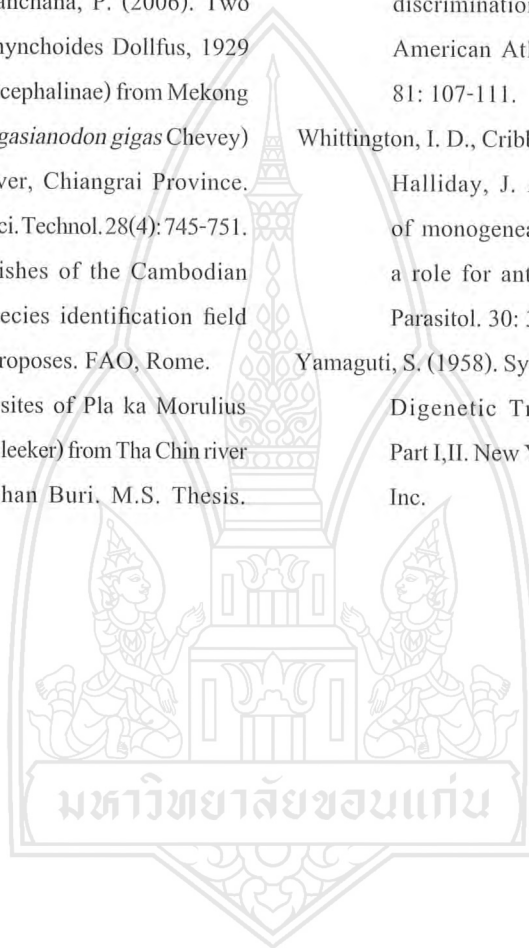
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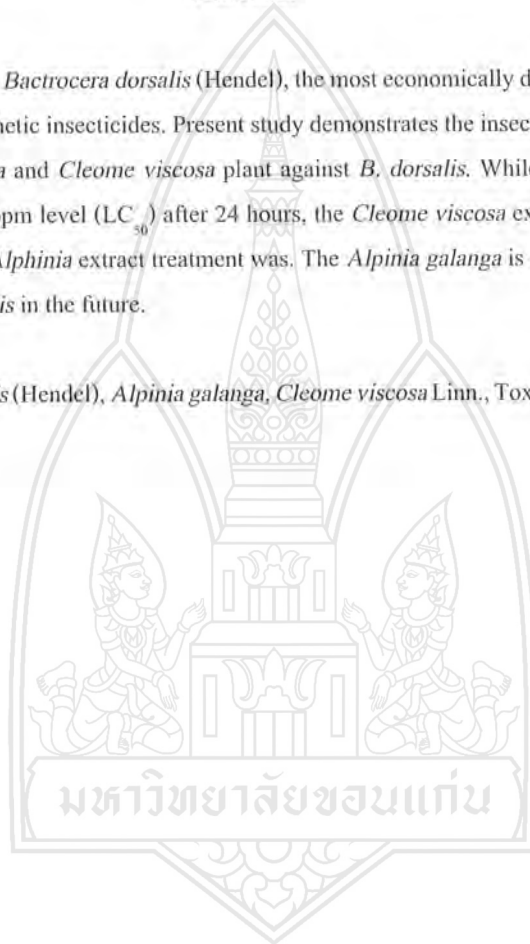
The Insecticidal Studies from *Alpinia galanga* and *Cleome viscosa* Extract as Alternative Control Tool to *Bactrocera dorsalis* (Hendel)

Nisakorn sukhirun¹, Vasakorn Bullangpoti^{1*} and Wanchai Pluempunupat²

Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), the most economically damaging pests in Thailand, is generally controlled by synthetic insecticides. Present study demonstrates the insecticidal activities of extracts prepared from *Alpinia galanga* and *Cleome viscosa* plant against *B. dorsalis*. While *Alpinia galanga* rhizome extract was toxic at 5,987.05 ppm level (LC_{50}) after 24 hours, the *Cleome viscosa* extract was much less active (LC_{50} = 27,867.80 ppm) than *Alpinia* extract treatment was. The *Alpinia galanga* is apparently good choice for alternative control of *B. dorsalis* in the future.

Keywords : *Bactrocera dorsalis* (Hendel), *Alpinia galanga*, *Cleome viscosa* Linn., Toxicity, Botanical insecticide



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Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) is serious pest of fruit trees in Thailand. These flies attack different fruit including rose apple, mango, banana, etc. The female fly lays their eggs under the skin of premature fruits. The larvae hatch within 1-3 days and feed inside the fruits causing premature drop and rot of whole fruits. Pupae are in soil under the host plant and then adults emerge after 1-2 weeks. Synthetic insecticides are the mainly controlling method for *B. dorsalis*; however, insects including other *Bactrocera* species, such as *Bactrocera oleae* have developed resistance (Brown and Payne, 1988) as has been shown in laboratory (Vontas et al., 2002).

The botanical insecticides are considered to have potentials in killing insects. It is unlikely to induce fast resistance problems and very friendly many animals and in Thailand. Many studies have reported efficacy of botanical against various pests. For instance, *Cymbopogon winterinus* (Jewitti) for the control of *Culex pipien quinquefasciatus* (Thummasarangkoon, 2000), *Capsicum frutescens* for *Sitophilus zeamais* (Motschulsky) (Bullangpoti et al., 2002), *Cleome viscosa* extract as the oviposition inhibitor for *Sitophilus oryzae* L. (Somboon and Pimsamarn, 2006).

Alpinia galangal or "Kha" is Thai spice plant (Larsen et al., 1999). Its essential oil from rhizomes have antimicrobial activity. Mayachiew and Devahastin (2007) reported that compounds like 1,8-cineole, β -bisabolene, β -caryophyllene and β -selinene, showed insecticidal activity against various pests (Areekul et al., 1987). *Cleome viscosa*, on the other hand, has been used as medicinal plant having active compound, like glucocapparin and glucocleomin (Song-sak and Lockwood, 2002). *C. viscosa* is also known to have contact insecticidal, repellent, antifeedant

and nematicidal properties (Ndungua et al., 1999; Williams et al., 2003; Lazzeri et al., 2004).

The objective of the present work is to determine the activity of these plants against Dipteran pest. *B. dorsalis* was chosen as it is dangerous fruit pest in Thailand.

Materials and Methods

1. Rearing method

B. dorsalis was received from the Division of Entomology and Zoology, Department of Agriculture Bangkok. Insects were reared on the artificial diet in the cage (30 cm x 30 cm x 30 cm) in the laboratory of the Zoology Department, Faculty of Science, Kasetsart University, Bangkok Campus, at 25 ± 2 °C and 70% RH. Adults were provided with 10% honey solution via a cotton pad and were allowed to lay eggs in a plastic jar.

2. Extraction method

Dried powder of *Cleome viscosa* leave and *A. galanga* rhizomes were received from Surat Thani (600 km from Bangkok) and were extracted using a modified method of Bullangpoti et al. (2006). The extraction was done with Soxhlet's extractor in 99% ethanol for 8 hrs. Extracts were concentrated using rotary evaporator (BUCHI B-850) under reduced pressure at 60 °C. The extracts were stored at 4°C until preparation of a stock solution. Stock solution was prepared by weighing a certain amount of the extracts and diluting in distilled water to give a series of concentrations.

3. Bioassay method

Bioassays were conducted using 5 concentrations in range of 5,000-40,000 ppm of crude extract along with control treatments (distilled

water). Topical mist sprays on 1 day old- adult *B. dorsalis* were made using 60 insects in 5 replicates. Mortality, were recorded after 24 and 48 hours. LC_{50} vaules was calculated using Probit analysis (StatPlus 2008). The data were analyzes with SPSS statistical software version 10.

Results and Discussion

The toxicity of *Alpinia galanga* rhizome and *Cleome viscosa* Extract against *B. dorsalis*

The rhizomes of *Alpinia galanga* extracts exhibited remarkable insecticidal activity against adult *B. dorsalis* in the topical mist sprayer application. After 24 hours, *Alpinia galanga* extract showed an LC_{50} of 5,987.05 ppm ($r^2 = 0.82$) and after 48 hours 5,652.53 ppm ($r^2 = 0.83$). Significant difference was observed between these two values (t-test, $p < 0.05$), suggesting that the exposure time might be correlated with the acute mortality and long exposure can give higher mortality.

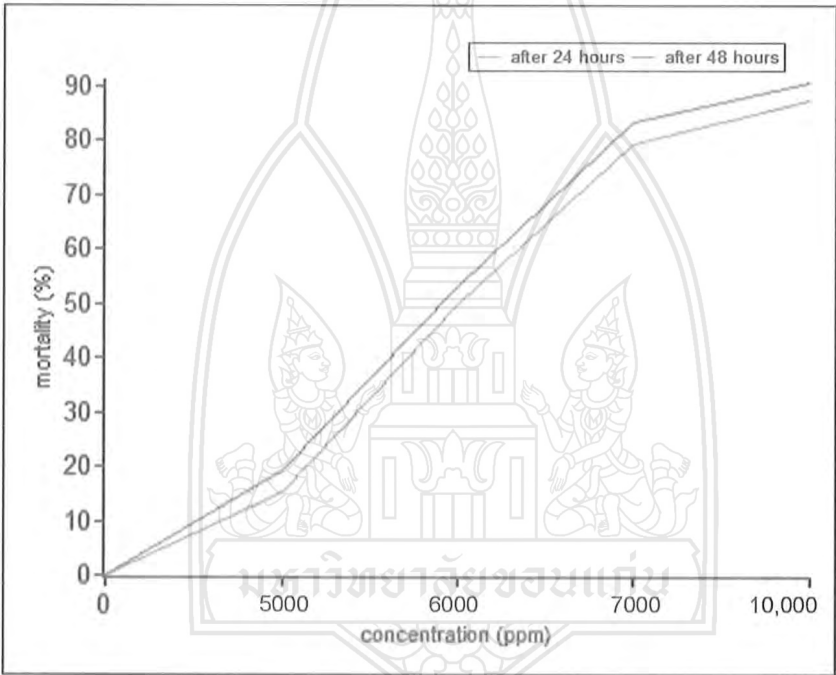


Figure 1.Mortality of *B. dorsalis* after *Alpinia galanga* application after 24 and 48 hours of treatment.

The concentrations report for kill of *B. dorsalis* by *Cleome viscosa* extracts was much higher (Figure 2). The LC_{50} values after 24 and 48 hours were 27,867.80 ppm ($r^2 = 0.96$) and 25,841.43 ppm ($r^2 = 0.97$) respectively. The significant difference between

these two values (t-test, $p < 0.05$) again suggest that the exposure time might be correlated with the acute mortality and long exposure can give higher mortality than short one, as was observed in case of *A. galanga* treatments.

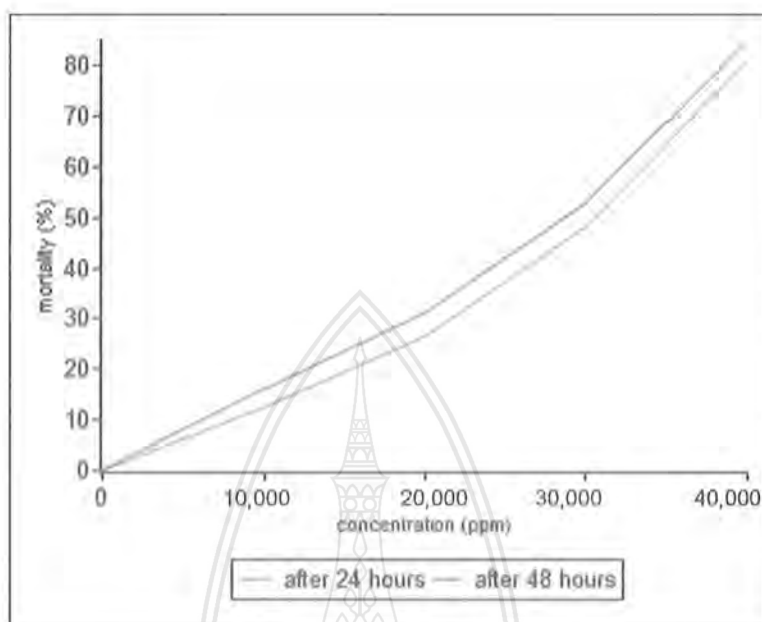


Figure 2. Mortality of *B. dorsalis* after 24 and 48 hours after treatment *Cleome viscosa* extracts.

However, both extracts have insecticidal activity similar to other botanical extracts, such as *Alpinia offinarum*, *Acorus calamus* or *Azadirachta indica* (Areekul et al., 1986). Earlier research show the efficacy of *Ternstroemia japonica* ($LC_{50} = 4,067$ ppm), *Cymbopogon citrates* ($LC_{50} = 8,150$ ppm) and *Citrus hystrix* ($LC_{50} = 31,000$ ppm) to *B. dorsalis*, which is lower than efficacy obtained with present extracts (Kitibumrungsuk, 1980).

Although the toxicity from both extracts is less than synthetic insecticide; the botanical products used here are biodegradable and safe to be used in any integrated pest management system.

Conclusions

The toxicity of *Alpinia galanga* and *Cleome viscosa* extract against *B. dorsalis* was determined using topical mist spray method. *Alpinia galanga*

showed higher insecticidal efficiency than that of *Cleome viscosa* extract. Both plant extracts, however, significantly increase in toxicity over the time. This is the first and preliminary research of insecticidal activity of *Alpinia galanga* and *Cleome viscosa* extract against *B. dorsalis*, suggesting that these botanicals could be use to control pest. However, detailed investigation of active ingredients and delivery system and the toxicity to non-target organisms are the future prospects of this research.

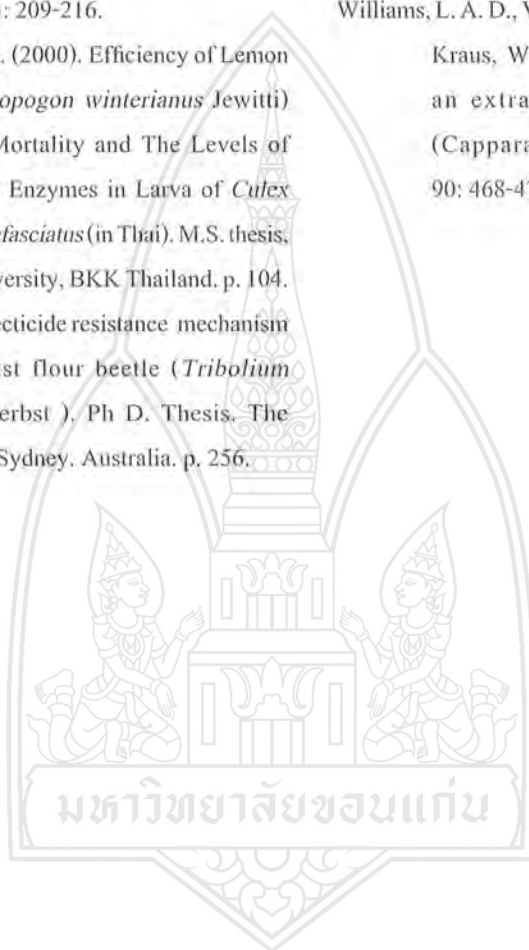
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Change of Microbial Population in Liquid Fertilizer

Nuntavun Riddech^{1*}, Wandee Bunyatrachata¹ and Yada Polsan²

Abstract

Changes of the following microbial communities in liquid fertilizer were studied: proteolytic, amylolytic, cellulolytic, lactic acid bacteria, total bacteria, molds and yeast. Microbes were detected inside three liquid fertilizer bins. The first bin was the control, having no starter culture. The second and the third bins were inoculated with starter cultures (KKU and Pordor 2, respectively). The inoculated liquid fertilizer was set for 15 days. The pH of the samples was measured. It was found that the pH values were at 3.2-5.8. Whereas amylolytic microorganism was 10^2 - 10^3 CFU/ml. Cellulolytic microorganism was 10^2 - 10^6 CFU/ml. Proteolytic microorganism was 10^2 - 10^5 CFU/ml. Moreover, molds, yeast, lactic acid bacteria, and total bacteria were 10^3 - 10^6 CFU/ml. Furthermore, the largest population of total microorganisms was observed during the first three days of the fermentation process. Thereafter, the number of total microorganisms noticeably decreased.

Keywords : Liquid fertilizer, Microbial population

Introduction

Organic waste is expected to increase due to a rapidly growing population in Thailand. In order to reduce this organic waste, the Thai government has developed a new waste reduction plan. Production of liquid fertilizer is an alternative recommended for treating organic wastes, and the end product can be reutilized for plants. Many soil problems result from chemical fertilizers. Liquid fertilizer is alternative to chemical fertilizer since it is not harmful to the soil. (<http://www.rin.ac.th/knowledge/agr013.asp>). Presently liquid fertilizer is a well-known and very important agricultural fertilizer. Various organic wastes such as vegetable, fruits, and other foods can be used as substrate for producing liquid fertilizer. Bacteria, fungi and yeasts involve in the fermentation process in changing organic waste to liquid fertilizer. Dark-brown liquid, which is rich in

growth hormones, amino acids, and other beneficial substances, is the end product of the process. (Rachain et al., 2007) General properties of liquid fertilizer are as follows: (1) the value of electrical conductivity is between 2-12 desicemen/meter (ds/m), (2) pH value is between 3.5-5.6, (3) C/N ratio is between 1/2 -70/1, (4) for the fermented plant extract: % total nitrogen is between 0.03-1.66%, % total P_2O_5 is between 0-4%, % water soluble K_2O is between 0.05-3.53%, (5) amino acids, (6) plant growth hormones (<http://www.doa.go.th/th/ShowArticles.aspx?id=174>); Chaisut (2007). The production process of liquid fertilizer is uncertain, and the information of microbes involving the fermentation process are not clearly understood. The aim of this study was to enumerate proteolytic, amylolytic, cellulolytic, lactic acid bacteria, total bacteria, molds, and yeast from three liquid fertilizer bins.

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Material and Methods

Starter culture

Two starter cultures were used in this experiment. KKK starter culture consisted of bacteria, yeast and fungi which were received from Faculty of Agricultural, Khon Kaen University, Thailand. Pordor 2 is the starter culture which produces by Land Development Department of Thailand. Yeast (*Saccharomyces* sp.), Lactic acid bacteria (*Lactobacillus* sp.) and Proteolytic bacteria (*Bacillus* sp.) are in Pordor. (http://www.ddd.go.th/Lddwebsite/WEB_r01/Website_station/cnt01/12wonder/wonder02/wonder2P2.htm) Both starters can degrade agriculture waste and enhance the fermentation process of liquid fertilizer.

Production of Liquid Fertilizer

Three kilograms of fresh fruits skin (pineapple, mango and watermelon, etc.) were used as substrates. Substrates were sliced into small pieces. One kilogram of molasses was added and mixed. Mixed substrates were divided into three liquid fertilizer bins. The fermentation process type was a septic fermentation in 10 liter bin. The first bin was the control (without starter culture). The second bin contained the starter culture KKK, and the third bin contained the starter culture Pordor 2. The inoculums size of starter culture was 3%. These three liquid fertilizers were incubated at room temperature for 15 days. Samples were taken on days 0, 1, 3, 5, 7, 10 and 15 of the fermentation process for enumeration of microbes.

pH of Liquid Fertilizer Analysis

Ten milliliters of Liquid fertilizer were taken on days 0, 1, 3, 5, 7, 10 and 15 of the fermentation process for pH analysis using pH meter.

Enumeration of Microbes

Liquid fertilizer at the 0, 1, 3, 5, 7, 10 and 15 days of the fermentation process were diluted to 10^{-7} in Ringer solution

1. Proteolytic Bacteria

Dilutes of Liquid fertilizer (10^{-2} - 10^{-4}) were spread on Calcium caseinate agar by the spread plate technique. Petri dishes were incubated at 30 degree Celsius for 2-3 days. Thirty to three hundred colonies that appeared clear zone around colony were enumerated on Petri dishes.

2. Amylolytic Bacteria

Diluted liquid fertilizer at 10^{-2} - 10^{-4} was spread on nutrient agar + 0.2% soluble starch by the spread plate technique. Petri dishes were incubated at 30 degree Celsius for 2-3 days. Thirty to three hundred colonies that appeared clear zone around colony after receiving a poured iodine solution were enumerated on Petri dishes.

3. Cellulolytic Bacteria

Diluted liquid fertilizer at 10^{-2} - 10^{-4} was spread on Cellulose agar by the spread plate technique. Petri dishes were incubated at 30 degree Celsius for 7-10 days. Thirty to Three hundred colonies that appeared clear zone around colony were enumerated on Petri dishes.

4. Mold and Yeast

Diluted Liquid fertilizer at 10^{-2} - 10^{-4} was spread on Potato dextrose agar + 10% lactic acid by the spread plate technique. Petri dishes were incubated at 30 degree Celsius for 2-3 days. Thirty to three hundred colonies were enumerated on Petri dishes.

5. Lactic Acid Bacteria

Diluted liquid fertilizer at 10^{-2} - 10^{-4} was spread on MRS agar + 0.5% CaCO_3 + 0.02% Sodium azide by the spread plate technique. Petri dishes were incu-

bated in candle jars at 30 degrees Celsius for 2-3 days. Thirty to three hundred colonies that appeared clear zone around colony were enumerated on Petri dishes.

6. Total Bacteria

Diluted liquid fertilizer at 10^{-4} - 10^{-7} was transferred to Petri dishes. Plate count agar was poured by the pour plate technique. Petri dishes were incubated at 30 degree Celsius for 2-3 days. Thirty to three hundred colonies were enumerated on Petri dishes.

Results

pH

pH of liquid fertilizer ranges 3.2-5.8. The pH was mildly acidic during 7 days of the fermentation and afterward it decreased to more in acidity. The pH in the bin which was inoculated with starter culture Pordor 2 was higher than in the control and KKU starter culture. (Figure 1)

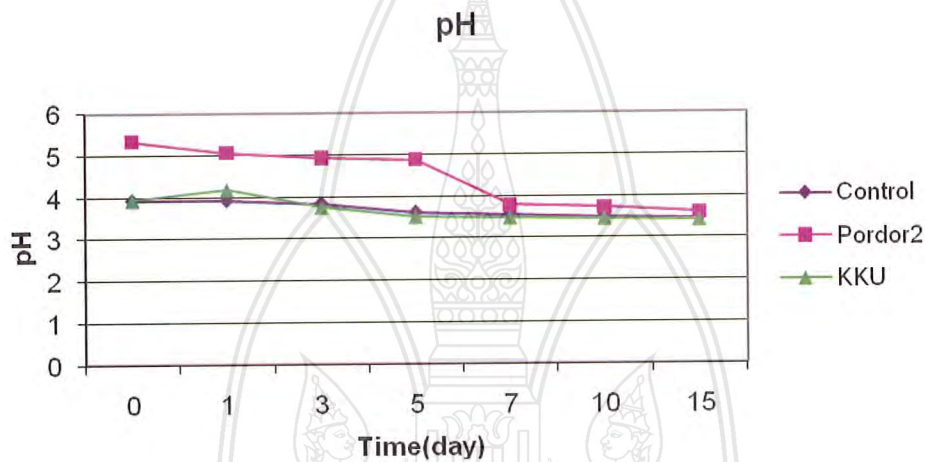


Figure 1. pH of Liquid fertilizer

Change of microbial population in liquid fertilizer

The microbe population increased with in three days of the fermentation process and afterward the numbers of microbes decreased by the aging of liquid fertilizer. The numbers of amyolytic bacteria, proteolytic bacteria and cellulolytic bacteria were

10^2 - 10^5 CFU/ml (Figures 2, 3 and 4), and the numbers of mold and yeast, lactic acid bacteria and total bacteria were 10^3 - 10^6 CFU/ml (Figures 5, 6 and 7). It was found that the number of total bacteria was highest in liquid fertilizer and conversely, the lowest in amyolytic bacteria.

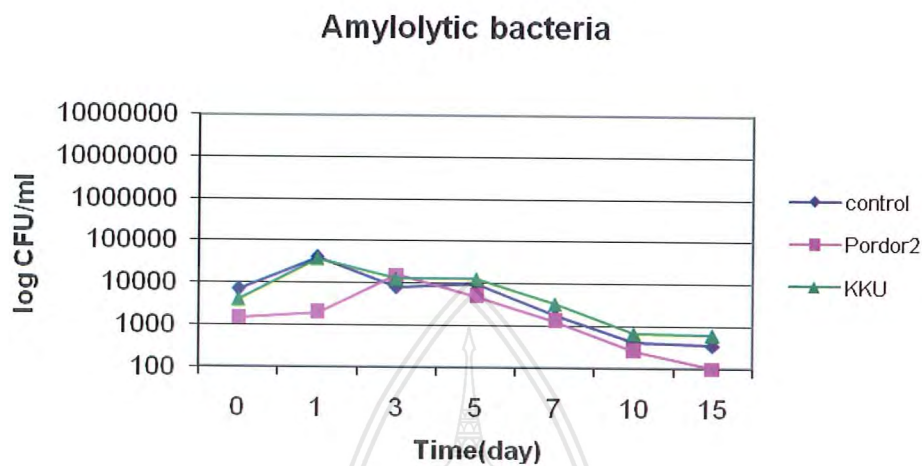


Figure 2. The numbers of Amylolytic bacteria (log CFU/ml) in Liquid fertilizer

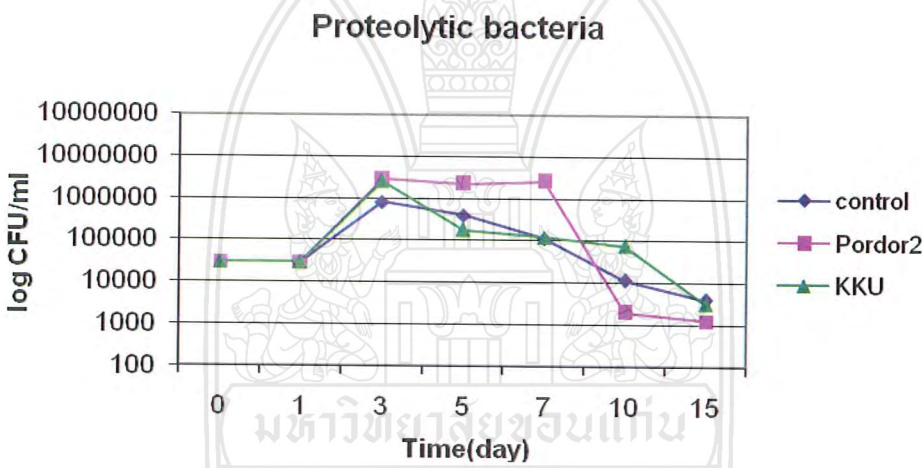


Figure 3. The numbers of Proteolytic bacteria (log CFU/ml) in Liquid fertilizer

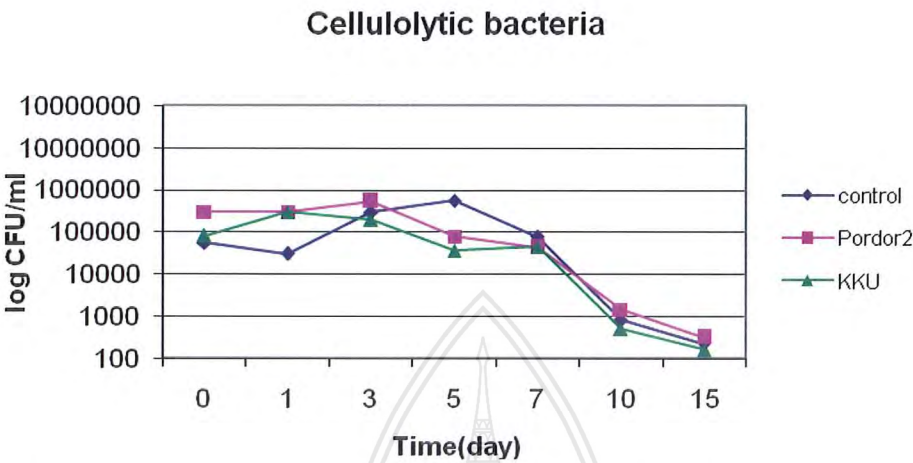


Figure 4. The numbers of Cellulolytic bacteria (log CFU/ml) in Liquid fertilizer

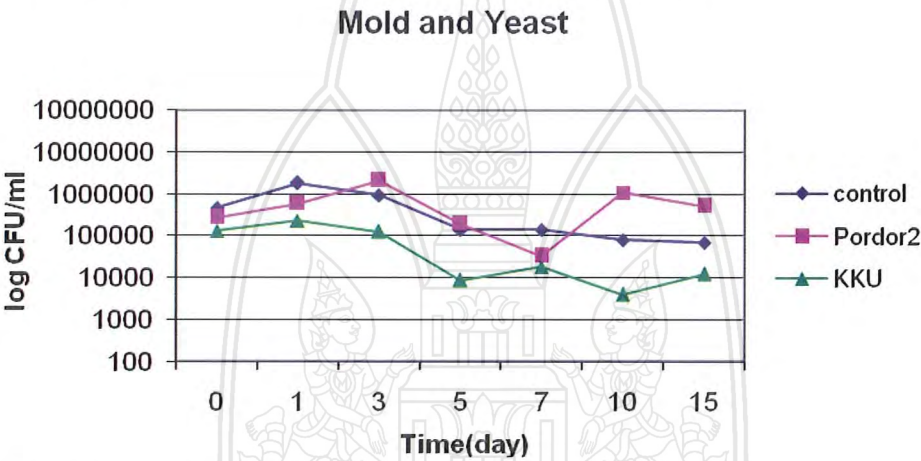


Figure 5. The numbers of Mold and yeast (log CFU/ml) in Liquid fertilizer

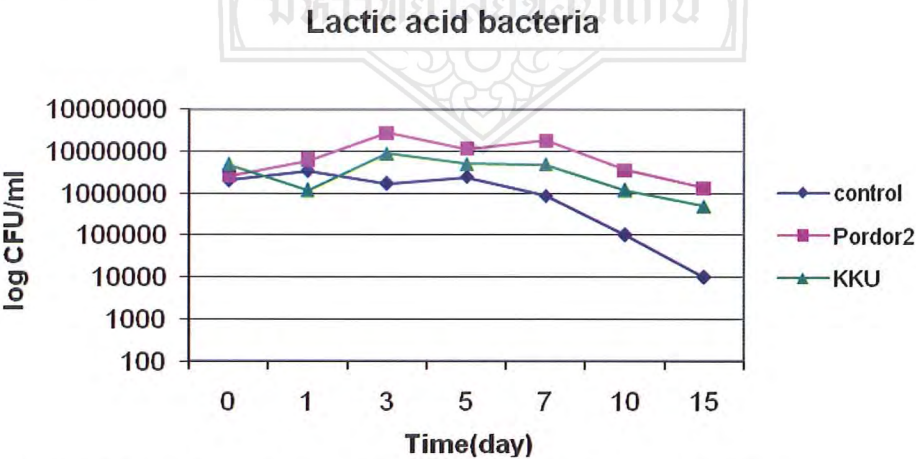


Figure 6. The numbers of Lactic acid bacteria (log CFU/ml) in liquid fertilizer

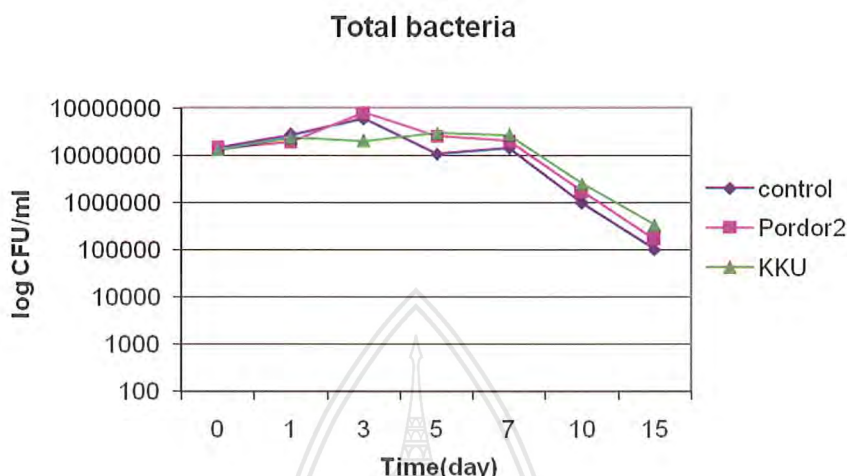


Figure 7. The numbers of Total bacteria (log CFU/ml) in Liquid fertilizer

Conclusion

The pH of liquid fertilizer from the control and KKU – cultured bins were acidic. Lactic acid bacteria which were found in liquid fertilizer had important role in the change to acidity. The acidic pH ($\text{pH} \leq 4.5$) indicated a good quality of liquid fertilizer (<http://www.petipomg.com/notice/index3.html>). The numbers of microbes increased in three days of the fermentation process and decreased during aging of liquid fertilizer. Total bacteria, yeast and mold were found in liquid fertilizer. These microbes were from the agricultural material and starter culture. They can produce alcohol, organic acids and enzymes and these substances are significant to growth of the plant. Agricultural wastes were degraded by these microbes (http://www.ddd.go.th/Lddwebsite/WEB_r01/Website_station/cnt01/12wonder/wonder02/wonder2P1.htm). Liquid fertilizer in this experiment was the fermented plant extract. Therefore, cellulolytic bacteria, lactic acid bacteria, yeast and mold had important role in the fermentation. Besides, we found fewer amounts of amylolytic bacteria in the bin. Proteolytic bacteria which produce enzyme to degrade protein in substrate that we used for the fermentation process were also found. Moreover, addition of starter cultures did not

affect the number of microbes in the liquid fertilizer.

Acknowledgements

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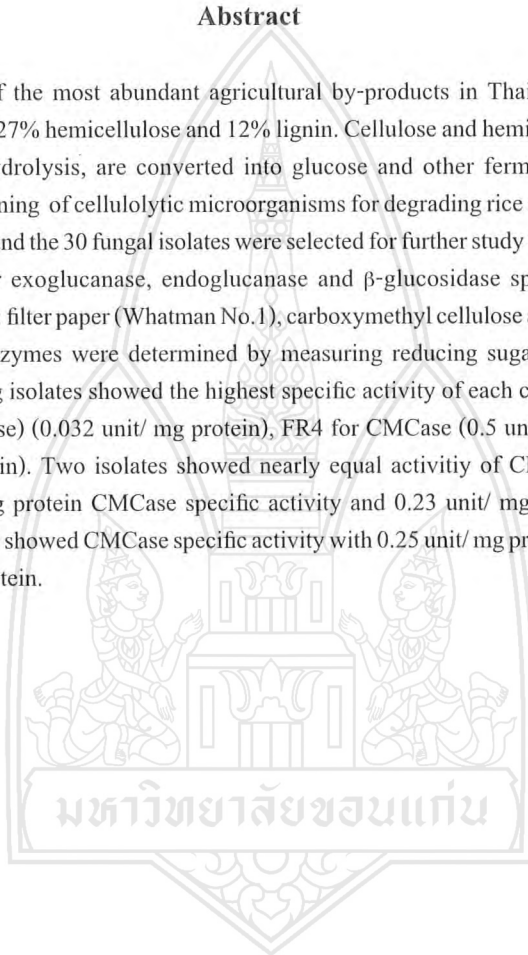
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Screening of Rice Straw Degrading Microorganisms and Their Cellulase Activities

Atcha Boonmee^{1,2*}

Abstract

Rice straw is one of the most abundant agricultural by-products in Thailand. It generally contains approximately 39% cellulose, 27% hemicellulose and 12% lignin. Cellulose and hemicellulose, when hydrolyzed by chemical or enzymatic hydrolysis, are converted into glucose and other fermentable sugars for ethanol production. In this study, screening of cellulolytic microorganisms for degrading rice straw has been investigated. Twenty nine bacterial isolates and the 30 fungal isolates were selected for further study on their cellulolytic activity. All isolates were assayed for exoglucanase, endoglucanase and β -glucosidase specific activities, by which following substrates were used: filter paper (Whatman No.1), carboxymethyl cellulose and cellobiose, respectively. Specific activities of those enzymes were determined by measuring reducing sugar released from substrates. From the results, the following isolates showed the highest specific activity of each category of cellulases; FR14 for Filter paper cellulase (FPase) (0.032 unit/ mg protein), FR4 for CMCase (0.5 unit/ mg protein) and FC1 for cellobiase (0.6 unit/ mg protein). Two isolates showed nearly equal activity of CMCase and cellobiase. The isolate FR3 had 0.22 unit/ mg protein CMCase specific activity and 0.23 unit/ mg protein cellobiase specific activity, while the isolate FR18 showed CMCase specific activity with 0.25 unit/ mg protein and cellobiase specific activity with 0.30 unit/ mg protein.



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Introduction

Due to global warming problem and continuous decrease of fossil fuel source, alternative energy such as gasohol or bio-diesel has been increasingly consumed. Therefore, bio-ethanol serving as gasohol additive has been consecutively produced and improved as well. At present, bio-ethanol production is based on sugar (from sugar cane) or flour (from maize or cassava) as substrates. Biomass feedstock represents about 40% of bio-ethanol production cost (Hamelinck et al., 2005) and makes the price of ethanol produced from those substrates much higher than those comparing to fossil fuel. Therefore, reducing cost of ethanol production by using lignocellulosic materials such as bagasse, wood scraps, corncob or rice straw as an alternative feedstock becomes a very interesting topic for researchers nowadays. The choice of the best technology for lignocellulose to bio-ethanol conversion should be decided on the basis of overall economics (lowest cost), environment (lowest pollutants) and energy (higher efficiency), i.e. comprehensive process development and optimization are still required to make the process economically viable (Chandel et al., 2007).

Rice straw is one of the most abundant agricultural by-products in Thailand. It generally contains approximately 39% cellulose, 27% hemi-cellulose and 12% lignin (Kaur et al., 1998). Cellulose and hemi-cellulose, when hydrolyzed by chemical or enzymatic hydrolysis, are converted by microorganisms into glucose and other fermentable sugars for ethanol production. Lignocellulose-degrading microorganisms such as bacteria, fungi, actinomycetes and protozoa generally exist in nature and yet remain to be discovered by researchers.

The mechanism of enzymatic cellulose hydrolysis involves synergistic actions by endoglucanase, exoglucanase or cellobiohydrolase, and β -glucosidase. Endoglucanases hydrolyze accessible intramolecular β -1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends; exoglucanases progressively cleave cellulose chains at their both ends to release soluble cellobiose or glucose; and β -glucosidases hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition. These three hydrolysis processes occur simultaneously as shown in Figure 1 (Zhang, 2006).

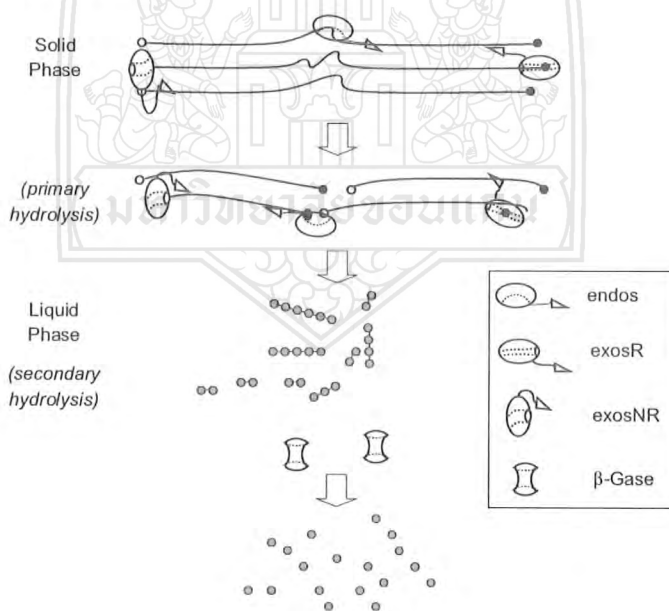


Figure 1. Mechanistic scheme of enzymatic cellulose hydrolysis by *Trichoderma* non-complexed cellulase system (Zhang et al., 2006).

In this study, screening of cellulase-producing microorganisms, considering various types of cellulases: endoglucanase, exoglucanase and β -glucosidase, was investigated in order to determine their potential use as agent to degrade rice straw which contains almost 39% cellulose and to provide preliminary data for further bio-ethanol production from lignocellulosic materials.

Material and methods

1. Screening of cellulose-degrading bacteria

Ten grams of each sample was diluted with 90 ml of 0.85% NaCl, resuspended well by using vortex mixer for 5 min. Ten-fold dilution series were performed with the suspension and 3 appropriate dilutions were selected for the screening. From each selected dilution, 0.1 ml of the suspension were spread on NA, 0.1% cellulose agar or 2% rice straw agar and incubated 3-5 days at 37°C. Colonies of bacteria and fungi were collected, thereby bacteria were streaked on screening agar and fungi were stabbed in screening agar.

2. Assaying cellulase specific activities

2.1 crude enzyme preparation

One loop-full of each bacteria isolate was inoculated in 25 ml 0.1% cellulose broth, incubated 24 hours at 37°C, 150 rpm. Cell suspension was measured by OD₆₀₀ and adjusted to OD₆₀₀ = 0.1, then 5 ml of the adjusted cell suspension were inoculated in 45 ml 0.1% cellulose broth and incubated for 24 hours at 37°C, 150 rpm. Ten milliliters of cell suspension were centrifuged for 10 minutes at 4°C, 5,000 rpm. Supernatant containing crude enzyme was used to assay enzyme specific activities.

2.2 cellulase specific activity assay

Total protein amount of crude enzyme was determined by Lowry method (Lowry et al., 1951) and protein concentration (mg/ml) was later used to calculate specific activity (unit/mg protein). Crude enzymes were then tested for their abilities to hydrolyze the following substrates: 1×1 cm filter paper (Whatman No.1), 1% carboxymethyl cellulose (1% CMC) and 0.1% cellobiose. All of these substrates were resuspended in 0.2 M sodium acetate buffer pH 5. Each reaction was prepared in 2 sets for incubation at 30°C and 50°C by mixing 1 ml crude enzyme with 1 ml buffer, incubated for 1 hr when filter paper was used as substrate and for 10 minutes when CMC or cellobiose were used as substrates. Reducing sugar was measured by Somogyi-Nelson method (Nelson, 1944) (for filter paper and CMC) or DNS method (Miller, 1959) (for cellobiose). Enzyme activities were reported in unit/ml of crude enzyme.

Results

1. Screening of cellulose-producing microorganisms

From eight samples of soils and waste water, 11 bacterial isolates (BC1-11) and 9 fungal isolates (FC1-9) were found on 0.1% cellulose agar; 19 bacterial isolates (BR1-19) and 20 fungal isolates (FR1-20) could grow on 2% rice straw agar containing pretreated rice straw as carbon and energy source. Thus, total number of isolates are 59, by which 30 of them are bacteria and 29 are fungi. Those isolates were then selected for further study on their cellulolytic activities. Figure 2 shows some of the isolates on screening agar.

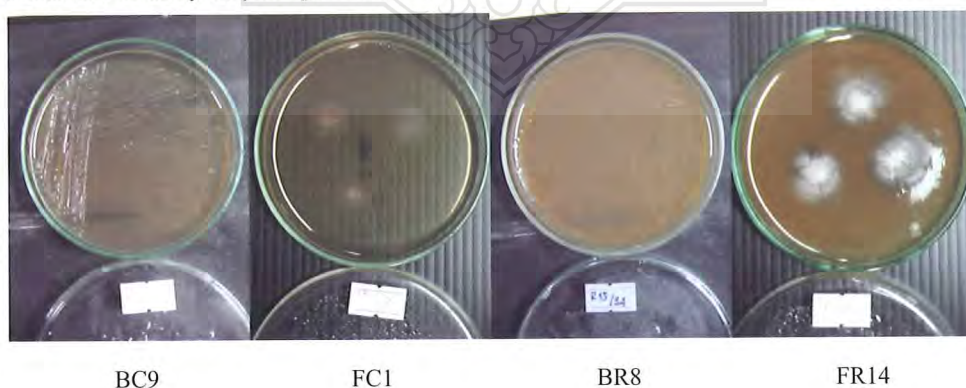


Figure 2. Some of the isolates grown on 0.1% cellulose agar (BC9 and FC1), or 2% rice straw agar (BR8 and FR14)

2. Assaying cellulolytic activities with various substrates

All isolates were assayed for exoglucanase, endoglucanase and β -glucosidase specific activity. The following substrates were used: filter paper (Whatman No.1), carboxymethyl cellulose, and cellobiose. Specific activities were determined by measuring reducing amount of sugar released from substrates. All reactions were performed at 30°C and 50°C

Filter paper cellulose activity (FPase activity)

In this work, we measured activity of enzyme which hydrolyzes filter paper. We referred to those

enzymes as exoglucanase. Some isolates showed high FPase specific activity at low temperature (30°C) and some showed higher activity at high temperature (50°C). However, overall activities of their filter paper cellulases were still very low, where the average of specific activity found in bacteria isolates was 0.0049 unit/ mg protein and in fungi isolates was 0.0041 unit/ mg protein. The isolates with high specific activity of filter paper cellulase (FPase) are shown in Figure 3, which are the isolates FR14, BR15, BR11, FR9 and BR4. The isolate FR14 showed the highest FPase specific activity with 0.032 unit/ mg protein.

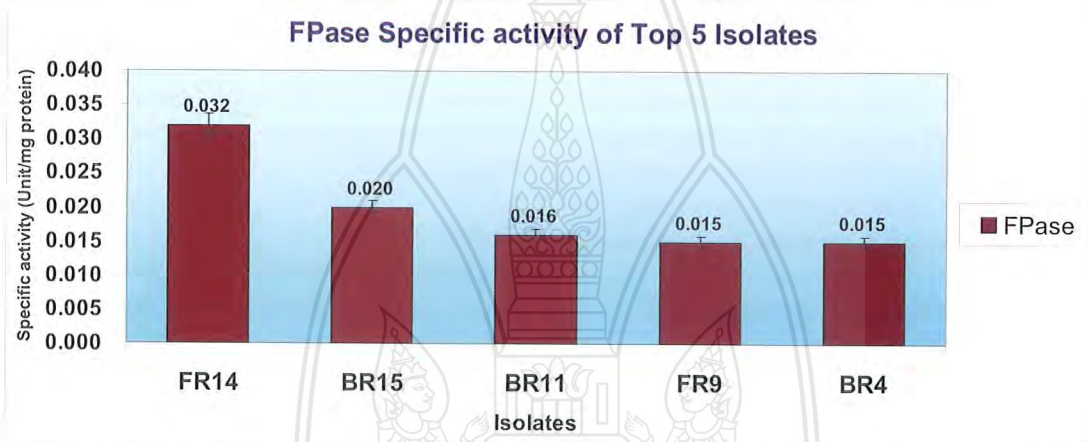


Figure 3. Summary of bacteria (B) or fungi (F) isolates with high specific activity of filter paper cellulase (FPase)

The screenings were done on 0.1% cellulose agar (C) and 2% rice straw agar (R) at 30°C and 50°C. Isolates FR14 and BR4 showed high activity at 30 °C, whereas BR15, BR11 and FR9 showed high activity at 50 °C CMCase and cellobiase specific activity. The term “carboxymethyl cellulase” (CMCase) and “cellobiase” represent endoglucanase and β -glucosidase, respectively. After screening on 0.1% cellulose agar and 2% rice straw agar at various temperatures, some isolates showed high cellulase specific activity at low temperature (30°C) and some showed higher activity at high temperature (50°C). Moreover, bacteria isolates tended to produce more cellulose enzymes hydrolyzing than carboxymethyl cellulose hydrolyzing enzymes (CMC),

whereas fungal isolates were able to produce various enzymes comparing to bacterial isolates. Some fungal isolates produced either CMCase or cellobiase, when some could produce both enzymes at the same level. The average of CMCase and cellobiase specific activity found in bacterial and fungi isolates were 0.06 and 0.062 unit/ mg protein, respectively. Isolates with high specific activity of CMCase and cellobiase are shown in Figure 4 and 5, respectively. The isolates with high CMCase specific activity are FR4, FR19, BC9, FR14, FR18 and FR3, where the isolate FR4 showed the highest CMCase specific activity with 0.5 unit/ mg protein. The isolates with high cellobiase specific activity are FC1, FC2, BR8, FC9 and BR14, where FC1 showed

the highest cellobiase specific activity with 0.6 unit/ mg protein. Furthermore, several fungi isolates moderately produced both enzymes. For example, FR18 had CMCase and cellobiase specific activity

of 0.25 unit/ mg protein and 0.30 unit/ mg protein, respectively and FR3 had CMCase and cellobiase specific activity of 0.22 unit/ mg protein and 0.23 unit/ mg protein, respectively.

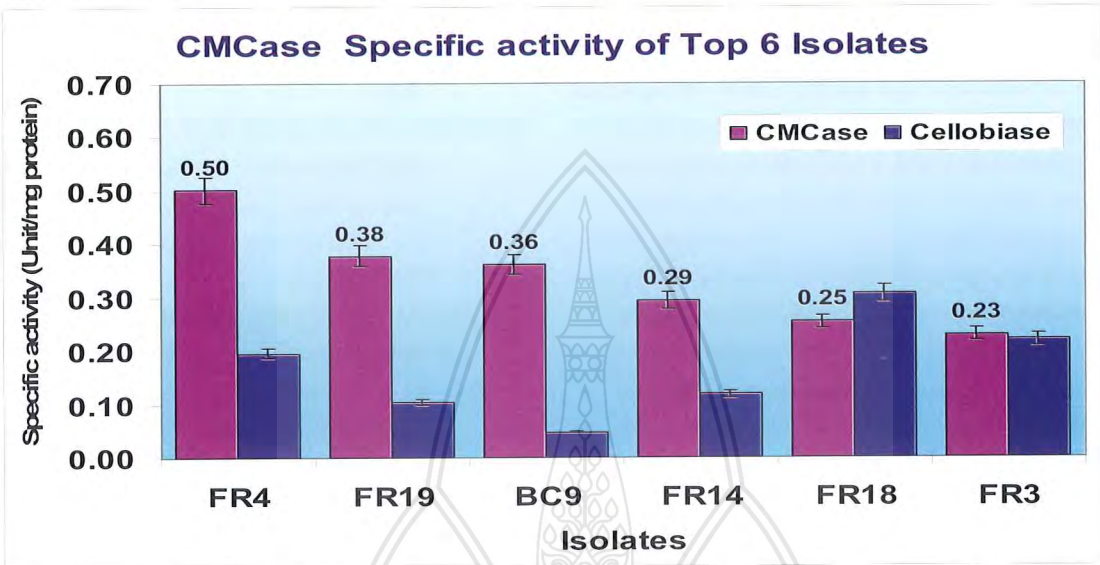


Figure 4. Summary of bacteria (B) or fungi (F) isolates with high specific activity of carboxymethyl cellulase. The screening were done on 0.1% cellulose agar (C) and 2% rice straw agar (R) at 30°C and 50°C. All isolates shown here performed their high activity at 50°C

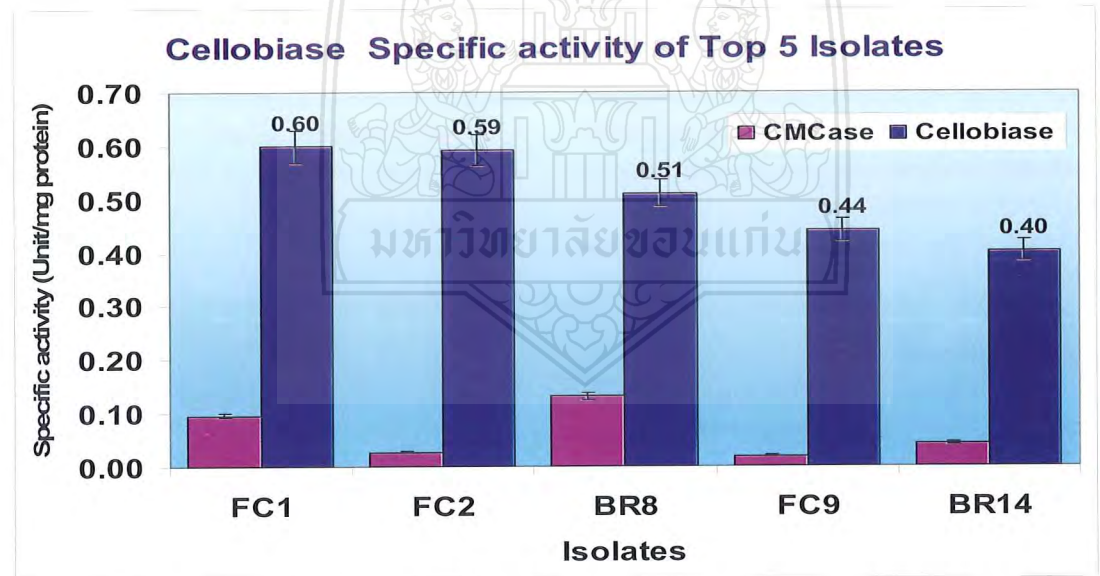


Figure 5. Summary of bacteria (B) or fungi (F) isolates with high specific activity of cellobiase. The screenings were done on 0.1% cellulose agar (C) and 2% rice straw agar (R) at and 50°C. Isolates FC1, FC2, BR8, FC9 showed their high activity at 30°C, whereas BR14 showed its high activity at 50°C

Discussion

The application of cellulolytic enzymes in degrading lignocellulosic materials needs synergistic actions of various types of cellulases. Thus, there are 2 feasible ways of making use of the enzymes from the isolates found in this study. The first way is to select the isolates with high specific activity of individual enzymes and use them as mix cultures. The other way is using only one isolate which is able to produce various types of enzymes, where the problem of optimal growth conditions of each isolates by co-fermentation could be avoided. Specific activities of cellulases assayed in this research are still very low comparing to others which have been reported. However, cellulase production from these isolates has to be further optimized under different conditions in order to enhance cellulose degradation and consequently make them useful for further applications.

Conclusion

Screening of cellulolytic microorganisms for degrading rice straw was investigated. The 29 isolated bacteria and the 30 isolated fungi were studied on their cellulolytic activities. The isolate FR14 showed the highest Filter paper cellulase (FPase) specific activity with 0.032 unit/ mg protein, while FR4 had the highest CMCase specific activity with 0.5 unit/ mg protein. The highest cellobiase activity was shown by the isolate FC1 with 0.6 unit/ mg protein. Cellulase production from these isolates has to be further optimized under different conditions.

Acknowledgment

This research has been supported by the Faculty of Science and the Protein & Proteomics Research Group (PPRG), Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. The author also thanks S. Saraihorm for her excellent assistance.

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Agricultural Modernization in Conjunction with Rural Development in Vietnam

Nguyen Xuan Trach¹

Abstract

To meet a rapid growth of demand for agricultural products, modernization of agriculture in Vietnam is indispensable. That is because there are now important socio-economic constraints to increasing agricultural production in the traditional mixed smallholder systems, although they have been reasonably productive and inherently sustainable. However, introduction of modern technologies in agricultural production operates for higher productivity may, on the other hand, result in consequences that are not in line with sustainable development targets such as alleviation of poverty, creation of jobs, more equitable life styles, protection of the environment, and conservation of biodiversity. This warrants search for appropriate ways of agricultural modernization coupled with sustainable rural development. The present paper aims to formulate with a conceptual framework for development of management, technical and policy approaches toward appropriate modernization of agriculture without the dilemma between unsustainable high productivity and low-productive sustainability to achieve both economic growth and rural development targets.

Keywords : Agriculture, modernization, rural development, Vietnam

Introduction

Traditionally, agricultural production has been based on mixed smallholder systems in Vietnam. In a low-income country, these integrated systems have considerable advantages compared with specialized cropping and animal production. Although each of these integral sub-systems may be an independently function, they are nevertheless complementary and synergistic. The integration and synergism of the sub-systems can produce a greater output than the sum of their individual effects. Such integrated crop-agricultural systems were allowed for minimizing wastes through recycling, which can reduce the need of raw materials from outside, minimizing risks for farmers. In addition, they can help to protect an environment and conserve

biodiversity owing to using indigenous inputs, which also require less agro-chemicals. Greater use of indigenous knowledge and more complete use of rural labor in these systems ensure high productivity, income and access to goods and services of the rural population. Thanks to these integrated farming systems, "if it were assessed in terms of sustainable agriculture it (Vietnam) would be among the leaders" (Preston, 1995).

The time-tested integrated systems, which have often been developed over hundreds of years, are reasonably productive and inherently sustainable. However, it should be recognized that there are now important socio-economic constraints to increasing agricultural production in such mixed smallholder systems (Ogle and Phuc, 1997).

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Especially, they are now challenged to decline farm size and raise a consume demand for agricultural products. As a result, modernization of intensive agricultural production is indispensable because modernized intensive production systems can produce more outputs per unit of land resource than can the traditional systems. Actually, middle-scale agricultural farms and large-scale agricultural production units have been recently developed in peri-urban areas, satisfying an important part of the consumer demand in the big cities (Le Viet Ly, 2000). Nevertheless, it is of increasing concern nowadays that undesirable ecological and sociological consequences may result from modernization of agricultural production. The present paper is, therefore, aimed to discuss possible negative impacts of inappropriate modernization of agricultural production and then propose a conceptual framework for modernization of agricultural production without penalizing sustainable development of the country.

Possible consequences of inappropriate modernization of agricultural production

As experienced in many developing countries, although intensification of agricultural production can bring more agricultural products per unit of resource, thus better meeting the increasing demands for domestic consumption as well as export, imported technologies for such intensive agricultural production have almost always failed to overcome the constraints imposed on local farming systems or to meet the socio-economic requirements of farmers (Sansoucy, 1997). The following consequences of introducing large-scale intensive production systems with imported modern technologies are possible and should be taken into account.

- Increased unemployment and impoverishment

An important question of concern is how

intensification of agricultural production should be undertaken in a densely populated country with the majority living in rural areas. Intensification of agricultural production with use of technologies imported from developed countries means minimum use of labor and maximum use of labor saving devices. The labor saving technologies would in turn require the use of large amounts of fossil fuels. Two main factors that would lead to such intensive agricultural production systems are high cost of labor and low cost of fossil fuels. This is not the case of Vietnam where around 80% of the population live in rural areas and approximately 70% of these rely on agriculture for their livelihoods.

In Vietnam, there is still a big problem of rural poverty which urgently needs to be alleviated. In the future the challenge will decrease a rural poverty in rural areas as only a limited amount of people can be absorbed into urban businesses (Orskov, 2001). Introduction of modern large-scale industrial livestock units would lead to problems for smallholder producers who cannot compete for available feed resources and markets (Ogle and Phuc, 1997) and may not have the skills for the more sophisticated management which is required (Preston, 1995). This will ultimately result in a reduction of rural employment opportunities and easily turn the problem of rural unemployment into an even greater problem of urban unemployment (Figure 1). So, intensive agricultural production may benefit a few capable people at the expense of the majority who will lose their shares in common resources, both tangible and intangible, and job opportunities. Consequently, the disadvantaged people will be faced with impoverishment, the gap between the few rich and the major poor will become larger and larger, and social evils will become a heavy burden. Clearly, modern intensive agricultural production models imported from developed countries are sociologically unsustainable under the circumstances of a developing country like Vietnam.

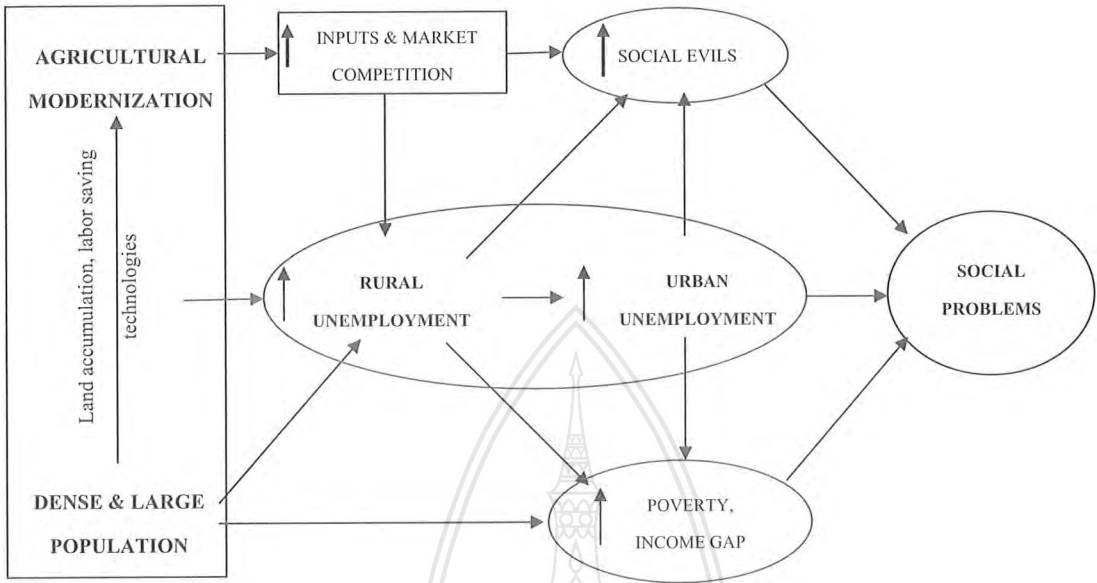


Figure 1. Increased social problems due to inappropriate modernization of agricultural production

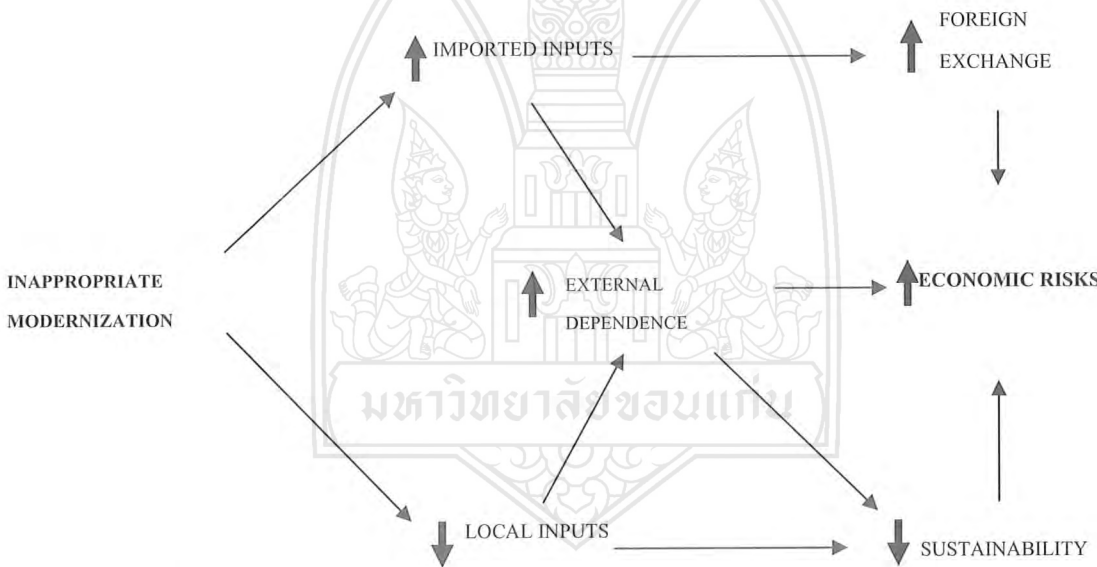


Figure 2. Increased economic risks due to inappropriate modernization of agricultural production

- Increased economic risks

Intensive agricultural production systems based on imported technologies are also economically unsustainable (Figure 2). They are first subjected to change in price and availability of inputs, to say nothing

of difficulty marketing products in changeable international markets. Under certain conditions animal producers can earn much profit by using low-priced imported feeds, but their businesses are vulnerable to any economic instability (Le Viet Ly, 2000). These

intensive systems will generally increase foreign exchange deficit due to high imports. In addition, the producers need to have high management skills and technical expertise, whereas the margins gained may be small. Another factor contributing to economic risks for these intensive systems is the introduced exotic breeds being highly susceptible to diseases and vulnerable to

hardship under unfavorable tropical climate conditions. The dangers of introducing inappropriate imported technologies have been well illustrated in the recent economic crisis in other South-East Asia countries. For example, in Indonesia, 80% of the poultry industry went bankrupt causing many problems of food supplies and malnutrition (Orskov, 2001).

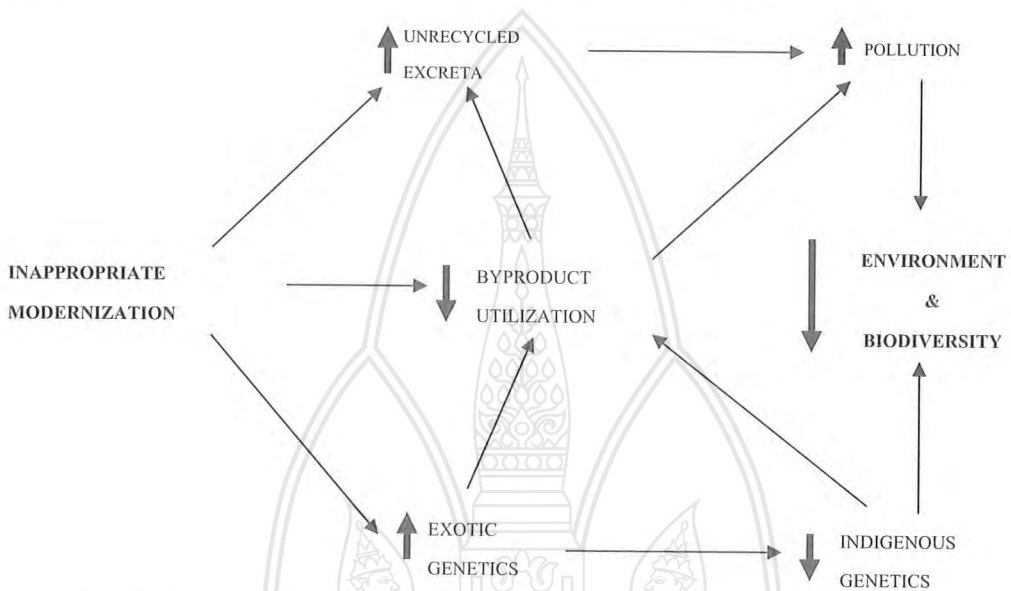


Figure 3. Negative impacts of inappropriate modernization of animal production on the environment and biodiversity

- Negative impacts on the environment and biodiversity

Protection of the environment and biodiversity is about increasing concern nowadays. That is the result of human activities that causing alarming levels of atmospheric contamination, deforestation, erosion, soil and water pollution and loss of biodiversity, which are all affecting people's livelihood and health, and threatening sustainable development (Preston, 1995). Modern large-scale industrial livestock units easily be problems of the environment and biodiversity (Figure 3). In such intensive production units, the high amount of animal population will produce too much excreta while there are not enough associated crops to recycle it. They will also result of a reduction in the numbers

of indigenous animals, which are highly tolerant to diseases and efficient in utilizing local feeds, especially agricultural by-products and household leftovers.

Proposed approaches for modernization of agricultural production in Vietnam

The above mentioned possible consequences of introducing intensive production systems with inappropriate modern technologies imported from developed countries should imply that they are not sustainable in social and ecological terms for Vietnam. Therefore, any strategies for modernization of agricultural production in the country must keep balance between the modernization targets and sustainable development targets (Figure 4).

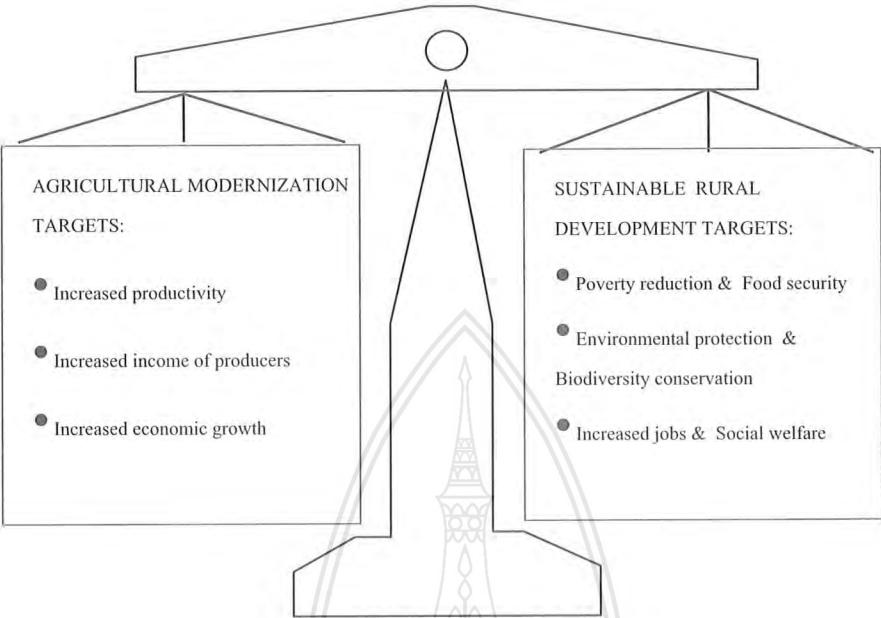


Figure 4. Needed balance between modernization targets and sustainable development targets

Modernization of agricultural production in Vietnam should thus be aimed at the following integrated targets:

- Increased productivity without compromising ecological benefits and job opportunities.
- Increased economic growth together with alleviation of rural poverty and attainment of food security.
- Increased income of the producers coupled with improved social welfare and equitability.

Putting all together, a conceptual framework for modernization and further improvement of agricultural production in Vietnam to be able not only to meet the rapidly increasing demand for agricultural products but also ensure sustainable development is recommended as follows (Figure 5).

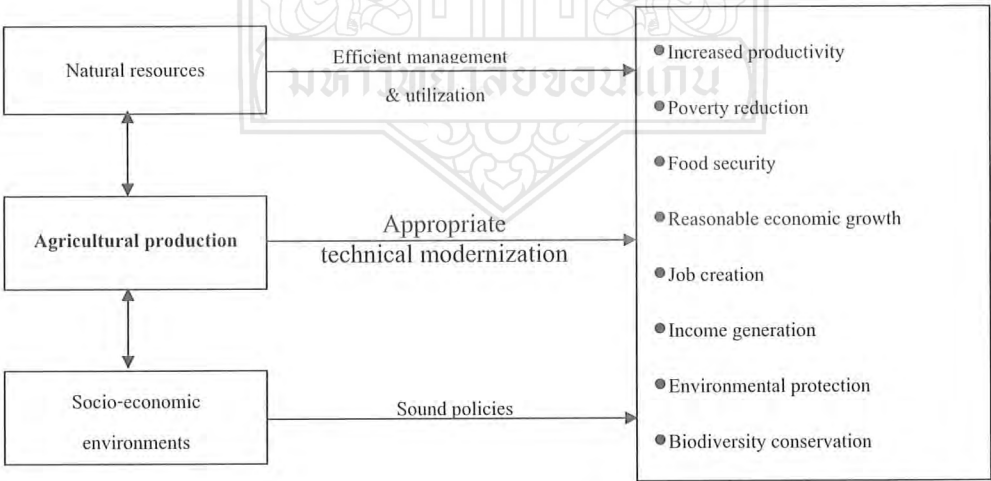


Figure 5. A conceptual framework for modernization and further improvement of agricultural production in Vietnam

To obtain the balanced targets, efforts to rationally modernize agricultural production need to take into account the available natural resources as well as the socio-economic environments. Appropriate technical modernization must be undertaken together with efficient management of natural resources and sound socio-economic policies. Researches for them are needed and should be focused on:

- Improving efficiency in the use and management of natural resources to optimize the capacity of the local ecosystem to produce increased agricultural products;
- Matching genetic potentials of animals and crops with local resources, climate conditions, and agricultural husbandry expertise;
- Maximizing the use of indigenous knowledge and local labor;
- Minimizing wastes through recycling and improved utilization of byproducts to reduce the

need for external raw materials and help to protect the environment;

- Intensification of small-scale agricultural production which can create more job opportunities;
- Developing technologies with farmer participation to ensure high levels of adoption and sustainability.
- Developing modernized large-scale production together with improving smallholder production;
- Increasing the value of agricultural production to smallholder producers by effective processing and marketing of their products ;
- Developing sound socio-economic policies for better use of common resources and fair commodity prices.
- Promoting rural industrialization together with civil and production services to absorb most redundant labor resulted from agricultural modernization (Figure 6).

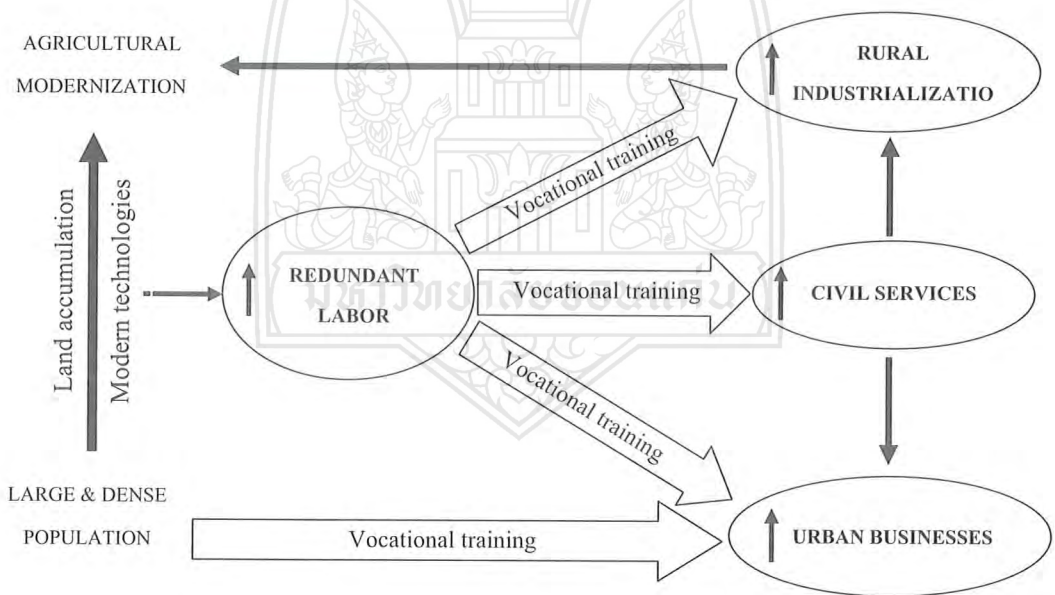


Figure 6. Use of redundant labor resulted from agricultural modernization

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Anti-Obesity and Body Weight Reducing Effect of *Fortunella japonica* Peel Extract Fractions in Experimentally Obese Mice

Do Ngoc Lien^{1*}, Nguyen Thuy Quynh¹, Nguyen Hoang Quang¹, Do Van Phuc¹ and Nguyen Thi Thanh Ngan¹

Abstract

The objective of the present study is to develop an experimentally obese mice model and to investigate anti-obesity and body weight reducing effect of *Fortunella japonica* peel extract fractions. Male mice (*Mus musculus*, Swiss race of 4 weeks, weighed at 14-15 g) were purchased from National Institute of Hygiene and Epidemiology (NIHE) and were put in a cage with a temperature of $25 \pm 2^\circ\text{C}$ and humidity ($80\% \pm 10\%$) under a 12 h light/12 h dark cycle. Diets and tap water were provided *ad libitum*. Experimental mice were divided into 5 groups, the first group was fed with normal pellet diet (NPD) (Standard chow from NIHE), and other groups (2-5 groups) were fed with high fat diet (HFD) containing 58% calories of fat for a period of 15 days. The HFD mice exhibited significant increase in body weight about 44% as compared with NPD group at that time ($p < 0.05$). Besides, the HFD mice showed significant increase in blood total cholesterol, blood triacylglycerols LDL and glucose concentrations to be 32.20%, 44.0%, 57.50% and 24.60%, respectively, as compared to NPD mice. In addition, the effects of daily repeated oral administration for 8 days of 800 mg/kg of ethyl acetate, chloroform and ethanol extract fractions from *Fortunella japonica* peels were studied. The obtained results showed that the body weights of HFD mice treated by different extract fractions were reduced by 9.2% (for ethyl acetate extract), 8.2% (for chloroform extract) and 4.7% (for ethanol extract) as compared with vehicle control, obese mice were treated by water replaced the extracts ($p < 0.05$). HFD mice were treated by different extract fractions exhibited significant decrease in blood total cholesterol to 13.2% (for ethanol extract), 1.4% (for chloroform extract), 0.7% (for ethyl acetate), in triacylglycerol 13% (for ethanol extract), 15.2% (for chloroform extract), 16.2% (for ethyl acetate extract), in glucose 0.9% (for ethanol extract), 23% (for chloroform), 18.5% (for ethyl acetate extract) as compared with control ($p < 0.05$).

Keywords : Anti-obesity, *Fortunella japonica*, Experimentally obese mice.

¹ Supported by Vietnam National University (Research project: QGTD 0809) Hanoi University of Science, Vietnam

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Introduction

Obesity can be considered as an abnormal state of lipid metabolism. The number of obese people are increasing in developed and even developing countries. Obesity could induce various live-threatening diseases, including type II diabetes, hypertension, coronary heart diseases stroke and certain cancers. The worldwide prevalence of obesity in adults is currently estimated to exceed 300 million [10]. Although altering eating and activity behavior, lifestyle is the cornerstone of anti-obesity and the pharmacotherapy for obesity is needed. However, most of the medications listed by United States Food and Drug Administration (FDA) were only approved for short-term use because patient’s complications to drug therapy usually occur. One of the novel strategies for anti-obesity is to exploit the natural products from traditional medicinal plants in form of plant extracts or functional food. However, information in pharmacologyl and action mechanisms of natural compounds oriental remedies is limited. For a long time, the kumquat (*Fortunella japonica* (Thunb.) Swingle Rutaceae) is used for traditional remedy preparing for treatment of respiratory and digestive diseases, but its anti-obesity effect has not been studied yet. In this

report the anti-obesity effect of *Fornunella japonica* peel extract was studied on experimentally obese mice.

Materials and methods

Kumquat (*Fortunella japonica*) peels were collected from plants growth in Hanoi. Characterization of some compounds was carried out by specialized techniques as specific colour reactions: shinoda, vanilin/ HCL, etc. and thin layer chromatography on silica gel sheets (Merck Flufolien 60 F254) with solvent system: Toluene: ethyl acetate: acetone: formic acid (5:3:1:1) ratio. Quantitation of total phenolic compounds was carried out by Folin-ciocalteau technique (Orthofer et al, 1999). Determination of some blood biochemical data such as: glucose, cholesterol, triacylglycerol, HDL and LDL concentrations was carried by Olympus Analyzer, Japan. The obtained data were analyzed by biological statistics with MS-excel.

Results and discussion

The solvent using such as ethanol, n-hexane, chloroform, ethyl acetate and water for extracting natural product from *Fortunella japonica* peels. The scheme of extraction processes is presented in the Figure 1.

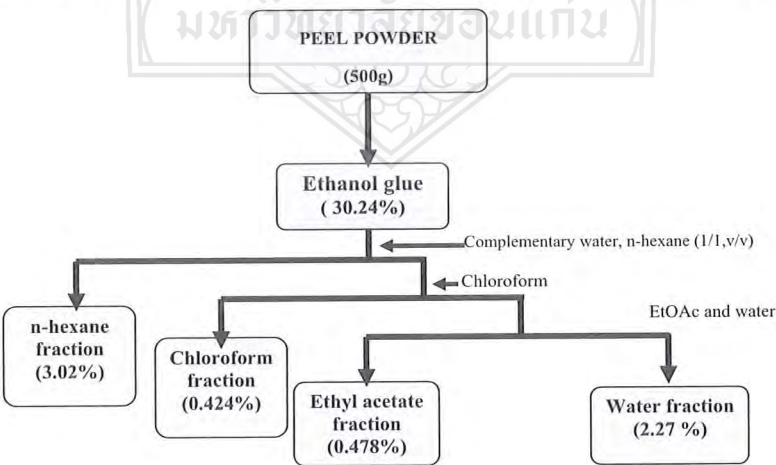


Figure 1. Process of isolation of natural products from Kumquat peels.

After that the extract fractions were evaporated under vacuum to prepare the fraction concentrates. In order to analyse the composition of extraction fractions we used specific reactions. The results are shown in the Table 1.

Table 1. Characterizations of natural products from *Fortunella japonica* peel extract fractions by specific reactions

Compounds		Specific reaction	Compound			
			Ethanol fraction	n-hexan fraction	Cloroform fraction	Ethyl acetate fraction
Phenolic	Flavonoid	Shinoda	+	-	+	+
		H ₂ SO ₄	+	+	+	+++
	Catechin	Vanilin/HCl	+	+	+	+
	Tannin	Vanilin/H ₂ SO ₄	+	+	+	+
		Lead Acetate	++	+	++	++
		Gelatin/NaCl	+	-	+	+
	Other Polyphenol	10% NaOH	+	+	+	++
		5% FeCl ₃	++	+	++	+++
Glycoside		Keller-killian	+	-	-	-
Alkaloid	Vans-Mayer	+	+	+	-	
	Bouchardat	+	+	+	-	
	Dragendroff	++	+	++	-	

(+) positive level

The results listed in the Table 1 showed the compositions of natural products from *Fortunella japonica* peel extract fractions consist of phenolic, glycoside and alkaloid compounds. However, phenolic compounds such as flavonoids, tannin, catechin were found principally in the ethyl acetate and ethanol fractions.

In order to determine relatively the composition of natural compounds we used the technique of thin layer chromatography on Silica gel sheets (Merck, Flufolien 60.F254) with solvent system Toluene/ ethyl acetate/ acetone/ formic acid (5:3:1:1 ratio). The results showed that kumquat peel extract fractions were composed from 6 to 12 bands of natural products (Figure 2).

Using the technique of Singleton et al. (1999) for quantification of phenolic compounds, we have determined phenolic contents of kumquat peel extract fractions. (Table 2)

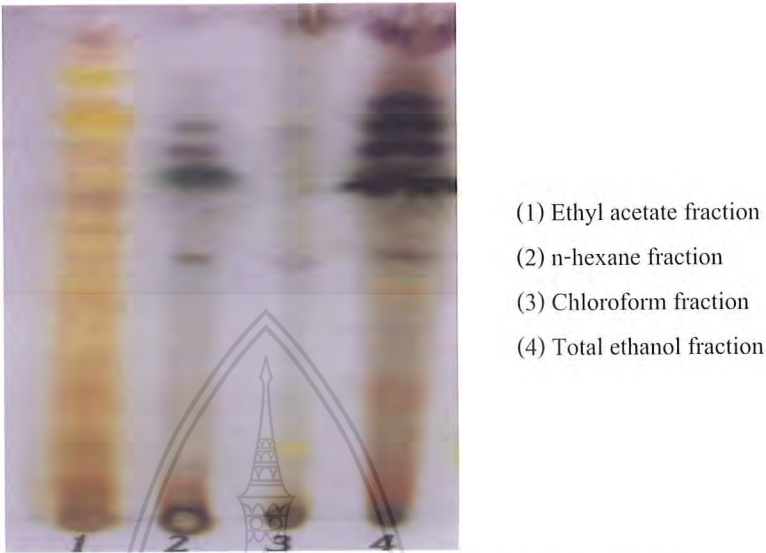


Figure 2. Thin layer chromatography on silica gel sheet by solvent system: Toluene-ethyl acetate-acetone-formic acid (5:3:1:1)

Table 2. Total polyphenol content determined by Follin- Ciocalteau technique

Fractions	Total polyphenol, g	% Material
EtOH concentrate	1.50	0.30
n-hexane concentrate	0.25	0.05
CHCl ₃ concentrate	0.65	0.13
EtOAc concentrate	0.68	0.14
Water concentrate	0.09	0.02

The results listed in Table 2 show that highest concentration (mg) of phenolic compounds from 500 g of dry material powder in ethanol (EtOH) fraction is 1.5 mg (0.3 % of dry material). The other fractions such as ethyl acetate and chloroform fractions also expressed high concentration of phenolic compounds, at 0.65 mg (0.13 % of dry material) and 0.68 mg (0.14 % of dry material), respectively. For designing the

model of experimentally obese mice, the mice, *Mus musculus*, Swiss strain, which weighed about 14-15 g were divided into 6 lots (6 mice/lot repeated three times)

Lot 1: control, mice were fed with standard diet (NIHE)

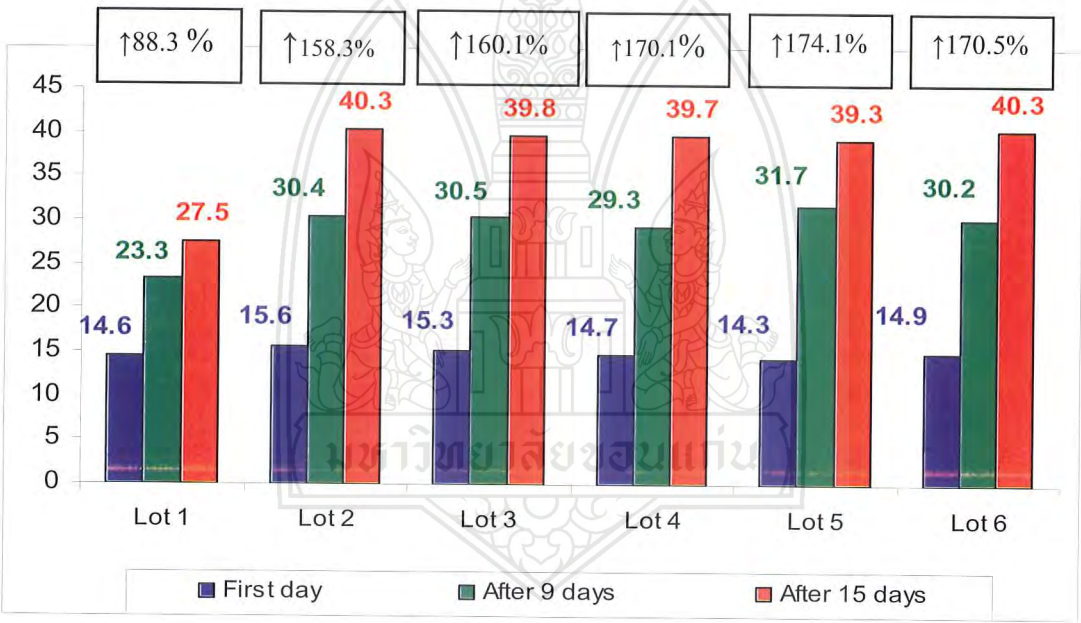
Lot 2 – 6: mice were fed with high lipid diet.

The composition of high lipid diet calculated by National Institute of Nutrition was represented in the Table 3.

Table 3. Composition of high lipid diet

Composition	%
Carbohydrate	30
Lipid	35
Casein	25
Cholesterol	3
Vitamin and minerals	3
Other compounds	4

Firstly, the body weight of experimental obese mice (fed standard diet). The result is shown in mice was determined in comparison with control Figure 4.



- Lot 1: fed standard diet

Lot 2: fed HLD

Lot 3: fed HLD
- Lot 4: fed HLD

Lot 5: fed HLD

Lot 6: fed HLD

Figure 3. A diagrammatic representation of weight increase of experimentally obese mice fed with high lipid diet after 15 days

The results obtained from the model of experimentally obese mice in Figure 4 showed that the body weight of experimentally mice fed with high lipid diet for 15 days clearly increase 158.3% to 174.8% as compared with the first day. While the body weight of control mice fed with standard diet only

increased 88.3% (Figure 3). In order to demonstrate the disorder of lipid metabolism of experimentally obese mice we proceeded to analyze blood lipid compositions of experimentally obese mice comparing to control mice. The results are presented in the Figure 4.

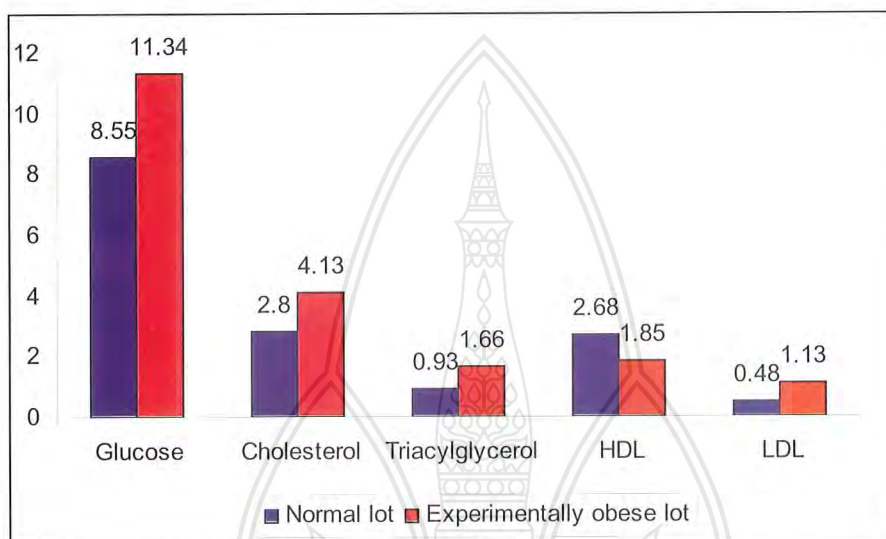


Figure 4. Diagrammatic representation of glucose and lipid concentration of normal and obese mouse blood

The blood glucose concentration of obese mouse blood increased 24.6% compared with normal mouse

The blood total cholesterol concentration of obese mouse blood increased 32.2% compared with normal mouse.

Blood HDL of obese mouse blood decreased 30.1% compared with normal mouse.

Blood LDL of obese mouse blood increased especially 57.5% compared with normal mouse.

Therefore, the blood lipid data indicated that the experimentally obese mice suffered from lipid metabolism disorder.

In order to treat the obese mice, we have designed the experimental schema for daily repeated

oral administration (8 days) of *Fortunella japonica* peel extract fractions (800mg fraction concentrates /kg).

The experimental schema was presented in

the following:

Lot 1: Control mice fed with standard diet and drinking water

Lot 2: Obese mice treated with ethanol fraction

Lot 3: Obese mice treated with chloroform fraction

Lot 4: Obese mice treated with ethyl acetate fraction

Lot 5: Obese mice treated with metformin (500 mg/kg)

Lot 6: Obese mice fed normal diet and drink water (no treatment)

The results obtained show that the effect of daily repeated oral administration of kumquat peel extracts clearly decrease of body weight. The body

weight of all the obese mice treated with kumquat peel extracts decreases from 4.7% to 9.2%.

The body weight of Lot 5 that treated with Metformin decreased 9.1% (Drug Metformin has effect on decreasing body weight and blood lipid, FDA).

- Lot 1 and lot 6 that were not treated with kumquat peel extracts increased body weight from 4% to 9.5%.

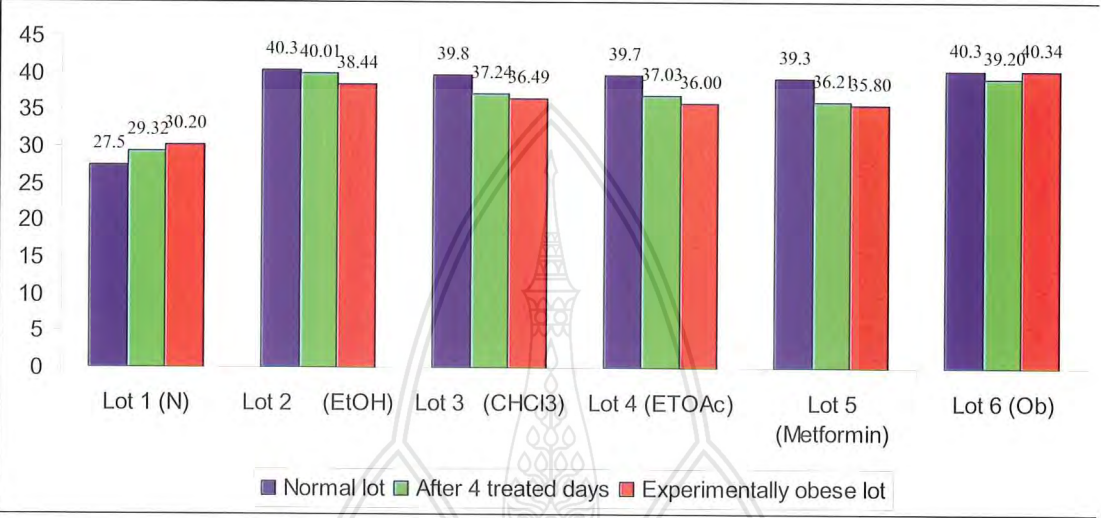


Figure 5. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate /kg) on body weight

In addition, the effect of kumquat peel extracts concentrations was demonstrated clearly (Figures6,7 and 8).

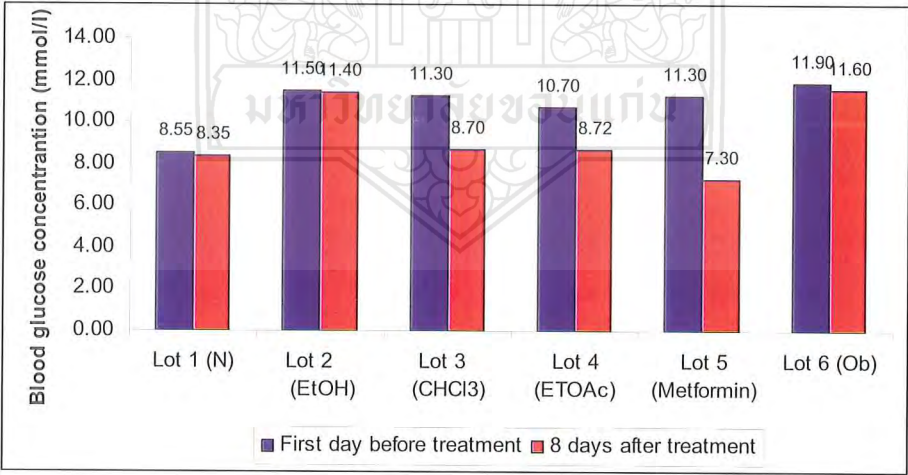


Figure 6. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood glucose concentration

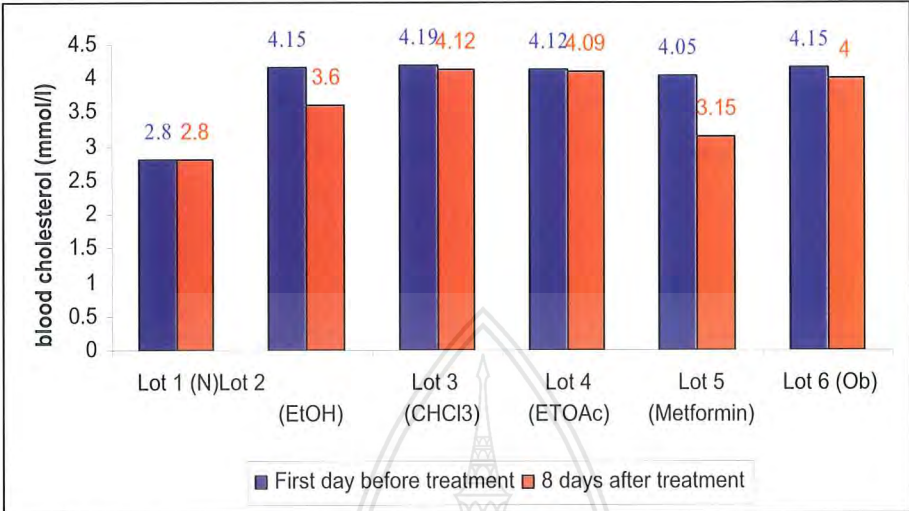


Figure 7. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood cholesterol concentration

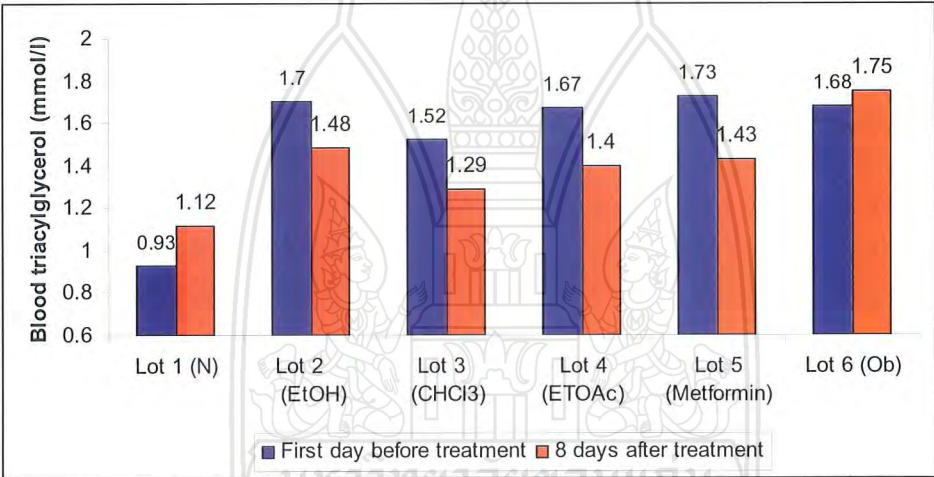


Figure 8. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood triacylglycerole concentration

Especially, the effect of daily repeated oral administration (8 days) of kumquat peel extracts on blood glucose concentration was proved clearly: Lot 3 decreases 23% (Chloroform fraction), Lot 4 decreases 18.5% (ethyl acetate fraction).

Conclusions

Natural products from *Fortunella japonica*

peel extracts essentially consist of polyphenol compounds, especially flavonoid. The model of experimental obese mouse (*Mus musculus* Swiss strain) was established based on high lipid diet for 15 days. The results show that body weight, blood lipid concentration such as total cholesterol, triacylglycerole and LDL of experimentally obese mice increased clearly when compared with control mice. Effect of repeated oral

administration in 8 days of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) decreased of body weight, blood glucose and lipid concentration was proved in experimentally obese mice.

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Survival and ovipositional capacity of Alien Invasive Species Bean Weevil *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in Vietnam

Nguyen Quy Duong¹* and Dang Thi Dung²

Abstract

Bean weevil *Acanthoscelides obtectus* Say (*A. obtectus*) was one alien invasive species in Vietnam before 1995. It expanded to many places in Vietnam by seed production traders, and has become a serious alien species in Vietnam. But the basic studies on this kind of alien invasive species were not enough investigated. *A. obtectus* can lay eggs on all kinds of seeds. However, it can attack varieties of seeds only that belonged to genera *Phaseolus*, *Vigna*, *Pisum* and *Cajanus*. Larvae of *A. obtectus* can survive in most kinds of cultivated bean host (exclusive soybean). The best host seed is white common bean (74.76% larvae survived), following by Chinese Lima bean (70.50%) and red common bean (66.84%). Whereas, wild Leguminosae grown in Vietnam, larvae of *A. obtectus* cannot survive. The density of *A. obtectus* adult has effected on oviposition capacity. When density of adult increases (1, 3, 5 and 7 couples of adults), the numbers of eggs laid by females decrease as followings: 67.25, 63.37, 52.34, and 46.09 (eggs/female), respectively. The similar effect of sex ratio to oviposition capacity of bean weevil, when density of females increases (from 1 to 3 and 5 females), oviposition capacity of bean weevil decreases as followings: 57.86, 40.37, and 41.22 (eggs/female), but there is no significant difference in the numbers of eggs laid by females at density of females between 3 and 5. When *A. obtectus* bred on mung bean seeds with the presence of *Callosobruchus chinensis* adults, it has effected on oviposition capacity of bean weevil (lower numbers of eggs), but there is no significant difference on the numbers of eggs laid by females of bean weevil at density of *C. chinensis* adults of 1, 3 and 5 couples. *A. obtectus* females prefer laying eggs on clean bean seeds and infected seeds.

Keywords : Bean weevil, *Acanthoscelides obtectus*, Insect, Store pests, Fabaceae.

Introduction

Bean weevil *Acanthoscelides obtectus* Say (Bruchidae, Coleoptera) has known as one of storage pests. Before 1975, this kind of pest was in the Vietnam List of quarantine pests in group 1. During 1975-1995,

it moved to quarantine pests group 2 and has become one invasive alien species in Vietnam since 1995. After 1995, this kind of bean weevil has expanded very fast all over the country by trade way. Nowadays, *A. obtectus* is one serious alien invasive species for bean storage.

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According to Vu Quoc Trung (1981), ability of survival, damage and development of *A. obtectus* on bean storage was very high. The basic biological characteristics of *A. obtectus* in Vietnam is not still enough investigated.

This paper presents some biological and ecological characteristics of *A. obtectus* affected by seeds in Vietnam.

Materials and Methods

Effects of 42 kinds of seeds of fabaceae (cultivated and wild seeds) on survival of *A. obtectus* were studied. On each kind of host seed, 5 couples of one day old adults were put in 1 mica box which contained 30 g host seed. Replications on each kind of host seed were 3 times. Infected seeds were observed until the last adults emerged.

Effects of 11 kinds of seeds of Fabaceae (cultivated seeds) on larval survival of *A. obtectus* were also conducted. On each kind of host seed, newly hatched larvae were put in 1 mica box contained 20 g ($n \geq 30$). Replications on each kind of host seeds were 5 times. Infected seeds were observed until the last adults emerged.

Effect of density of adults on oviposition capacity was also observed. One day old adults were released in box containing white common bean seeds which were sterilized at 70°C in 2 hrs. Treatments were arranged with 4 levels of density of adults (1, 3, 5 and 7 couples). Eggs laid on the same day were collected and put in another box. Egg hatch was observed every day. Each treatment was replicated 10 times.

Effect of sex ratio to oviposition capacity of bean weevil was calculated. One day old adults were released in box containing white bean seeds which were sterilized at 70°C in 2 hrs. The treatments were arranged with 3 levels of density of females (1, 3 and 5 females/male). Eggs laid on the same day were collected and put in another box. Egg hatch was observed

every day. Each treatment was replicated 10 times.

Effect of presence of *Callosobruchus chinensis* on oviposition capacity of bean weevil. One day old adults were released in box containing mung bean seeds which were sterilized at 70°C in 2 hrs. The treatments were arranged with 3 levels of density of adults (1, 3 and 5 couples of *Callosobruchus chinensis*/couples of *A. obtectus*). Eggs of *A. obtectus* laid on the same day were collected and put in another box. Egg hatch was observed every day. Each treatment was replicated 3 times.

Reproductive behavior of different stages of bean weevil on seeds was investigated. The treatments were arranged with white common bean that infected by bean weevil at 4 levels as follows: clean bean seed, infected seed by egg, infected seed by larvae and seed with hole of adult emerged. These seeds were laid separately in glass box 24 × 10 (diameter × height). A couple of *A. obtectus* was released in these boxes. Numbers of eggs were counted every day until females died. The treatments were replicated 8 times.

Results and Discussion

Effect of food on survival of *A. obtectus*

The dynamic of insect population was depended on larvae survival, and the larval survival was effected by many ecological factors. Food is a very important role in larval survival as well as its development. The results of food effecting on survival of *A. obtectus* are showed in Tables 1, 2 and Figures 1, 2. Table 1 shows that *A. obtectus* can survive on four genera such as *Phaseolus*, *Vigna*, *Cajanus* and *Pisum*. This result is similar to Hamraoui and Regnault-Roger (1995); Haines (1991); Udayagiri and Wadhi (1992); Lyon (2000). In addition, Hamraoui and Regnault-Roger (1995); Tucic et al. (1997) showed that *A. obtectus* can survive on other genera such as *Vicia*, *Lathyrus* and *Cicer*.

Table 1. Effect of food (cultivated Fabaceae) on survival of *A. obtectus*

No.	Common name	Scientific name	Observed criteria			
			Female laid egg	Egg hatched	Larvae entered in seed	Adult emerged
Order Fabaceae						
Genus <i>Phaseolus</i>						
1	White common bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
2	Red common bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
3	Black common bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
4	Green common bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
5	Chinese bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
6	Yellow common bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
7	Chinese bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
8	Chinese bean	<i>Phaseolus vulgaris</i> L.	+	+	+	+
9	Pink bean 1	<i>Phaseolus vulgaris</i> L.	+	+	+	+
10	Pink bean 2	<i>Phaseolus vulgaris</i> L.	+	+	+	+
11	Chinese Lima bean	<i>Phaseolus lunatus</i> L.	+	+	+	+
12	White Lima bean	<i>Phaseolus lunatus</i> L.	+	+	+	+
13	Hue Lima bean	<i>Phaseolus lunatus</i> L.	+	+	+	+
Genus <i>Vigna</i>						
14	White cowpea 1	<i>Vigna unguiculata</i> var. <i>unguiculata</i>	+	+	+	+
15	Red cowpea	<i>Vigna unguiculata</i> var. <i>unguiculata</i>	+	+	+	+
16	White cowpea 2	<i>Vigna unguiculata</i> var. <i>unguiculata</i>	+	+	+	+
17	Cowpea	<i>Vigna unguiculata</i> var. <i>Sesquipedalis</i>	+	+	+	+
18	Black cowpea	<i>Vigna catjang</i> Walp.	+	+	+	+
19	Mung bean	<i>Vigna radiata</i> (L.)	+	+	+	+

Table1. (continued)

No.	Common name	Scientific name	Observed criteria			
			Female laid egg	Egg hatched	Larvae entered in seed	Adult emerged
Genus <i>Cajanus</i>						
20	Pigeon pea 1	<i>Cajanus cajan</i> L.	+	+	+	+
21	Pigeon pea 2	<i>Cajanus cajan</i> L.	+	+	+	+
Genus <i>Glycine</i>						
22	Soy bean	<i>Glycine max</i> L.	+	+	+	-
Genus <i>Pisum</i>						
23	Garden pea	<i>Pisum sativum</i> L.	+	+	+	+
Genus <i>Lablab</i>						
24	Lablab bean	<i>Lablab purpureus</i>	+	+	+	-
Genus <i>Psophocarpus</i>						
25	Wing bean 1	<i>Psophocarpus tetragonolobus</i>	+	+	+	-
26	Wing bean 2	<i>Psophocarpus tetragonolobus</i>	+	+	+	-
Genus <i>Arachis</i>						
27	Groundnut	<i>Arachis hypogaea</i>	+	+	+	-
Other seeds						
28	Rice	<i>Oryza sativa</i> L.	+	+	-	-
29	Maize	<i>Zea mays</i> L.	+	+	+	-
30	Ornamental gourd	<i>Cucurbita pepo</i> L.	+	+	+	-

Note: + = present; - = absent



Figure 1. Seeds of cultivated plants

Table 2. Effect of food (wild Fabaceae) on survival of *A. obtectus*

No.	Common name	Scientific name	Observed criteria		
			Female laid egg	Larvae entered in seed	Adult emerged
1	Thailand velvet bean	<i>Mucuna</i> sp.	+	+	-
2	Striped velvet bean	<i>Mucuna</i> sp.	+	+	-
3	Wild legume mucuna	<i>Mucuna bracteata</i> A.DC	+	+	-
4	Gotani bean	<i>Canavalia ensiformis</i> (L.)	+	+	-
5	Crotalaria	<i>Crotalaria anagyroides</i> Kunth	+	-	-
6	Coffee senna	<i>Cassia occidentalis</i> L.	+	-	-
7	Sunnhemp	<i>Crotalaria juncea</i> L.	+	-	-
8	Sicklepod	<i>Cassia tora</i>	+	-	-
9	White hoarypea	<i>Tephrosia candida</i> DC	+	-	-
10	Sesbania	<i>Sesbania canabia</i> Pers.	+	-	-
11	Koa haole	<i>Leucaena leucocephala</i> (Lam.) deWit	+	-	-
12	Black mimosa	<i>Mimosa pigra</i> L.	+	-	-

Table 2 shows that *A. obtectus* laid their eggs on 12 kinds of wild Fabaceae seeds. Larvae of *A. obtectus* can born and enter inside of *Mucuna* sp. 1., *Mucuna* sp. 2., *Mucuna bracteata* and *Canavalia*

ensiformis. Anyway, all of them died before emerged to adults. It means that wild Fabaceae are not suitable food for *A. obtectus* to survive. They can develop in cultivated legume only.

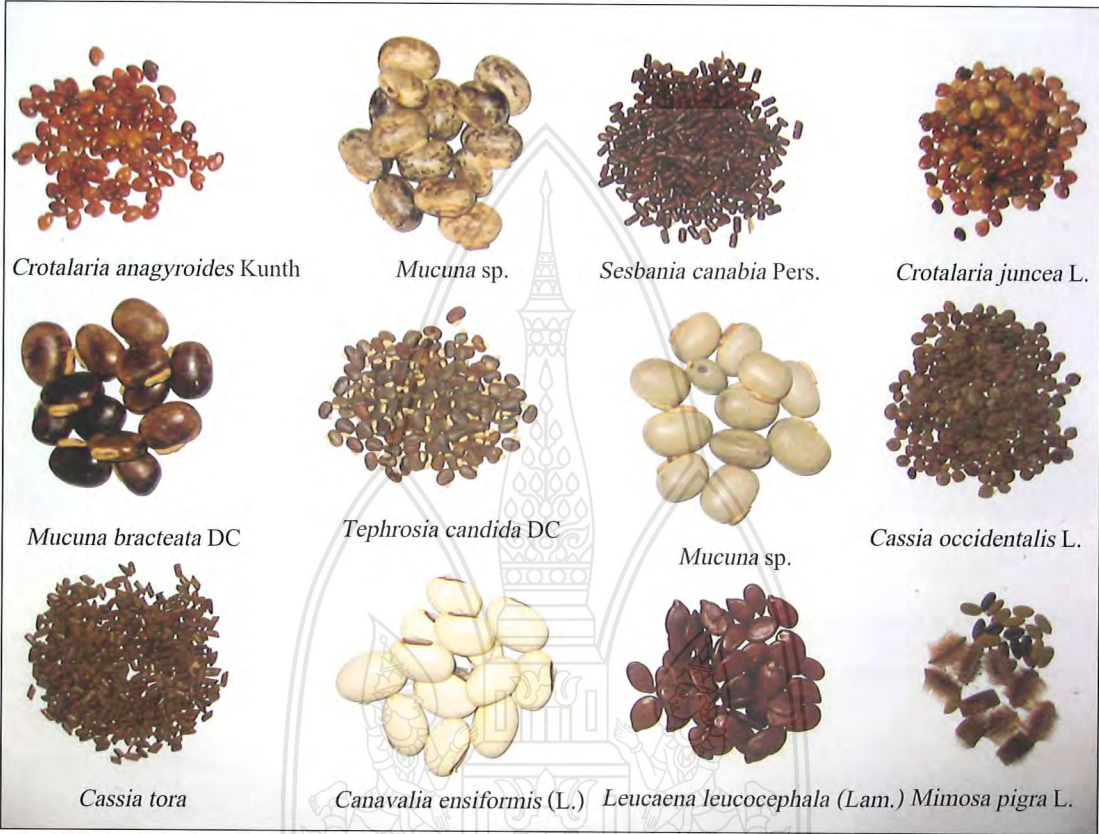


Figure 2. Seeds of wild plants

Effect of food on larval survival of *A. obtectus*

Table 3. Effect of food (cultivated Fabaceae) on larval survival of *A. obtectus*

No.	Name of Host seed	Percentage of survival larvae (%)		
		Min.	Max.	Average
Genus <i>Phaseolus</i>				
1	Red common bean	62.50	75.86	66.84 ab
2	White common bean	72.73	75.86	74.76 a
3	Pink common bean	42.11	88.89	65.54 ab
4	Hue Lima bean	44.44	72.00	59.55 b
5	Chinese Lima bean	55.00	86.11	70.50 ab
6	White Lima bean	42.86	59.57	48.94 c

Table 3. (continued)

No.	Name of Host seed	Percentage of survival larvae (%)		
		Min.	Max.	Average
Genus <i>Vigna</i>				
7	Black cowpea	31.25	68.00	54.88 bc
8	Red cowpea	30	71.43	53.77 bc
9	White cowpea 1	36.37	70.83	49.65 c
10	White cowpea 2	34.21	63.64	48.49 c
11	Mung been	40	66.67	51.24 c
Genus <i>Glycine</i>				
12	Soy bean	0	0	0

Note: Average temperature and relative humidity: 30.0 ± 0.5°C and 70.8 ± 2.2% RH.

With in column, means following same letter are not significant difference at α=0.05 (by DMRT)

Table 3 and Figure 3 show that larvae of *A. obtectus* can consume and survive on most of cultivated bean tested (11/12 kinds). The most suitable food is white common bean (74.76% larvae survived) following by Chinese Lima bean (70.5%) and Red common bean (66.84%), respectively. The left beans

are less suitable for the survival of bean weevil larvae except soybean. It means that *A. obtectus* has a wide range of cultivated bean host, so it can survive and develop on most kinds of cultivated beans in Vietnam. Thus, its capacity to destroy bean storage is very high.

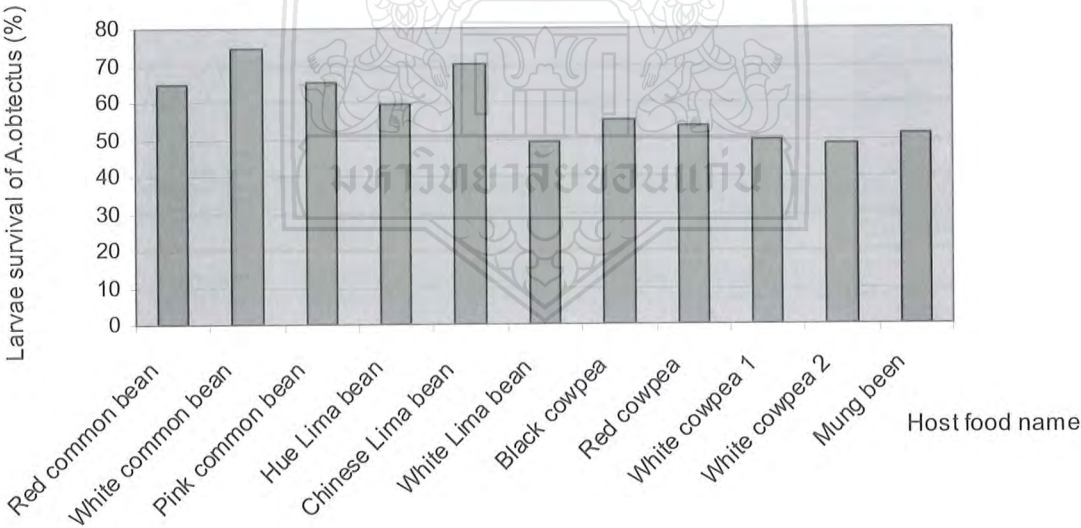


Figure 3. Effect of food to larval survival of *A. obtectus*

Effect of density of *A. obtectus* adult to oviposition capacity on white common bean

Table 4 shows that the density of *A. obtectus* adults has oviposition capacity on white common bean. When the numbers of adults increased from 1, 3, 5 and 7 couples, the numbers of eggs laid by females decreased as following: 67.25, 63.37, 52.34 and 46.09

(eggs/female), respectively, but there was no significant difference in the numbers of eggs laid by females at density of adults between 1 and 3, as well as 5 and 7 couples. Similarly, the numbers of eggs laid by females per day decreased when the numbers of adults increased. On the other hand, laying time was increased when adults increased in density.

Table 4. Effect of density of *A. obtectus* adult to oviposition capacity on white common bean

Treatment	Observed criteria		
	Laying time (Days)	Numbers of eggs/female	Numbers of eggs/female/day
1 Couple	6.25 b	67.25 a	10.79 a
3 Couples	7.11 b	63.37 a	8.94 b
5 Couples	8.70 a	52.34 b	6.18 c
7 Couples	8.30 a	46.09 b	5.66 c

Note: Average temperature and relative humidity: 30.0 ± 0.5°C and 70.8 ± 2.2% RH.
With in column, means following same letter are not significant difference at $\alpha=0.05$ (by DMRT)

Effect of density of *A. obtectus* female to oviposition capacity on white common bean

Density of *A. obtectus* female has oviposition capacity. In general, when the density of female increased (from 1 to 3 and 5 females), numbers of eggs laid by females decreased as followings: 57.86, 40.37,

41.22, respectively. However, there is no significant difference in the numbers of eggs laid by females between 3 and 5 females. Tables 4 and 5 show that density of *A. obtectus* adults as well as females have similarly oviposition capacity.

Table 5. Effect of density of *A. obtectus* females to oviposition capacity on white common bean

Treatment	Observed criteria		
	Laying time (Days)	Number of eggs/female	Number of eggs/female/day
1 male/1female	5.00 b	57.86 a	11.94 a
1 male/3 females	6.00 b	40.37 b	7.01 b
1 male/5females	6.80 a	41.22 b	6.09 b

Note: Average temperature and relative humidity: 30.0 ± 1.5°C and 78.4 ± 5.5% RH.
With in column, means following same letter are not significant difference at $\alpha=0.05$ (by DMRT)

Effect of presence of *Callosobruchus chinensis* to oviposition capacity of *A. obtectus*

When *A. obtectus* bred on mung bean seed with the presence of *Callosobruchus chinensis* adults, it has effected on oviposition capacity of bean weevil (lower numbers of eggs), but there is no significant difference on the numbers of eggs laid by females of bean weevil at

density of *C. chinensis* adults of 1, 3 and 5 couples. The presence of *C. chinensis* adults has also effected on the numbers of eggs laid by females of *A. obtectus* per day. When *C. chinensis* adults presented, the numbers of eggs laid by females of *A. obtectus* per day were decreased. However, there is no significant difference in laying time of *A. obtectus* among all of the treatments (Table 6).

Table 6. Effect of presence of *C. chinensis* to oviposition capacity of *A. obtectus*

Treatment	Observed criteria		
	Laying time (Days)	Numbers of eggs/female	Numbers of eggs/female/day
1 <i>A. obtectus</i> : 1 <i>C. chinensis</i>	6.00 a	34.00 b	5.38 b
1 <i>A. obtectus</i> : 3 <i>C. chinensis</i>	5.67 a	32.00 b	5.12 b
1 <i>A. obtectus</i> : 5 <i>C. chinensis</i>	6.00 a	29.00 b	4.81 b
1 <i>A. obtectus</i>	6.00 a	42.67 a	7.19 a

Note: Average temperature and relative humidity: 29.5. ± 0.8°C and 72.3 ± 5% RH.
With in column, means following same letter, are not significant difference at $\alpha=0.05$ (by DMRT);
Food is mung bean seed Number 1,3,5: one, three and five couple of adults

Reproductive behavior of different stages of *A. obtectus* on seeds

Table 7 shows that *A. obtectus* females preferred laying eggs on clean bean seed and infected seeds by egg stage than other host seeds. Numbers of

eggs laid by females on the clean bean seeds and the infected seeds by egg stage are similar (25.0, 26.88 eggs/female), and are more than other treatments (5.13, 0.13 eggs/female). This result was similar to Parsons and Credland (2003).

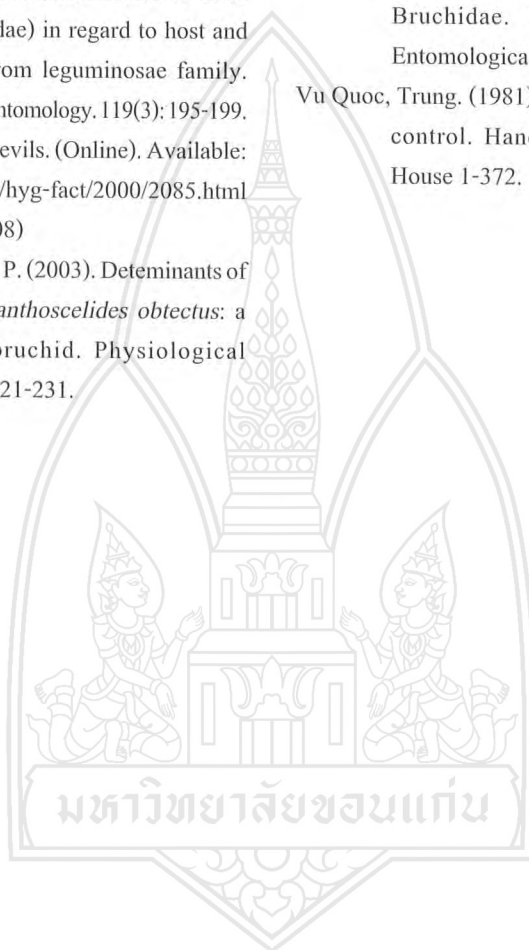
Table 7. Reproductive behavior of different stages of *A. obtectus* on seeds

Treatment	Observed criteria		
	Laying time (Days)	Numbers of eggs/female	Numbers of eggs/female/day
Clean bean seed	4.28 a	25.00 a	5.27 a
Seed infected by egg	4.37 a	26.88 a	5.59 a
Seed infected by larvae	1.88 b	5.13 b	1.96 b
Seed with hole of adult emerged	0.13 b	0.13 b	0.13 b

Note: Average temperature and relative humidity: 26.7 ± 0.5°C and 75.2± 2.3% RH.
With in column, means following same letter, are not significant difference at $\alpha=0.05$ (by DMRT)

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Synthesis Assessing Changes in an Agricultural System at Tat Hamlet in Vietnams' Northern Mountain Region

Pham Tien Dung¹, Tran Duc Vien² and Nguyen Thanh Lam²,

Abstract

This study was carried out to assess how agricultural system changes over 5-year period and to evaluate how the system was developed in tat hamlet, Da Bac district, Hoa Binh province, north Vietnam. The survey was initiated in 1999 and 2004 by using the same standard questionnaire for interview, semistructured interview, group discussions, and direct observation. Radar net and simple statistic methods were used for analysis, which were based on individual indicators and indicator groups such as (1). Human capital; (2). Household assets; (3). Production activities; (4). Economic activities; and (5). Environment. indicator group (6). The results revealed that almost measurements of indicators and groups of indicators show positive development trends following the time from 1999 to 2004. The system in 2004 was more sustainable as the area of radar graph was larger than that in 1999. The changes of indicator groups presented on a radar graph that the graph area of 2004 is larger than that of 1999. This change in 2004 was 2.06 times larger to compare with that in 1999. Thus, in terms of sustainability assessment, the Tat agricultural system was considered more stable in 2004 comparing with itself in 1999 even though population pressure increased to 65 persons km⁻² in 2004.

Keywords : Changes in agricultural system, Indicator group, Synthesis assessment, Vietnam uplands

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Introduction

There is a worldwide trend towards development of sustainable agricultural systems. There is no exception for Vietnam, but rapid population growth in the Northern mountainous region of the country has high pressure on agrosystem resources. In the period from 1990 to 2000, the average population density of this region increased to over 20% from 52 to 64 persons km^{-2} . Hoa Binh province, which is a part of this region, reached a population density of 165 people km^{-2} in 2002 (Chu Huu Quy, 2002). Because of this increase in population, the demand for food has increased as well. In order to meet nutritional and other basic needs, such as fuel wood and building material, farmers of this region exploit the forest. As a result, forest quantity and quality have been reduced drastically. In just 30 years (1963-1993), the percentage of total land area covered by forests in this region has declined dramatically from 52% to 21% (Dao The Tuan, 1995). The common practice of shifting agriculture or swidden not only reduces vegetative cover, but also degrades the land. The validity of the previous statement can be described as common wisdom exemplified by the following quote from Rambo (1998): "Deforestation is listed as the leading cause of land degradation, followed by agriculture, over-grazing, and fuel wood collection." Nevertheless, while this common wisdom is true on a world wide scale, it may not be true for small-scale agroecosystems. Obviously, population density is an important factor that determines the sustainability of this practice. Le Trong Cuc (2002) argued that the population density should not exceed 5 - 10 persons km^{-2} for the effective regeneration and conservation of forest resources.

This paper focuses on the Tat hamlet of the Tay minority, Da Bac district in the mountainous northwestern region of Vietnam. The agricultural system in this hamlet can be described as a composite swiddening agroecosystem defined by Rambo (1998) as "a unique type of agroecosystem that integrates permanent wet rice fields and rotating swidden plots into a single household resource system." The author

also noted that this system has persisted for generations, perhaps even centuries, in the mountain and valleys of the highlands of northwestern Vietnam, and thus may be considered stable. Yet in light of recent assessment of the limitations of such systems (Le Trong Cuc, 2002) and the high average population density (65 persons km^{-2}), it is worthwhile to look at the specific situation in this hamlet, investigate how it is changing.

The objectives of this research were to assess the changes in the present system over a five-year period and to evaluate whenever the system was developed under high population pressure.

Conceptual Framework

This study is based on the agricultural systems and agroecosystem approaches. The minimum requirements for a proper study of agricultural systems must include a definition of the purpose, system boundaries, context, system components, interactions, resources, inputs, products or desired output, and by-products (Spedding, 1975). System boundaries decrease in the following order: Global $\rightarrow$ National $\rightarrow$ Regional $\rightarrow$ Community $\rightarrow$ Farm $\rightarrow$ Field (Cocklin et al., 1997).

In this study at Tat hamlet, the agricultural system has the community boundary. The farm household systems that are subjects of the whole system often make the decisions of all the activities in the system to create other components, and interactions among components. Furthermore, they decide inputs and outputs (what and how), and how these control the activities of the system. Therefore, to assess the household component in order to evaluate the sustainability of an agricultural system at the community level, Stevens (2003) suggested that any attempt to measure sustainability requires a set of quantifiable indicator criteria which satisfactorily represent the state of household quality of life at the present time and the desired state of quality of life at various future times.

This study includes two main of agricultural production systems: Crop production and livestock production. They are highly important for agricultural

system products and, hence, need to be evaluated. The third sub-system is the agro ecosystem. It indicates which kinds of natural resources are used to produce the desired output in the agricultural system, but it is also affected by production activities of the system in the output producing process. However, the state of the agro ecosystem needs to be monitored and evaluated.

The economic system often presents the activity resulting from the production systems and other activities of the agricultural system through monitoring and evaluating the input-output of the systems. In order to know whether an agricultural system is satisfying the increasing living standard requirements of their subjects, it should be based on the characteristics of the economic systems.

The last sub-system is the social system. It could be said that the social system can integrate and represent all aspects of the agricultural system. For example, when farmers' income increases and they have enough food for their living, it is often the case that relationships among people in the community become better; they have fewer social problems and more friendships among the community. However, in some cases there is a relatively strong positive association between the level of development and the incidence of social problems (Rambo et al., 2001). Briefly, in order to assess an agricultural system one should evaluate some important sub-systems (components) as mentioned above, and one also needs to find indicators that represent these sub-systems. Then, we can measure indicators for comparing them by time or between systems.

Research Methods

General method

In order to evaluate the sustainability of the system, this study was based on time trend so that the indicator measurements can be compared from time to time. We have selected two points of time, 1999 and 2004, with a time gap of five years. Limitation of research finances in our case was also a constraint. Despite

this, in 1999 we had conducted an integrated survey on development trends of economic, social, and human ecology development in the study area. This primary data base was selected for comparison with the data collected during the year 2004. In the year 2004, the survey was again conducted for 42 households, which were selected randomly. The same questionnaires were used for both the surveys. The survey was conducted not only through questionnaires but also through semi-informal interviews with key informants.

Selection of research indicators

System indicators were selected based on the guide of Department of Environment and Rural Affairs, London (2004), using USDA concept of sustainable agriculture, indicators which represent development trend of economic, social, and human ecology and the concrete research conditions of Tat agricultural system. These selected indicators represent characteristics of the system components. Furthermore, they should be available and sensitive to temporal change, and capable of being quantified. Indicator groups were selected as follows:

(1) Human capital group indicators include a structure of community population, literacy rate, and number of school attendance by adults.

This group represents a quality of a farmer's life. Farmers are subjects of the system. Good human capital in an agricultural system will make it easier for the system to develop and provide access to sustainability. In an undeveloped area these three indicators also measure its development.

(2). Household assets group indicators include value of houses, ownership of valuable possessions, and land holding.

This indicator group is selected to evaluate sustainable communities in terms of housing and assets.

(3). Production activities group indicators include the number of livestock, crop area, crop diversity index, and land productivity.

These indicators denote system capacity to

make the most efficient use of nonrenewable resources and on-farm resources.

(4). Economic activities group indicators include: cash income, income diversity index, cash expenditure, balance of cash income and cash expenditures, total income, equality in total income, and food security.

This indicator group is supposed to ascertain whether or not the system can satisfy human food and fiber needs, and sustain the economic viability of farm operations.

(5). Environmental indicator group includes two indicators: land cover, and farmer's perception of the changing environment.

These indicators represent the sustainable agricultural system with the aspect of enhancing environmental quality and the natural resource base upon which the agricultural economy depends.

(6). Cultural and social indicator group includes three indicators: social problems, perception of the quality of intra-community social relations, and change in farmer's life. These indicators present characteristics of developed agricultural system in terms of enhancing the quality of life for farmers and society as a whole.

The same method was used to measure indicators (indicators in money regulated in one time period, 1999) in two time periods of the study. We then compared every group at two time periods by radar graphs to evaluate the development (growth) of each indicator group through the area of graphs. If the graph area of the last period of time is larger or equal to that of the previous one, it is considered as more development. Beside we also change indicators measurements into numeric form without unit for comparing them between two time periods.

After evaluating the changes in indicator

groups, we quantified the changes of each group of indicators to establish a new radar graph for integrated assessment of the development trend of the whole system.

Research Results and Discussion

Assessing the Human Capital

In order to assess human capital, the subject of the system, three indicators that represent quality of human resources were measured. Research results (Figure 1, 2) reveal that there are differences of age structure of community populations between two time periods. The proportion of adults (above 40) has visibly increased in 2004 compared to 1999. At the age level of 60 or above, we have similar realization as in age level of 40-59. In contrast, with the age level of 0 - 9 the proportion is higher in 1999 compared to that in 2004. When focusing at the age level from 20 to 59 (main working age) it has increased from 45.8 percent in 1999 to 48.3 percent in 2004. This proves that the quality of populations is weaker in 1999 than that in 2004. The changing population age structure has led to the changes in the dependency ratios of these two periods. In 2004, the dependency ratio was lower than that of 1999, which is a good signal for more sustainable development in the area. This may be attributed to the fact that the farmers here did not like giving birth to more children, which has led to a decreased birth-rate. Besides that, the farmer paid more attention to their life by increasing quality of meals. Furthermore, with the help of army doctors from a just established clinical station in the village must have protected farmer's health better.

The quality of population, which is developing with a better trend, proves inner potential of the system becoming stronger and represents the integrative development of the whole system.

Another indicator concerns about the literacy of the people in the system. This is one of the Vietnamese

government’s targets for sustainable development of the mountainous regions in order to come out of hunger,

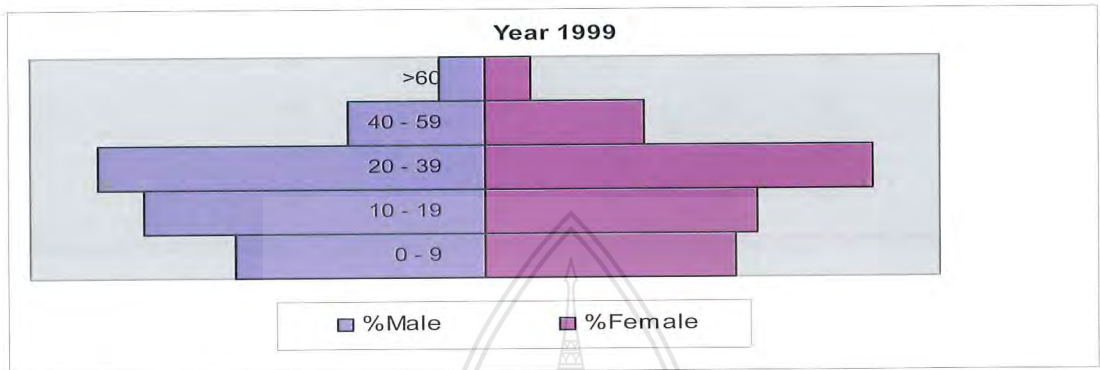


Figure 1. Population Pyramid in year 1999

decrease poor people ratio living in remote areas of the country. The number of people that can read and write common Vietnamese language was presented in Table 1. In the age of 7 to 18, the literacy rate of females is always higher than males for both two time periods. However, at the ages of 19 to 35 and above 35, the literacy rate of males is higher than females, which may be due to the fact that females got married and had to spend time to take care of their family and their children more than their husbands. Females then do not concern themselves with reading or writing any

more and as time passes they almost forget how to read and write altogether. While, the husbands still have time to watch film, read newspapers, and some time to attend meetings. In the meetings, they often use the common language, so their literacy capability is higher (according to discussion with some key persons in the hamlet).

Looking at the two time periods for comparing this indicator, we can see that almost all age levels literacy rates in year 2004 are higher than that of 1999. This difference of literacy rate of the people in

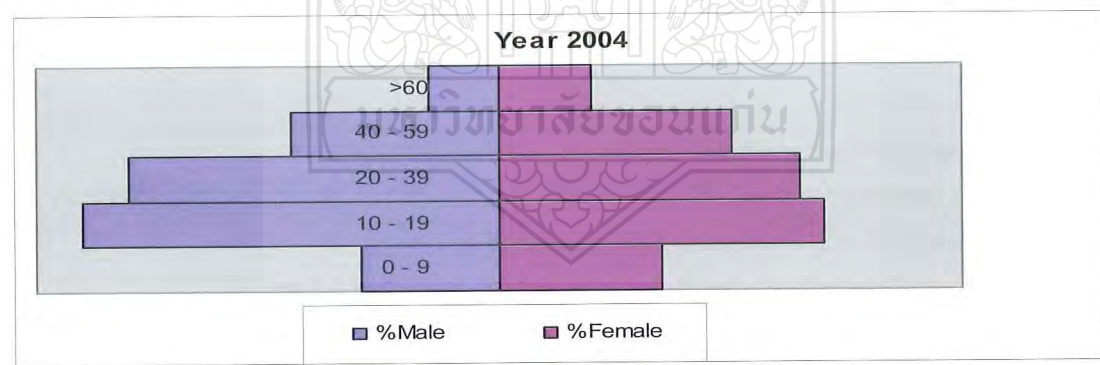


Figure 2. Population Pyramid in year 2004

Tat between the two time periods is not much. The above mentioned realizations prove that there is slight development trend of literacy indicator over the period of time, from 1999 to 2004

Conclusions and Suggestions

Conclusions

In order to assess (or to compare) the changes of characteristics of the system between two time

Table 1. Literacy Rates by Age and Sex (percentage)

Age levels	Year 1999			Year 2004			Compared(*)
	Male	Female	Total	Male	Female	Total	
7-18	95	100	97	90	100	95	-
19-35	100	85	93	100	95	98	+
>35	91	75	82	89	84	87	+
Total	96	87	92	93	93	93	+

(*)Marks of(-)or(+)represent the trends of the negative or positive changes in total column beetwen two years

The third indicator is the number of school year attendance by adults. This indicator reflects understanding of the people in the study site, which presents the quality of human capital. This related to subjects' decision making of the system in their production activities. Normally, the subjects of the system have good understanding and they have good decision making for their production, which helps them to get higher labor productivity and higher economical efficiency. This contributes to making

the system reaching sustainability.
The results displayed on Table 2, reveal that in 2004 there was a noticeable improvement in adult school attendance. If we compare adult school attendance at these two time periods, it can be proved that attendance clearly increased in 2004. The difference is statistically significant at $p < 0.05$. This improvement may be due to a recently constructed middle school in the village, and also the farmer's awareness.

Table 2. Number of Year of School Attendance by Adults (Percentage of adults in each category)

Number of year of school attendance	Year		Compared (P)
	1999	2004	
0	9.7	7.5	-
1 – 5	60.2	45.8	-
6 – 9	23.9	30.8	+
10 – 12	6.2	15.8	+
Average number of year of school attendance	4.7	5.8	p=0.022*

* P value got during to compare two data lists by Z test

Thus, this indicator also represents the development itself. This is signal for better development of the Tat hamlet agricultural system at 2004 to 1999.
In order to integratively compare the indicator groups of human capital between two time periods, three typical indicators were selected for quantitation and making a radar graph, as follows:

(1) Proportion of persons from 20-59 year old, which represents population quality; (2) Average literacy rate of population; (3) Average number of adult school attendance years, which represents the knowledge of the people. All of them represent the quality of human capital or the quality of life of farmers (Table 2-a) and (Figure 3).

Table 2-a. Comparison of Indicators which Represents Population Quality at Two time Periods

Items	Years		Compared 2004/1999 (times)
	1999	2004	
Proportion of Persons from 20-59 years old (%)	45.8	48.3	1.05
Literacy Ratio of Adults (%)	87.5	92.5	1.06
Average Number of School Attendance of Adults	4.7	5.8	1.23
Average (times)			1.11

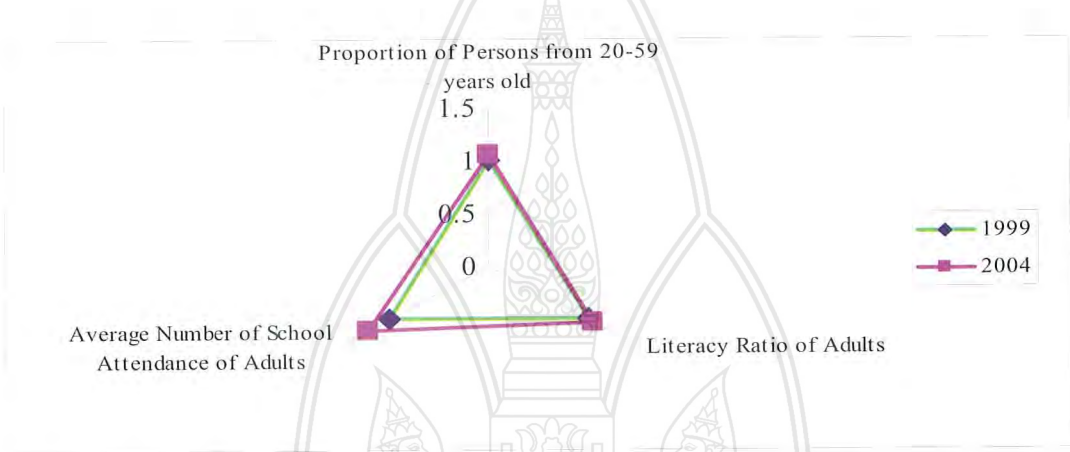


Figure 3. Integrated Evaluation of the changes in the system on human capital aspect

From table 2-a and Figure 3 we see that the graph area for year 2004 is larger than the area of the year 1999. The change average increases of 1.11 times from 1999 to 2004. This proves that indicators of human sources of the system to be more development by the time period.

Households Assets Aspect

In practice, farmers in the North and in the Northwestern mountainous regions of Vietnam use money savings to repair or build their houses, buy their possessions and production instruments, and even to buy land. When their buying increases, we can say that their life is improving.

This portion of the study looks at housing, possessions, and land holding of the farmers in the study area through two time periods to see how they have changed. For this purpose, four indicators have been used to describe characteristics of household assets. The calculated results for house value, which were assessed from farmer’s estimations during interviews, are presented in Table 3. Looking at the highest and lowest values of houses we realize that they all have the trends of higher value in 2004 than in 1999. In evaluating the average value of houses, it has a lower value in 2004 than in 1999, but the difference is not statistically significant (p=0.50). Therefore, if looking at general situation of house values, we conclude that it remains the same situation as before.

Table 3. House Values of Households (thous. VND)

Categories	Year		Compared
	1999	2004	(P)
Highest value	40,000	62,892	+
Lowest value	100	279	+
Average value	8,245	7,793	p=0.50

Regarding household assets, data on Table 4 shows that only after five years there were enormous changes on household's valuable possessions in Tat hamlet. Household rates include motorbikes/bicycles increasing from 17 percent in 1999 to 74 percent in 2004 with a statistically significant difference at $p < 0.05$. The same realization is for valuable tools and electrical appliances. This means that family's possessions, like

valuable tools and electrical appliances, increased clearly from 1999 to 2004. The large change on household's valuable possessions can be explained by increase in the farmer's income from additional sources such as increased livestock raising, better exploitation of non-timber forest products, even wood, and subsidy from the government for afforestation (it will be revealed more exactly in the following sections).

Table 4. Ownership of Valuable Possessions by Households (percentages of households owning items)

Items	Year		Compared
	1999	2004	
Motorbikes/bicycles	17.0	74.4	$p < 0.05$
Valuable tools (water pumps, carts, rice husking, etc)	0.0	17.9	$p < 0.05$
Electrical appliances (TV, radio, VCR, fan, etc)	67.0	79.5	$p < 0.05$
Large furniture	86.0	97.4	+
Wet rice field (Mean per Capita, m ²)	370	357	-
Gardens (Mean per Capita, m ²)	211	249	+

One of the possessions that is especially valuable and cannot be insufficient for farmer's production is land. In Tat hamlet, composite swiddening agro- ecosystem is mainly practiced. Although farmers have some types of land holding, only two types of land that have close relation to the system sustainability, such as wet rice field and garden areas. In general intensive farming on these land types to produce big product amounts, in practice, does not affect much of the environment.

It can also be seen from Table 4 that the

average land area per person in these categories did not change much. Eventhough the village population increased by 44 persons, villagers were able to extend their wet rice field and garden areas. Generally, it can be concluded that the ownership of valuable possessions by households has been increasing with time.

Briefly, in order to evaluate the changes of this indicator group between two time periods, Integrated comparison of indicators performed on a table 4-a and a radar graph was used and displayed as Figure 4.

Table 4-a. Integrated Comparison of Indicators representing Households Assets Aspect
(percentages of households owning items)

Items	Year		Compared 2004/1999 (time)
	1999	2004	
Average Value of House (Thous.VND)	8,245	7,793	0.95
Motobikes/Bicycles	17.0	74.4	4.38
Valuable tools (water pumps, carts, rice husking, etc,...)	0.0	17.9	17.90
Electrical Appliances (TV, Radio, VCR, Fan, etc,...)	67.0	79.5	1.19
Large Furniture	86.0	97.4	1.13
Wet Rice Field (Mean per Capita, m ²)	370	357	0.96
Garden (Mean per Capita, m ²)	211	249	1.18
Average (time)			3.96

In this graph we realized that the graph area was limited by lines connecting to marks of indicators of year 2004, which is larger than that of in 1999.

The graph area on this figure for the two time periods estimated by 3.96 times larger of year 2004 to 1999 (table 4-a)

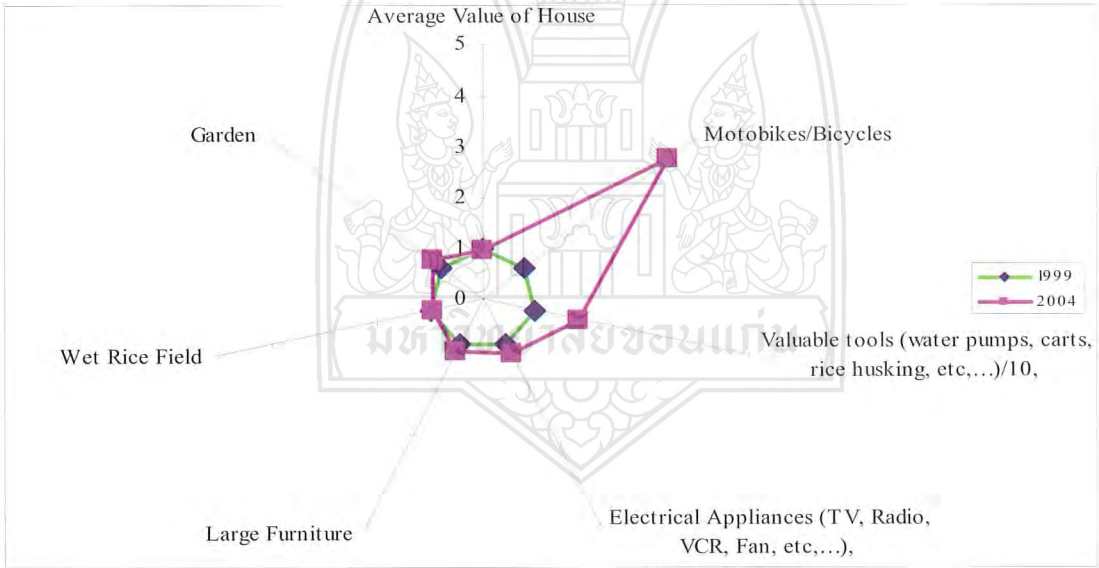


Figure 4. Comparison of Household Assets changes in two periods

Production Activities of Households

There are two sub-systems that produce the main agricultural products of this composite swiddening cultivation system: crop production system and

livestock production system.

Crop Production

The first sub-system mentioned is crop production with two proposed indicators.

Table 5. Different Crops Area of the Hamlet in 1999 and 2004

Crop	Mean Area per Household, in 1999 (m ²)	Mean Area per Household, in 2004 (m ²)	Compared (P)
Wet Rice	1,889	1,659	0.159
Swidden Rice	4,228	412	1.76E-05
Cassava	1,910	2,856	0.089
Swidden Maize	754	1,312	0.154
Swidden Cana	900	51	0.001
Swidden Ginger	271	53	0.078
Home Garden	111	531	0.114
Tree Garden	571	564	0.520
Tea garden	377	20	0.013
Others	4	89	0.334
Vegetable Garden	0	42	+
Peanut	0	25	+
Potato	0	55	+
Total	11,009	7,669	0.35
Crop Diversity Index (H)(*)	0.76	0.75	

(*) Calculating formula is described in Appendix (1).

The first indicator is Crop Diversity Index (H), which is calculated based on the area of different crops in two time periods. In both time periods, there are nine categories of different crops with change in their area in which the cropped area under cassava, swidden maize, and home garden increased. However, it is not statistically significant, as the P value is greater than 0.05, where as area under other crops has been reduced. Only areas of swidden cana and tea garden were reduced differently by the time with statistically significant at $p < 0.05$. Three crops just appeared in 2004, such as vegetable, peanut, and potato due to the advices of CARES' staffs but their area is still small (each household has an average of 25 – 55 square

meters). Comparing the total cropped area between the two time periods, the number in 2004 is smaller than the number in 1999 (7669 and 11009 square meters respectively), which is also not statistically significant. Further, the crop diversity index in two time periods is approximately similar (Table 5).

The second indicator considered is the value produced from a piece of land. This is a very important indicator as it represents land production capability. Research results were presented in Table 6 and Figure 5. Swidden land productivity was reduced from 3,310 thousand VND per ha to 1,367 thousand VND, from 1999 to 2004, respectively. This is a degradation signal of swidden land. The main reason of degraded swidden

land is erosion during the swidden cultivation process. For example, in Tat hamlet, the amount of soil loss was 87 tons/ha in the third year of rice swidden field (Tran Duc Vien, 1998). In contrast, the productivity of wet rice land, cultivated land, and natural land are all statistically significant increasing in 2004 compared to that in 1999. This is because farmers in the studied area have protected their forest better in recent years under the effect of forest land allocation policy of Vietnam

Government; and, some new and improved techniques, such as new application method of fertilizer to wet rice, and improved wet rice varieties that have been applied into crop production of Tat farmers. For example, in 2003 new rice varieties used at Tat were Nhi uu 838, Q5, Khang dan. Chemical fertilizer used in 2004 was much more than in 1999: Nitrogen dose applied increased from 40 to 68 kg N, Potassium increased from 0 to 33 kg K₂O per ha (Nguyen Van Dung et al., 2003).

Table 6. Value produced^(*) per hectare of land (Thous.VND per ha, year)

Land Type	Year		Compared
	1999	2004	(P)
Swidden land	3,310	1,367	7.21E-08
Wet rice Land	9,435	12,246	0.004
Cultivation Land	4,108	4,176	0.014
Natural Land	1,280	2,616	4.9E-08

(*). Calculating methods are described in appendix (2.)

In short, the crop diversity index is considered not to change, land productivity with the trend was higher than before, except swidden land

productivity; therefore, we can say that this indicator group has the signal of more development in 2004 to 1999.

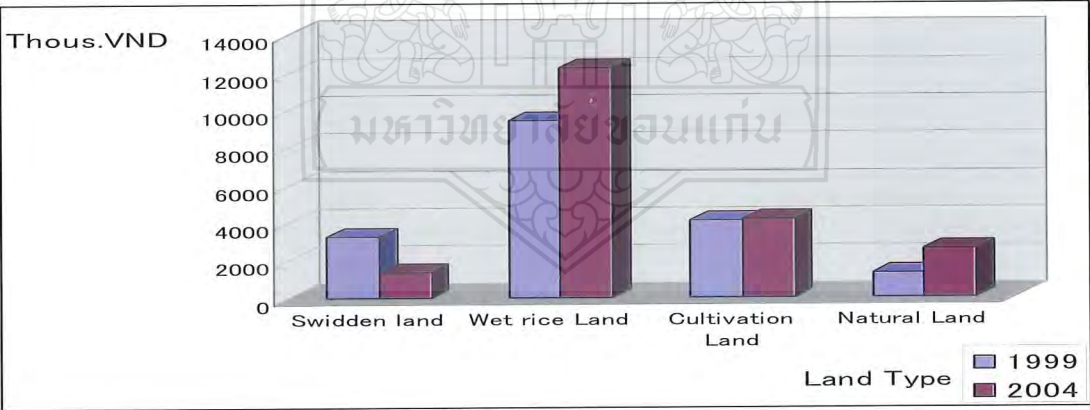


Figure 5. Value produced per hectare of land

Livestock Production

Three categories (indicators) of livestock production were proposed for analyzing and evaluating their changes. The results were presented in Table 7. The first indicator considered here is the large livestock raising situation. In 2004, both the number of households owning large livestock and the average number of large livestock head per household are bigger than those in 1999 (87% compared to 74% and 4.7 compared to 2.2 head on both periods, respectively). This difference is statistically significant. The trend of large livestock production development in the area is strong because the farmers nowadays understand well that raising large livestock is a way to accumulate wealth and it

also brings them high economical efficiency; so, they are very active in finding ways to raise large livestock. The second indicator is the small livestock feeding situation. This indicator is in the state opposite the first one, but this difference is not statistically significant. So, it may not be considered a change.

The last indicator mentioned here is poultry production. In this case, it follows the same trend as the first one. The increase is also statistically significant, and the average number of poultry per household increased from 13.2 in 1999 to 43.8 heads in 2004. Generally, all of indicators in livestock production represent either development, or no change after a time period of 5 years.

Table 7. Ownership of Livestock

Type of Livestock	Year		Compared	
	1999	2004	(P)	
Large Livestock	% of Household	74.0	87.2	+
	Average(head)	2.2	4.7	(0.0007)
Small Livestock	% of Household	71.0	64.1	-
	Average (head)	3.2	2.9	(0.6995)
Poultry	% of Household	88.0	92.3	+
	Average (head)	13.2	43.8	(2.25E-5)

In short, when comparing activities of production sub-systems, all the proposed indicators reveal the trend of development over time. Remarkably, some new crops such as potato, soybean, vegetables, and the development of large livestock or poultry are found in the studied site. The above achievements in crop and livestock production activities are with the help

of CARES’s staff in extension works and the economical reform movements of the Vietnam Government.

In order to better realize development trend of the system by these indicators group, table 7-a for integrated comparison of indicators at two time periods established and a radar graph with five main indicators was drawn on Figure 6.

Table 7-a. Comparison of Production Activities Changes of Households

Items	Year		Compared 2004/1999 (time)
	1999	2004	
Head of Large Livestock (Head per HHs)	2.2	4.7	2.14
Small Livestock (Head per HHs)	3.2	2.9	0.91
Poultry (Head per HHs)	13.2	43.8	3.32
Crop Diversity Index (H)	0.76	0.75	0.99
Values Produced on a Ha of Natural Land (Thous.VND)	1,280	2,616	2.04
Average (time)			1.92

It clearly reveals that the area of graph in 2004 is larger than that of 1999. The area of the graph is quantified on table 7-a, it is 1.92 times bigger in 2004 to 1999. It can be concluded that the production systems here to be more development in 2004 to 1999.

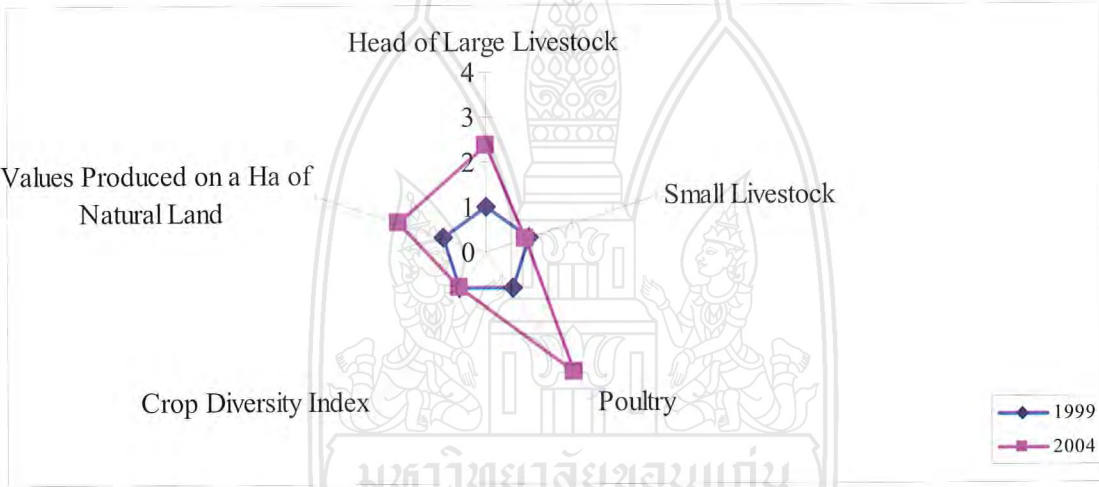


Figure 6. Synthesis Comparison of the changes of Production Activities

Comparing of Economic Activities

Economic activities are the scale of input and output of the system. It is also an integrated representation of the system activity results. Survey results were calculated and presented in Table 8. From the table we can observe cash income of farmers in all sectors in the village increased in 2004.

The highest increase is government salaries, pensions, and other assistance. These increases are almost 5 times higher than that of 1999. This is because in year 2004, the Vietnamese Government had an investment project for farmers to grow bamboo on barren hills at this site for which farmers received a good amount of money.

Table 8. Kinds and Average of Cash Income of Households (Thous.VND per HHs)

Sources	Year		Compared
	1999	2004	(P)
Crop products	754	437	-
Livestock Products	703	2,054	+
Forest products	1,012	2,131	+
Wage Labor	56	181	+
Handicrafts	17	0	-
Services	325	729	+
Government	324	1,529	+
Total	3,191	7,060	1.5E-06
Income Diversity Index ^(*)	0.69	0.67	

(*) Calculating formula is described in Appendix (1.)

The second source of cash income comes from the increase in livestock products. As mentioned above, farmers in this area understood that raising large livestock has high economic efficiency; hence, they used every opportunity for raising cattle. It is almost three folds bigger in 2004 compared with in 1999. All cash income increasing from every source has performed a total cash income increase more than twice in 2004 compared to 1999. The difference is highly statistically significant ($P=1.5E-06$). The cash income diversity index reveals that there is no difference between two time periods because two values are approximate. Thus, in this case we can say that the cash income indicator is increasing and the income diversity index is not changed and, therefore, it is considered a little more development generally.

The second indicator proposed here is cash expenditure. Research results of this study are presented in the Table 9. It is similar to the previous indicator (cash income indicator), in which expenditure of farmers in all the sectors increased from 1999 to 2004. The biggest expenditure is for house building and repairing, which strongly increased from 1999 to 2004 (almost 7 times), because house construction was only in the year 2004. The second increase belonged to animal husbandry expenditure as in 2004 farmers developed to raise livestock very strong (Table 7), so they used a lot of money for feed and seedlings. All expenditure components of the system in 2004 have built up their total expenditure being 4 times bigger compared to that in 1999 with highly statistically significant ($P=1.57E-10$). Thus, this indicator group shows a development trend of the system

Table 9. Types and Cash Expenditure of Households (Thous.VND)

Sources	Year		Compared
	1999	2004	
Grain	654	2,419	+
Animal Husbandry	128	1,795	+
Clothes	214	322	+
Tax and Contribution to funds	74	26	-
School fees	57	493	+
Health Care	83	160	+
Ritual Occasions	203	217	+
Interest on Debts	262	720	+
House building and Repair	262	1,879	+

Cash income and cash expenditure balancing results in two time periods are presented in Table 10. Although both cash income and cash expenditure increased from 1999 to 2004 as highly statistically significant, while negative balancing of two these categories occurred because cash expenditure was bigger than cash income. The balance is presented by S

index and it has shown that in 2004 S is smaller than 1.0 and smaller than S in 1999. However, this is all not bad. There are two reasons for explaining this: (1). Capital for house building is not only accumulated in one year but is also accumulated from many years. (2). Farmers can foresee their economical development in the future so they dare to invest for themselves.

Table 10. Balance of Cash income and Cash Expenditure for Households

Items	Year	
	1999	2004
Total Cash income (Thous.VND)	3,191	7,060
Total Cash Expenditure (Thous.VND)	2,341	9,163
Balance (Thous.VND) (income-expenditure)	850	- 2,103
S: (Total Income/Total Expenditure)	1.36	0.77

Total income (cash and non-cash income) was presented in Table 11 and Figure 7, and it was calculated by average value per capita of each household per year through two time periods (1999 and 2004). The average total income of each capita

clearly increased from 1,316 thousands VND in 1999 to 2,113 thousands VND in 2004 with the difference being highly statistically significant (p=0.001). This changing trend is the same of households having total income at the lowest level as well as the highest level.

Table 11. Average Total Income per Capita of Households per year

Income Levels (Thous. VND)	Year		Compared
	1999	2004	(P)
Lowest Income	211	318	+
Highest Income	2,888	6,247	+
Mean	1,316	2,113	0.001
Medial	1,210	1,533	
Gini Coefficient	0.22	0.33	

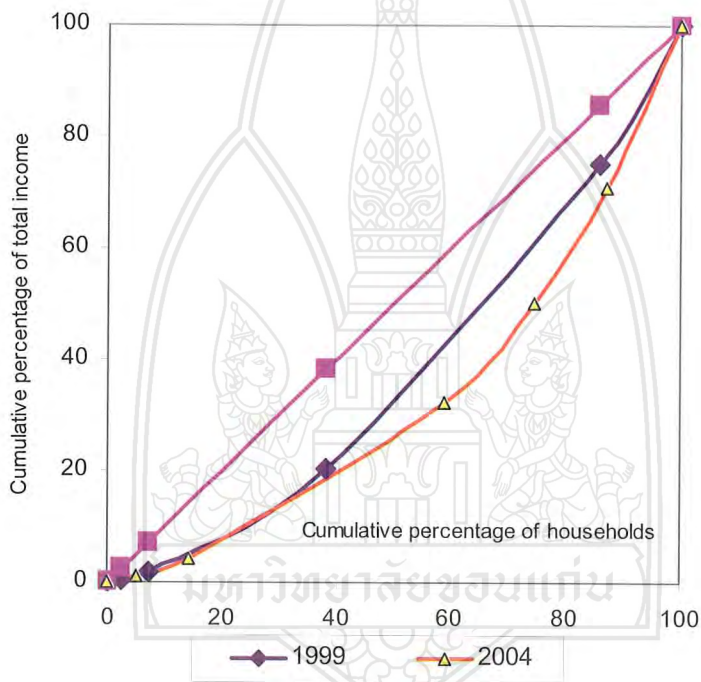


Figure 7. Lorenz curve for average total income of households

Gini coefficient value and Lorenz curve in Figure 7 reveals the equality in total income distribution among households trend decreased since the Gini coefficient in 2004 is bigger than the Gini coefficient in 1999 (Gini values are 0.33 and 0.22 respectively).

Food security is one of the indicators that have been considered by many international development organizations not only for economic but also for social development.

Table 12. Number of months in which the Households Suffer from Hunger (Number of household and percentage)

Number of months	Year	
	1999	2004
1-2	1 (2.3)	0 (0.0)
3-4	3 (7.1)	1 (2.6)
7-8	1 (2.3)	0 (0.0)

The measured results for this indicator were presented in Table 12 indicating that in 1999 that 12 percent of households had suffered from hunger from 1-2 to 7-8 months, while in 2004 only 2.6 percent

households had still suffered from hunger for 3-4 months. This is a big improvement for the development of this agricultural system.

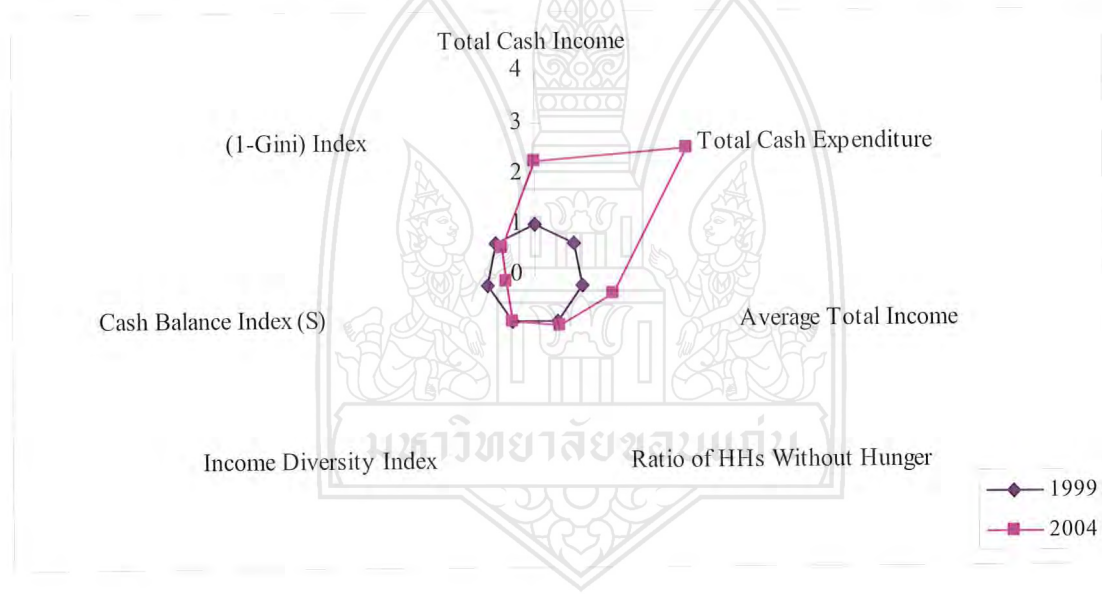


Figure 8. Comparison of Economical Changes of the System

In order to have a synthesis evaluation of the development on the economic aspect of the system, synthesis table 12-a established in order to compare indicators at two times periods and then all these indicators were entered to draw the radar graph (Figure 8).

Table 12-a. Comparison of Economical Changes of the System

Items	Year		Compared 2004/1999 (time)
	1999	2004	
Total Cash Income (Thous.VND)	3,191	7,060	2.21
Total Cash Expenditure (Thous.VND)	2,341	9,163	3.91
Average Total Income (Thous.VND)	1,316	2,113	1.61
Ratio of HHs Without Hunger (%)	88.3	97.4	1.10
Income Diversity Index	0.69	0.67	0.97
Cash Balance Index (S) (Total income/Total Expenditure)	1.36	0.77	0.57
(1-Gini) Index	0.78	0.67	0.86
Average (times)			1.60

In Figure 8, it appears that although there were some indicators (cash balance index, 1- Gini Index, and Income Diversity Index) to be under development in 2004, generally all other indicators represent their growth from 1999 to 2004, which still makes the graph area of 2004 larger compared to that of 1999. The graph area was quantified of 1.60 times in 2004 to 1999 (table 12-a). In this state we can say that the agricultural system is more development on the economical aspect.

Environmental Evaluation

In order to evaluate the environmental aspect of the systems, two representative indicators of the system were selected for measuring the changes of the system and given as follows: (1) Land cover of the study site through the selected time. It is considered to be one of the very important indicators for evaluating the environment because it is affected by other characteristics, such as soil type, soil fertility, rainfall, human behavior, social policy, etc... It also affects the environment. For example, when vegetative cover is good the water source and soil fertility will

be better, and crop production activities will be more effective, etc. All of this would make the systems sustainable. (2) Indicator of general perception of farmers about the changes of natural environment. This indicator is selected based on a farmer's viewpoint, the one who has lived for a long time in their country sites; who knows more of their natural environment conditions, and they can compare it from time to time more exactly than others can.

The land cover indicator is represented by five types of land use of which on rice paddy cover is the same from 1998 to 2003 (2.4 percent), up land cultivated area's variation is not much, it ranges from 9.9 percent in 1998 to 11.0 percent in 2003, the same variations for land cover types of young and medium fallow but with old fallow it has variation rather clearly with trend increasing land cover, it's variation of land cover ranges from 14.5 percent in 1998 to 17.0 percent in 2003. However, generally it can be considered as no change for all of the land cover types in Tat from 1998 to 2003 (Table 13 and Figure 9, 10).

Table 13. Land Cover type in Tat Hamlet from 1998 to 2004

Land use/land cover type	1998	%	2000	%	2003	%
Rice paddy	17.7	2.4	17.7	2.4	17.7	2.4
Current cultivated upland	71.9	9.9	76.3	10.5	79.7	11.0
Young fallow (grasses)	150.4	20.7	171.4	23.6	150.5	20.7
Medium fallow (bamboo and bush)	382.2	52.5	308.8	42.5	356	48.9
Old fallow (tall tree and giang)	105.4	14.5	152.3	21.0	123.8	17.0

The interviewer showed a 10 step ladder scale to the respondents in order to receive the farmer’s perception of the changing natural environment. Questions included thinking about their natural environmental state at present, five years ago, and five years into the future. The respondents then put each concrete natural environmental state at a fixed time into the appropriate ladder step. There are three fixed points, which are 1999, 2004, and 2009. The research results showed that 68 – 71 percent respondents confided that the environment changed for the better; so, the average score on natural environmental quality gradually

increased from 1999 to 2004 then 2009 to be of 4.24; 5.06; and 6.03 respectively (Figure 11). This proves that natural environment at the site has been improving and in the future it is hoped to be better. The reason for this is that farmer’s awareness of environmental protection is getting better day by day and under the effectiveness of forestry allocation policy to every farmer of Vietnam Government; so, the forest is better protected. The last correspondents (26 – 31 percent) think that the natural environment was still degraded because of population increase, forest exploitation has no plan, and swidden cultivation is still occurring.

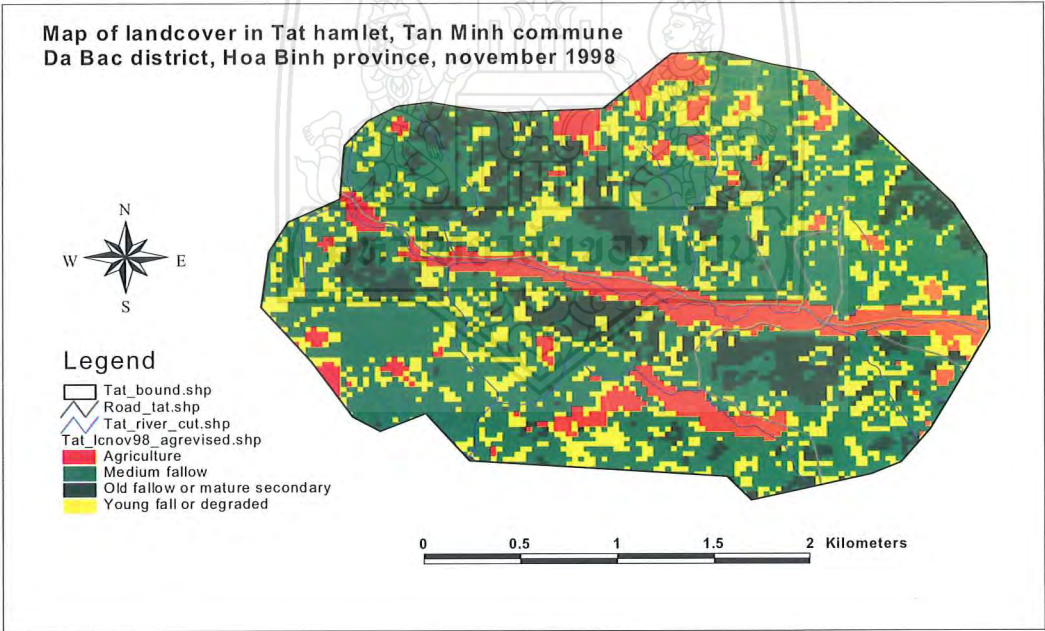


Figure 9. Map of Land Cover in Tat Hamlet, November 1998

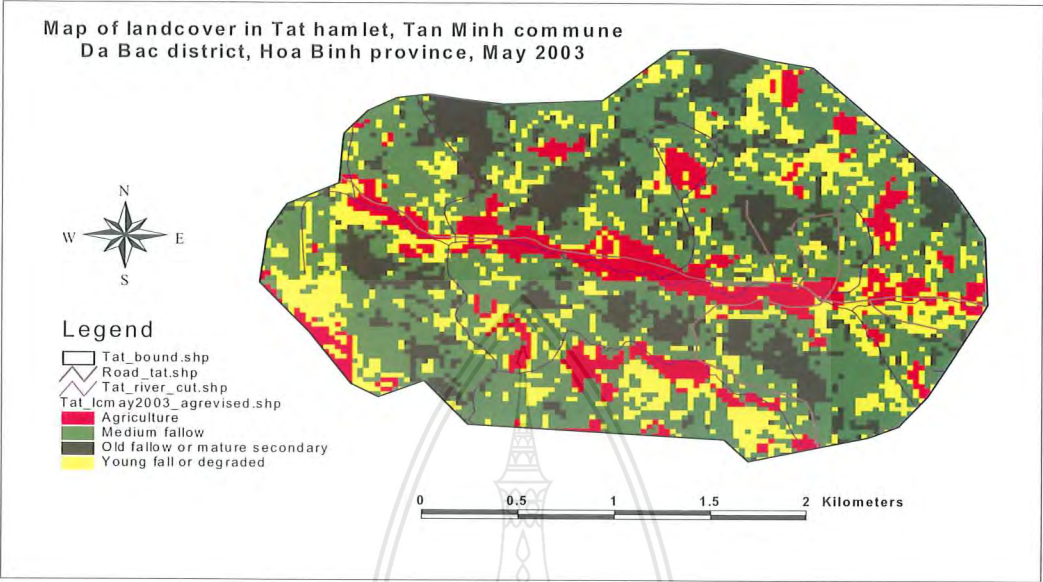


Figure 10. Map of Land Cover in Tat Hamlet, May 2003

Thus, general evaluation of the changing environment is because the forest is better protected from time to time. The majority of respondents said that the environment has been becoming better and the minority of them said it was had not changed nor would be degraded continuously. So, generally, we can conclude that the environment has been changing following a better trend.

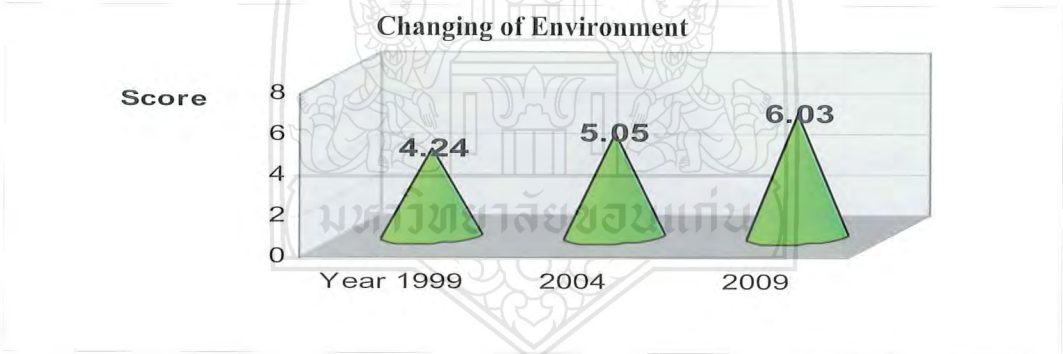


Figure 11. Perception of the Farmers on Changing Environment

Briefly, in order to evaluate the changes of environment of the system from 1999 to 2004, based on the results of measuring land cover and farmer’s perceptions on the changing of natural environment, we can put forward that the natural environment of the system has been gradually changing following the improving trends and is more development. This change is quantified from 1 to 1.19 times respectively to 1999 and 2004.

Cultural-Social Conditions and the Life

According to the theory of system's components, the cultural and social life is the spirit and soul of the system. It represents cultural understanding, behavior between people, and the subjects of the system, in the system so it can be considered as a spiritual scale of the system subjects by sets of different indicators in material and unmaterial things. In the conditions of this study, the indicator group was

selected and proposed included the following: (1) Social problems including gambling, drunkenness, theft of crops or livestock, domestic violence, quarrels with neighbors, and fighting; (2) Quality of intra-community social relations, includes friendly and cooperative, neither friendly nor cooperative, and no change; (3) Perceptions of the farmers on their quality of life in the past, at present, and in the future (5 year time periods each compared to at present).

Table 14. Social Problems Perceived as Occurring Frequently (%)

Sources	Year		Compared
	1999	2004	
Gambling	35.7	15.4	-
Drunkenness	28.6	56.4	+
Theft of crops or livestock	4.8	7.7	+
Domestic violence	7.1	7.7	+
Quarrels with neighbors	4.8	0.0	-
Fighting	9.5	7.7	-
No thing	9.5	2.6	-

For the social problems: The results in table 14 showed that in a total of seven studied sub-indicators three of them had increasing trend by the time. They are drunkenness (the frequency of the answer “yes” increased from 28.6% to 56.4%). Theft of crops or livestock (the frequency of the answer “yes” increased from 4.8 to 7.7 percent), and domestic violence (its decrease means that the problem increasing). They are represented in the compared column by a plus sign and this is a signal of less development. The rest are four other sub-indicators with decreasing trends, and are as follows: gambling, quarrels with neighbors, fighting and no thing. They are presented in the compared column by a minus sign. Generally speaking, in this indicator of social problems group there are four indicators developed following the better trend and

three indicators presenting worst and can be considered to be negative changes.

The second indicators group of cultural-social aspects of the system is the quality of intra-community social relations. In Table 15, there are three sub-indicators evaluated. Most (84.6 percent) of the respondent’s answers were “yes” belonging to the sub-indicator of friendly and cooperative, for the year 2004, while in 1999 it was only 7.1 percent. This means that in 2004, people in the village were friendlier with each other. This is a characteristic representing “the way of life” for peasants in the Vietnamese countryside. When the economy is growing, peasants often have better behavior with each other. Thus, it can be said that the quality of intra-community social relations of Tat Hamlet has been developing with the improving trend.

Table 15. Evaluate Perceptions of the Quality of Intra-Community Social Relations (% correspondents)

Quality of relationships	Year	
	1999	2004
Friendly and cooperative	7.1	84.6
Neither friendly nor cooperative	26.2	5.1
No change	59.5	10.2
Don't know	7.1	0.0
Total	100.0	100.0

To evaluate the changes of farmer’s life, which is based on a ten steps ladder scale, each respondent thought of their community’s life at three different time points of the past (1999), present (2004) and future (2009) to compare and see at which time point the life is better, worse, or medium. Then each of them is put in to the most suitable step on the scale. Ordinal number on step scale is the score number that the life received at the fixed time point. The results are represented in Figure 12. It indicates that over ninety percent of respondents believe their quality of life increases over time. The scored average on quality of life at each time point varies and increases from the lowest score of 2.36 in 1999 to 4.64 in 2004, and the highest score of 6.87 belonging for 2009. This reveals the fact that the life quality of the community in this agricultural system is increasing. This is a trend of better development. These changes can be explained by peasants in the area knowing how to farm on their land better since they have new production technique information from outsiders. They have also received some government subsidies for their life and production. This includes land allocation policy, extension services program of the district, some extension works from CARES, policies to help poor people, grow plants to cover barren land, and 135 program to improve infrastructure of remote communities.

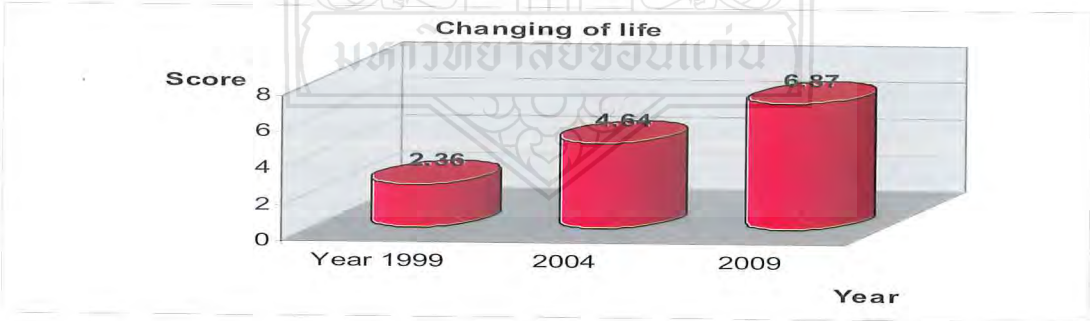


Figure 12. Evaluation of the changes of Farmer’s Life

In order to measure the changes of the system on the cultural, social conditions and the life aspect of the system subjects, table 16 and a radar graph were established to evaluate the changes of indicators mentioned (Figure 13).

Table 16. Comparison of indicators of Cultural - Social Condition and Farmer's - Life

Items	Year		Compared 2004/1999 (time)
	1999	2004	
Gambling (% answer to be no)	64.3	84.6	1.32
Drunkenness (% answer to be no)	71.4	43.6	0.61
Theft of Crop or Livestock (% answer to be no)	4.8	7.7	1.60
Domestic Violence (% answer to be no)	7.1	7.7	1.08
Quarrel with Neighbours (% answer to be no)	95.2	100.0	1.05
Fighting (% answer to be no)	90.5	92.3	1.02
Friendly and Cooperative (% answer to be yes)	7.1	84.6	11.92
Life quality improved (Score)	2.36	4.64	1.97
Average (time)			2.57

On the graph we can see the graph area for 2004 is larger than that of 1999. It could be quantified by table 16 with 2.57 times bigger of indicators average in 2004 to 1999. These changes are a signal for better development of the system on this aspect.

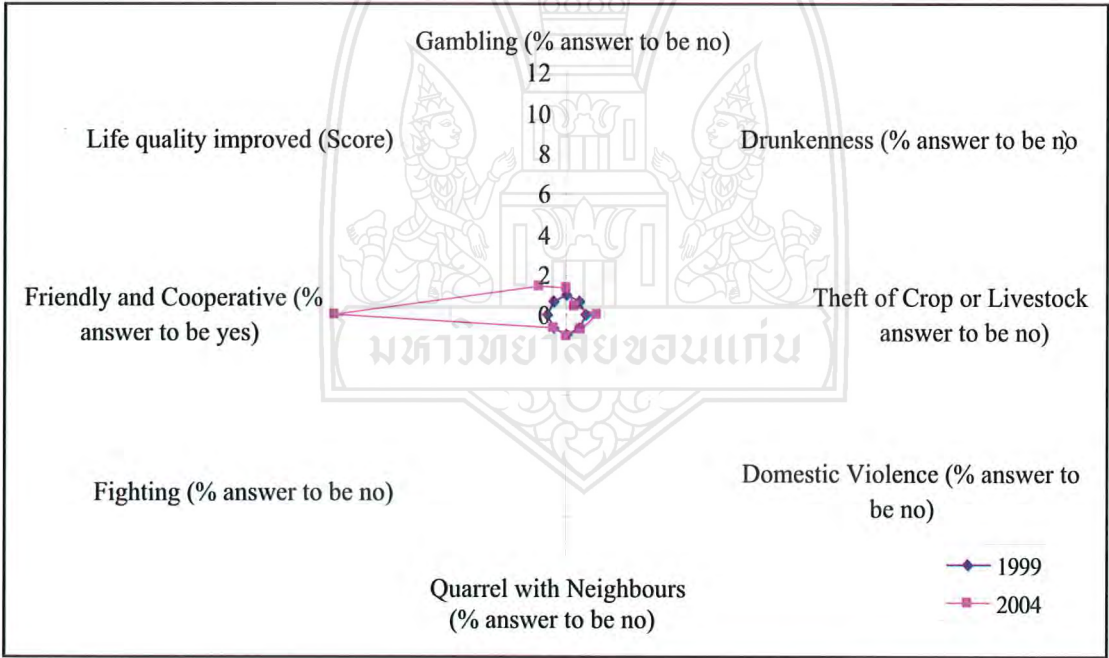


Figure 13. Integrated Evaluation of the Social and the life changes

Integrated Evaluation of the System’s Changes

In order to have a general view on the changing of the Tat agricultural system after 5 years, it needs

to be established an synthesis comparison table (17) and integrated graph from the evaluated results of 6 indicator groups mentioned above.

Table 17. Synthesis Comparison of the Indicator Groups change (score)

Items	1999	2004
Human Capital	1	1.10
Household Assets	1	3.96
Production Activities	1	1.92
Econmic Activities	1	1.60
Environment	1	1.19
Cultural,Social, and Life	1	2.57
Average	1	2.06

From the graph in Figure 14, we can distinguish more clearly of the changes of the measured indicators or indicator groups. All of the measured indicators reveal their changes following the time from 1999 to 2004 by the trend of non – negative; so, the graph area for 2004 is larger than that for 1999.

It is worth noting changes in Household Asset; Cultural, Social, and the Life; and Production Activities in the system. These are indicator groups exhibiting big changes. These changes are effects of the system development due to several causes. One of the causes is the role of some CARES’ staffs who have been working in this community. They held meetings with farmers discussing their production direction, training courses of agricultural production techniques, demonstrating models of wet rice fertilizer application, VAC (Garden, Ponds, and Livestock) production model. They studied in order to introduce to farmers techniques to grow soybean, groundnut on the hills and wet rice field fallowed in spring season, how to apply fertilizer to wet rice, and how to develop winter crops

on wet rice field. They also advised farmers on how to develop raising livestock to help them prevent diseases in livestock. All of the activities that CARES’ staffs had carried out in Tat Hamlet have brought good results in the production activities of farmers. This is often presented by a saying of Mr. Chom (one of progressive farmers of Tat) that “tu ngay co cac anh o trung tam sinh thai (CARES) len day nong nghiep cua chung em khac xua nhieu lam,” which means that thanks to the help from CARES to Tat hamlet farmers that their agricultural production has been improving now.

Other causes that are mentioned are Vietnamese government economic reform policy, land and forestry allocation policy, credit and poor subsidy policy, changes of local infrastructure, the big capability of accessing marketing, and so on.

In terms of development, with the general evaluation results, the system can be considered to be better development in 2004 to 1999. It is also proved by the average change of indicator groups from 1999 to 2004 to be 2.06 times (table 17).

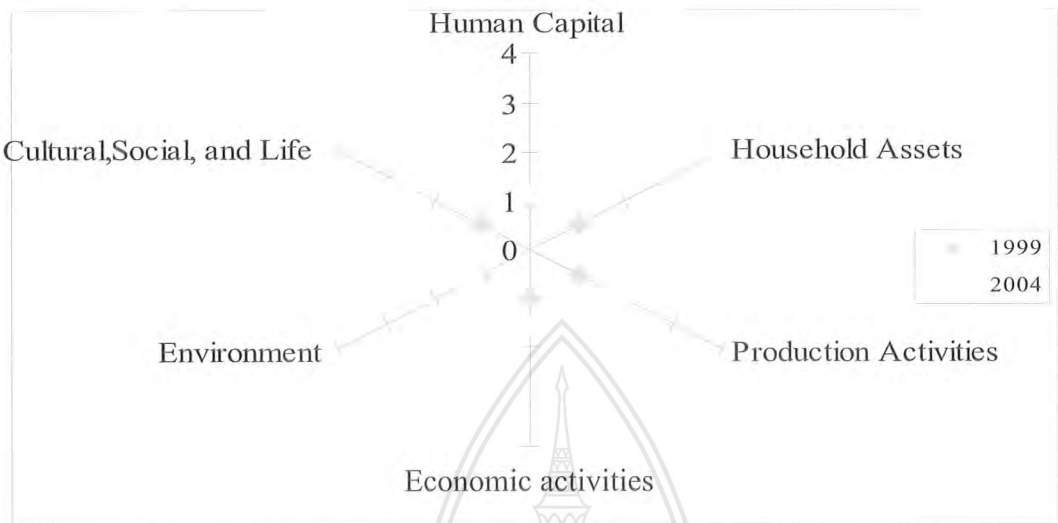


Figure 14. Synthesis Evaluation of the System's Changes

periods, the authors have measured changes of the system components with 6 indicator groups representing characteristics of the system components based on actual research conditions and the research objectives. Some conclusion are follows:

Generally, the research results have indicated that almost all measurements of indicators and groups of indicators changed showing the positive trends, development trends following the time from 1999 to 2004. Thus, in terms of the comparison of the changes, it can be concluded that these indicators are big changes in the period of time from 1999 to 2004. The changes of indicator groups presented on the final radar graph show the graph area of 2004 is larger than that of 1999. This change is 2.06 times larger in 2004 to 1999. Thus, eventhough population pressure increased to 65 persons per square kilometer in 2004, the system still developed.

With the changed indicators, indicator groups of Household Assets; Cultural, Social, and the Life; and Production Activities in the system have revealed the larger changes following more development trend.

The human capital indicator group and environmental indicator group were showed a little change during the period from 1999 to 2004. It is only estimated from 1 to 1.10 scores (times) in 1999 and 2004 respectively, because in practice these indicators

are slowly changing and they can be depended partly on changes of others.

The exchange of commodities, information between the system and outside environment, is also necessary for the changes of characteristic in Tat agricultural systems following the improving trend.

This research results also confirm the composite swiddening agroecosystem having good development trend in the northwestern mountains of Vietnam, as mentioned above. With the achieved results above, the authors have shown that the research results reflect the present status of the Tat hamlet agricultural system correctly.

The obtained results above have only meaning for the selected time periods of this research. In order to have a more complete conclusion on development trend of this agricultural system, there needs to be a regular assessment for every time period later on.

Suggestions for sustainable development of the system in the future

- Based on the revealed research results, the quality of the subjects of the system (human capital) has changed the smallest. However, according to system theory the farmers are subjects of the agricultural system and deciders of all the activities in the system.

Thus, good conditions should be created to improve the quality of human capital more rapidly so that they can make decisions for agriculture activities of the system more effectively.

- Household assets indicator group has had the biggest change due to the main part of Vietnamese people's shopping custom, especially it is shopping transport means. Therefore, increased livestock development in the right production direction for this region was good condition to accumulate capital for shopping. However, it needs to have a plan for grazing in order to avoid contradiction with other production activities.

- There are strong advantages of material for development of handicraft in this region, but the ratio of cash income from a household's handicraft is the smallest. Therefore, This job should be developed for local farmers to increase their income and have access capability to sustainability with respect on economical aspect

- New crops that have appeared in 2004 such as vegetable, peanut, potato are active production activities for the system, but their areas are still small so it should be a concern for development with these kinds of crop.

Acknowledgement

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APPENDIX

1. The formula for calculating the crop diversity Index and cash income diversity index is adapted from the diversity index formula of Shannon (E.P. ODUM, 1978) as the following:

$$|H| = \sum p_i \log p_i$$

Of which: $p_i = \frac{n_i}{N}$
 n_i is value (role) of each component

N: Total value (role) components

p_i is probability of each component

2. (1). Value produced on a piece of swidden land (P_i) (Thous. VND per ha, year).

$$P_i = \frac{\sum \frac{X_{il} * 10000}{S_{il}}}{n_i}$$

Of which: X_{il} is total of money income from swidden land per year (rice, cassava, maize, etc.,) of each household interviewed

S_{il} is total swidden crop areas of each household interviewed per year (m^2)

n_i is total households interviewed that have swidden cultivation

2. (2). Value produced on a piece of wet rice paddy (P_2) (Thous. VND per ha, year).

$$P_2 = \frac{\sum \frac{X_{i2} * 10000}{S_{i2}}}{n}$$

Of which: X_{i2} is total money income from paddy per year of each household interviewed

S_{i2} is wet rice paddy area of each household (m^2)

N_2 is total households interviewed that have wet rice production

2. (3). Value produced on a piece of cultivation land (P) (Thous. VND per ha, year).

$$P = \frac{\sum \frac{X_i * 10000}{S_i}}{n}$$

Of which: X_i is total money income from cultivation land of each household interviewed ($X_i = X_{i1} + X_{i2}$)

S_i is total cultivation areas of each household

N is total households interviewed that have crop production

2. (4). Value produced on a piece of natural land (Q) (Thous. VND per ha, year).

$$Q = \frac{\sum \frac{T_i * K}{K_1}}{S}$$

Of which: T_i is total money income on natural land of each household (income from forest, crops, livestock, and handicraft)

K_1 is total capital in survey sample

K is total population of the site

S is total natural land of the site

3. SAS (Self-Anchoring Scale) is used to measure perceptions of correspondents on the changes of their life or the environment in which they are living

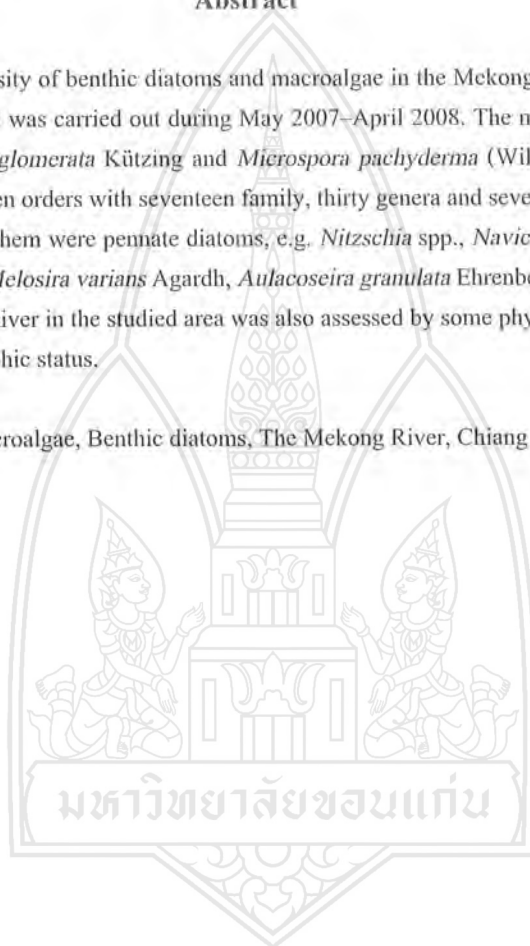
Diversity of Benthic Diatoms and Macroalgae and Water Quality in the Mekong River Passing Chiang Rai Province, Thailand

Pongpan Leelahakriengkrai¹, Supattira Pruetiworanan¹ and Yuwadee Peerapornpisal¹

Abstract

A study on the diversity of benthic diatoms and macroalgae in the Mekong River passing Chiang Rai Province in Northern Thailand was carried out during May 2007–April 2008. The majority of macroalgae was green algae, e.g. *Cladophora glomerata* Kützinger and *Microspora pachyderma* (Wille) Lagerheim in Division Chlorophyta. In addition, eleven orders with seventeen family, thirty genera and seventy-nine species of benthic diatoms were found. Most of them were pennate diatoms, e.g. *Nitzschia* spp., *Navicula* spp. and *Gomphonema* spp. and centric diatoms e.g. *Melosira varians* Agardh, *Aulacoseira granulata* Ehrenberg and *Cyclotella* spp. The water quality of the Mekong River in the studied area was also assessed by some physical and chemical factors, it was moderate with mesotrophic status.

Keywords : Biodiversity, Macroalgae, Benthic diatoms, The Mekong River, Chiang Rai Province.



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Introduction

The Mekong river is one of the world's major rivers which originates from Himalai Mountain. It is regarded as the 10th longest river in the world with an estimated length of 4,880 kilometers. It drains an area of 810,000 km². From the Tibetan Plateau, it runs through 6 countries: China, Myanmar, Thailand, Laos PDR, Cambodia and Vietnam.

Although the Mekong River passes Thailand in the Northern and Northeastern regions, this research focuses on the section of the River which passes the Northern region because Kai (*Microspora* spp. and *Cladophora* spp.), the local economic algae and Giant

Catfish (*Pangasianodon gigas* Chevey) are found in this area. For these reasons, this research ultimately aims to monitor changes in water quality and Kai in this area. Moreover, records of benthic diatoms which are good bioindicators for water quality assessment as well as some physical and chemical factors were obtained.

In this research, benthic diatoms and macroalgae samples were collected from the Mekong River passing Chiang Khong District, Chiang Rai Province (Figure 1). Samples were collected in summer, rainy and cool dry seasons during May 2007-April 2008. Physical, chemical and biological factors of water were also analyzed.



Figure 1. Map of Thailand and the location of sampling sites in Chiang Rai Province.

Materials and Methods

Samples were collected from the Mekong River passing Chiang Khong District, Chiang Rai Province which is located at 20°15'125" N, 100°24'832" E and 345 meters above sea level in summer, rainy and cool dry seasons during May 2007-April 2008.

Some physico-chemical factors were analysed in field and laboratory such as water temperature, velocity, pH, and conductivity were measured directly by portable meters. Chemical analyses of water such as turbidity, alkalinity, TDS, DO, BOD, nitrate nitrogen, ammonium nitrogen, soluble reactive phosphorus

(SRP) were carried out and followed by Greenberg et al. (2005). The water quality was classified according to trophic status using the methods of Lorrain and Vollenweider (1981); Wetzel (2001) and Peerapornpisal et al. (2004)

The benthic diatom samples were scraped from 5-10 stones (or other hard substrates) per site. The stones were brushed with toothbrush. A plastic sheet with a hole area of 10 cm² cutout was placed on the upper surface of the selected stone, benthic diatoms were brushed from the squared hole. In the laboratory, the samples were cleaned by concentrated acid digestion method (Rott et al., 2003). The benthic diatom samples were identified and classified according to Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b); Metzeltin and Lange-Bertalot (1998); Rumrich et al. (2000) and Lange-Bertalot (2001).

Macroalgae samples were collected from the

rocks, cobbles, tree branches and artificial substrates in the water and fixed in 2.0% glutaraldehyde. The identification and classification of macroalgae were carried out according to Van Den Hoek (1963); Whitford and Schumacher (1969); Necchi et al. (1997); John (2002) and Kumano (2002).

Results and Discussion

The water quality in the Mekong River passing Chiang Rai Province was moderate and the trophic status was mesotrophic. High conductivity, turbidity and TDS were observed in all seasons, especially in August 2007 the highest turbidity was recorded. BOD₅ in both summers was lower than that in August 2007 and January 2008. Total coliform bacteria showed high level in January 2008 and April 2008 and was in negative correlations with turbidity (Table 1).

Table 1. Mean values of some physico-chemical factors, trophic status and dominant species in each month (n=3).

Month	May 2007	August 2007	January 2008	April 2008
/Parameter	(summer 1)	(rainy)	(cool dry)	(summer 2)
Water Temperature (°C)	27.6	26.9	21.1	26.3
pH	7.7	7.3	7.4	7.6
Conductivity (µs/cm)	212.3	179.7	240	231.3
TDS (mg/l)	99.7	83.7	115	110
Turbidity (NTU)	196.3	589.3	118.3	172.3
Velocity (m/s)	0.2	0.3	0.3	0.3
DO (mg/l)	6.1	6.2	7.9	6.7
BOD ₅ (mg/l)	0.2	2.9	2.5	0.5
Alkalinity (mg/l as CaCO ₃)	118.3	115	122.7	93.7
NO ₃ -N (mg/l)	0.5	0.5	0.3	0.4
NH ₄ -N (mg/l)	0.3	0.5	0.8	0.6
SRP (mg/l)	0.1	0.1	0.3	0.1
Total coliform bacteria (MPN/100)	460	460	24000	24000
Trophic status	mesotrophic	mesotrophic	mesotrophic	mesotrophic
Water quality	moderate	moderate	moderate	moderate

A total of 80 species of benthic diatoms were found (Table 2 and Figure 2). *Nitzschia palea* (Kützing) W. Smith was found to be the dominant species in both summer seasons. *Navicula symmetrica* Patrick was a dominant species during May 2007-January 2008. In May 2007, 36 species were found and the dominant species were *Nitzschia palea*, *Navicula symmetrica* and *Achnantheidium minutissimum* (Kützing) Czarnecki. In August 2007, 27 species were found and the dominant species were *Nitzschia intermedia* Hantzsch, *Navicula symmetrica* and *Gomphonema minutum* (C. Agardh) C. Agardh. In January 2008, 32 species were found and

the dominant species were *Nitzschia dissipata* (Kützing) Grunow, *Cymbella turgidula* Grunow and *Navicula symmetrica* and in April 2008, 40 species were found with *Nitzschia palea* and *Navicula* sp.1 as the dominant species. Four Species of macroalgae were found (Table 3 and Figure 3). In May 2007, 3 species were found and the dominant species were *Cladophora* sp.1 and *Microspora pachyderma* (Wille) Lagerheim. In August 2007, no macroalgae were found. In January 2008, only one species, *Cladophora glomerata* Kützing was found and *Cladophora* sp.1 was found in April 2008.

Table 2. List and distribution of benthic diatoms in the Mekong River passing Chiang Rai Province.

* = common species, *** = dominant species

Species list of benthic diatoms	May 2007 (summer 1)	August 2007 (rainy)	January 2008 (cool dry)	April 2008 (summer 2)
Division Bacillariophyta				
Order Aulacoseirales				
Family Aulacoseiraceae				
<i>Aulacoseira granulata</i> Ehrenberg	*		*	
<i>Melosira varians</i> Agardh				*
Order Thalassiosirales				
Family Stephanodiscaceae				
<i>Cyclostephanos invisitatus</i> (Hohn& Hellermann)Theriot, Stoermer&Håk				*
<i>Cyclotella atomus</i> Hustedt	*			
<i>C. cyclopuncta</i> Hakansson & Carter				*
<i>C. meneghiniana</i> Kützing		*	*	*
<i>C. stelligera</i> Cleve	*		*	*
<i>Cyclotella</i> sp.1	*			
<i>Cyclotella</i> sp.2	*			
<i>Thalassiosira</i> sp.1	*			
<i>Thalassiosira</i> sp.2			*	
Order Triceratiales				
Family Triceratiaceae				
<i>Pleurosira laevis</i> (Ehrenberg) Compare				*
Order Achnanthales				
Family Achnanthaceae				
<i>Achnanthes altaica</i> (Poretzky) A.Cleve-Euler				*
<i>A. minutissima</i> Kützing var. <i>minutissima</i>				*
<i>Achnanthes</i> sp.1	*			

Table 2. (continued)

Species list of benthic diatoms	May 2007 (summer 1)	August 2007 (rainy)	January 2008 (cool dry)	April 2008 (summer 2)
<i>Planothidium lanceolatum</i> (Brébisson) Round & Bukhtiyarova	*	*	*	*
<i>P. lanceolatum</i> var. <i>abbreviate</i> C.W.Reimer				*
<i>P. rostratum</i> Round&L.Bukhtiyarova	*			
Family Cocconeidaceae				
<i>Cocconeis placentula</i> Ehrenberg			*	*
<i>Cocconeis</i> sp.1				*
Order Thalassiophysales				
Family Catenulaceae				
<i>Amphora ovalis</i> (Kützing) Kützing			*	
<i>Amphora</i> sp.1		*	*	
Order Naviculales				
Family Naviculaceae				
<i>Caloneis bacillum</i> (Grunow) Mereschkowsky			*	
<i>Geissleria decussis</i> (Østrup) Lange-Bertalot&Metzeltin				*
<i>Geissleria</i> sp.1	*			
<i>Mayamaea atomus</i> (Kützing) Lange-Bertalot	*	*		*
Family Diploneidaceae				
<i>Diploneis</i> sp.1	*			
Family Pleurosigmataceae				
<i>Gyrosigma scalproides</i> (Rabenhorst) Cleve	*	*	*	
<i>G. spencerii</i> (Quekett) Griffith&Herfrey		*	*	
Family Diadesmidaceae				
<i>Luticola cohnii</i> (Hilse) D.G. Mann				*
<i>L. mutica</i> (Kützing) D.G. Mann		*		
<i>L. ventricosa</i> (Kützing) D.G. Mann	*			
<i>Luticola</i> sp.1	*			
<i>Navicula capitatoradiata</i> Germain		*		
<i>N. caterva</i> Hohn & Hellermann		*		
<i>N. cryptotenella</i> Lange-Bertalot		*		
<i>N. germainii</i> Wallace	*			*
<i>N. reichardtiana</i> Lange-Bertalot	*		***	*
<i>N. rostellata</i> (Kützing) Cleve			*	*
<i>N. symmetrica</i> Patrick	***	***	***	*
<i>N. viridula</i> (Kützing) Kützing			*	
<i>Navicula</i> sp.1	*	*		***
<i>Navicula</i> sp.2	*			

Table 2. (continued)

Species list of benthic diatoms	May 2007 (summer 1)	August 2007 (rainy)	January 2008 (cool dry)	April 2008 (summer 2)
<i>Navicula</i> sp.4				*
<i>Naviculadicta nanogomphonema</i>			*	
Lange-Bertalot & U.Rumrich				
Family Sellaphoraceae				
<i>Sellaphora gibbula</i> D. Metzeltin & H. Lange-Bertalot			*	
<i>S. pupula</i> (Kützing) Mereschowsky	*	*		*
Order Cymbellales				
Family Gomphonemataceae				
<i>Gomphonema lagenula</i> Kützing	*	*	*	
<i>G. minutum</i> (C. Agardh) C. Agardh		***		
<i>G. parvulum</i> (Kützing) Grunow			*	*
<i>Gomphonema</i> sp.1	*			
<i>Gomphonema</i> sp.2	*			
Family Cymbellaceae				
<i>Cymbella tumida</i> (Brébisson) Van Heurck			*	*
<i>C. turgidula</i> Grunow		*	***	*
<i>Cymbella</i> sp.1	*			
<i>Encyonema</i> sp.1	*		*	*
<i>Encyonema</i> sp.2			*	
Order Fragilariales				
Family Fragilariaceae				
<i>Asterionella formosa</i> Hassall				*
<i>Diatoma</i> sp.1	*			*
<i>Fragillaria</i> sp.1				*
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	*	*		*
Order Eunotiales				
Family Eunotiaceae				
<i>Eunotia subarcuatoidea</i> Alles, Norpel & Lange-Bertalot				*
Order Bacillariales				
Family Bacillariaceae				
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow in cleve et Grunow				*
<i>Nitzschia acicularis</i> (Kützing) W.Smith	*			
<i>N. angustata</i> (W. Smith) Grunow				*
<i>N. clausii</i> Hantzsch	*	*	*	
<i>N. dissipata</i> (Kützing) Grunow	*	*	***	*
<i>N. fonticula</i> Grunow in Cleve et Möller	*			*

Table 2. (continued)

Species list of benthic diatoms	May 2007 (summer 1)	August 2007 (rainy)	January 2008 (cool dry)	April 2008 (summer 2)
in Van Heurck				
<i>N. palea</i> (Kützing) W. Smith	***	*	*	***
<i>N. sociabilis</i> Hustedt		*		
<i>Nitzschia</i> sp.1	*	*		
<i>Nitzschia</i> sp.2	*			
<i>Nitzschia</i> sp.3	*			
Order Surirellales				
Family Surirellaceae				
<i>Surirella</i> sp.1			*	

Table 3. List and distribution of macroalgae in the Mekong River passing Chiang Rai Province.

* = common species, *** = dominant species

Species list of benthic diatoms	May 2007 (summer 1)	August 2007 (rainy)	January 2008 (cool dry)	April 2008 (summer 2)
Division Chlorophyta				
Order Cladophorales				
Family Cladophoraceae				
<i>Cladophora glomerata</i> Kützing			***	
<i>Cladophora</i> sp.1	***			*
Order Ulotrichales				
Family Microsporaceae				
<i>Microspora pachyderma</i> (Wille)	***			
Lagerheim				
Division Rhodophyta				
Order Nemalionales				
Family Erythotrichaceae				
<i>Compsopogon</i> sp.	*			

From this research, the diversity of benthic diatoms and macroalgae could be used as a biomonitor for assessing the water quality. It was found that the dominant species of benthic diatoms such as *Nitzschia palea*, *Navicula symmetrica*, *Cymbella turgidula* and *Achnantheidium minutissimum* and macroalgae such as *Cladophora glomerata* and *Microspora pachyderma* could be used to monitor the moderate water quality

which were in agreement with the reports by Krammer and Lange-Bertalot (1986) Inthasotti (2006) and Kunpradid (2005), respectively.

The water quality in the Mekong River passing Chiang Rai Province was similar to the water quality of Mekong River Commission (MRC) report (2005). However, the number of macroalgae (Kai) of the report was higher than this study.

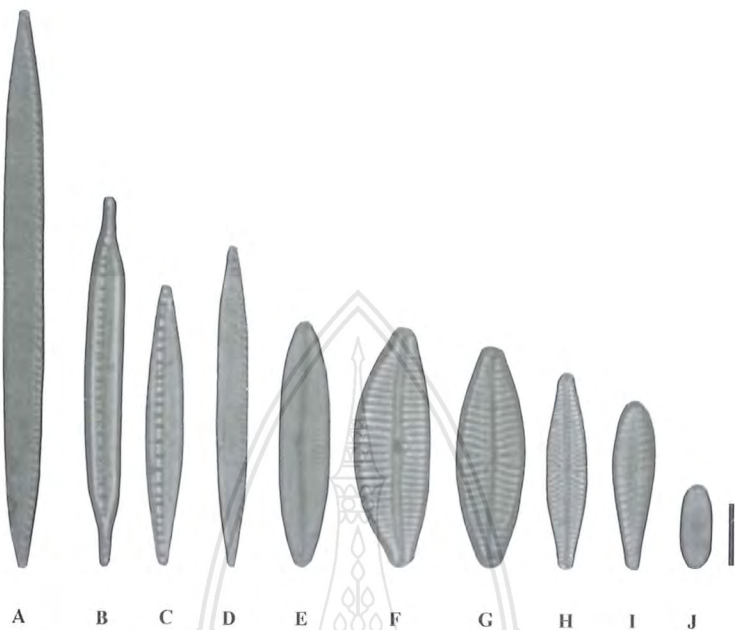


Figure 2. Light micrographs showing dominant species of benthic diatoms in Mekong River passing Chiang Rai Province. (A) *Nitzschia intermedia* Hantzsch, (B-C) *Nitzschia dissipata* (Kützing) Grunow, (D) *Nitzschia palea* (Kützing) W. Smith, (E) *Navicula symmetrica* Patrick, (F) *Cymbella turgidula* Grunow, (G) *Navicula* sp.1, (H) *Navicula reichardtiana* Lange-Bertalot, (I) *Gomphonema minutum* (C. Agardh) C. Agardh, (J) *Achnantheidium minutissimum* (Kützing) Czarnecki. scale bar = 10 μ m

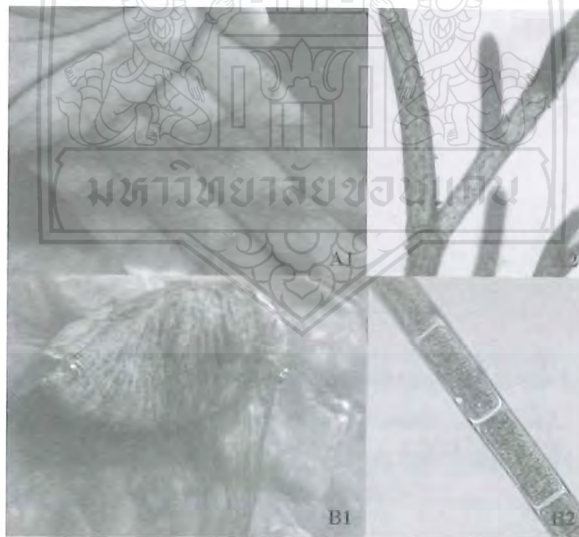


Figure 3. Light micrographs showing dominant species of macroalgae in Mekong River passing Chiang Rai Province. 1: in the field 2: under light microscope (A) Division Chlorophyta: *Cladophora glomerata* Kützing, (B) Division Chlorophyta: *Microspora pachyderma* (Wille) Lagerheim. scale bar = 20 μ m

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Rate of Composting and Quality of Compost Under Different Passively Aerated Composting

Sayan Tanpanich¹, Rewat Chindachia¹, and Soonthorn Duriyaprapan¹

Abstract

Study on composting efficiency was carried out in passive aeration using perforate pipes in comparison with static and turning piles. The seven treatments under investigation are one horizontal pipe, twin horizontal pipes, one vertical pipe, twin vertical pipes, upside T pipe, turning pile and static pile. There were no significant difference in terms of temperature increasing and decreasing rate, time to reach the peak temperature, peak temperature and duration of temperature above 55°C. The completion of composting as indicated by time to reach ambient temperature at 32°C was significantly different. The shortest duration of 72 days was recorded in treatment with twin horizontal perforate pipes in comparison with 111 days in turning pile and 124 days in static pile. There was no significant difference in peak temperature. There showed no significant different in duration above 55°C which indicating that sanitation of compost pile was adequate in all treatments. There was no significant difference in the most of chemical property parameters under investigation except higher nitrogen content in treatment with twin horizontal perforate pipes. Perforate pipes can effectively promote passive aeration in compost piles and can be employed in the production of organic fertilizer. However, further studies are needed to compare composting efficiency between passive and active aeration as well as the study of passive aeration for different raw materials and improvement of passive aeration.

Introduction

Composting is a biological process in which starting carbon to nitrogen ratio, moisture content and oxygen levels as well as temperature are important factors affecting composting process. At farm level, appropriate technology is employed in composting farm waste to produce organic fertilizer or soil conditioner for domestic use. However, increasing demand for high quality organic fertilizer to replace chemical fertilizer in commercial crop production, especially for organic agriculture, requires proper techniques and management to maintain the quality and standard as stipulated by law or regulations.

In western world, there are number of composting method for large amount of organic waste

at commercial level such as windrows, enclosed aerated windrow, aerated static pile, enclosed aerated static pile, modular in-vessel containers, modular in-vessel tunnels, in-vessel bays, in-vessel vertical silos, rotary drums as well as anaerobic digestion (Composting Council of Canada, 2008). Increasing attention has been given to vermi-composting within the past years but in general it is still in the early stage and the production of vermi-compost only in small scale.

Anonymous (2008) has indicated that composting rate depends on physical as well as chemical factors. Aeration of composting pile is an important factor in composting. Both pile turning and active aeration using forced air are labor intensive and require relatively high capital investment (Martin, 2005). In

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order to reduce composting cost, attention has been given to passive aeration or natural ventilation in compost pile. Fernandes et al. (1994) confirmed the effectiveness of passive aeration in composting poultry manure slurry and peat or chopped straw. The lower and variable flow rate in passive aeration is more energy efficient and produce compost that is richer in nitrogen than actively aerated compost (Solano et al., 2001)

Turning compost pile is generally recommended and practical in Thailand. However, due to the lack of labor and inconvenience conditions during pile turning the number and frequency of pile turning in real practice is inadequate for optimal aeration. Active aeration through forced provides an alternative to pile turning (Sawangpanyangkura et al., 2001) but the additional investment resulting in increasing production cost may hamper acceptance.

The objective of this study is to investigate the effectiveness of passive aeration of dairy cow manure and rice straw through plastic perforated pipes as compared to turning pile and static pile.

Materials and Methods

The 1.25 m. x 1.50 m. x 1.50 m. (H x W x L) composting bins (Figure 1) were constructed with

interlocking blocks. The 5 – inch diameter perforate pipes were inserted into compost bins in 5 different patterns. Composting efficiency was investigated in comparison with turning pile and static pile. The 7 treatments (Figure 2) under investigation are as follows:

T1 = one horizontal perforate pipe;

T2 = twin horizontal perforate pipes 0.75 m. apart;

T3 = one vertical perforate pipe;

T4 = twin vertical perforate pipes 0.75 m. apart;

T5 = upside T perforate pipe;

T6 = turning pile every 7 days;

T7 = static pile.

Each compost bin contains 825 kg of raw materials at the beginning of the experiment. In order to adjust C:N ratio of raw materials to be close to 30:1, 200 kg of rice straw was added to 625 kg of dairy cow manure for each bin. Each treatment contains the mixture of 625 kg dairy cow manure and 200 kg rice straw. Moisture content of the mixture was adjusted to approximately 60% at the beginning of the study. The Land Development Department's composting microorganism was used in all treatments in accordance with the instruction.

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Figure 1. Models of compost bin using in the experiment

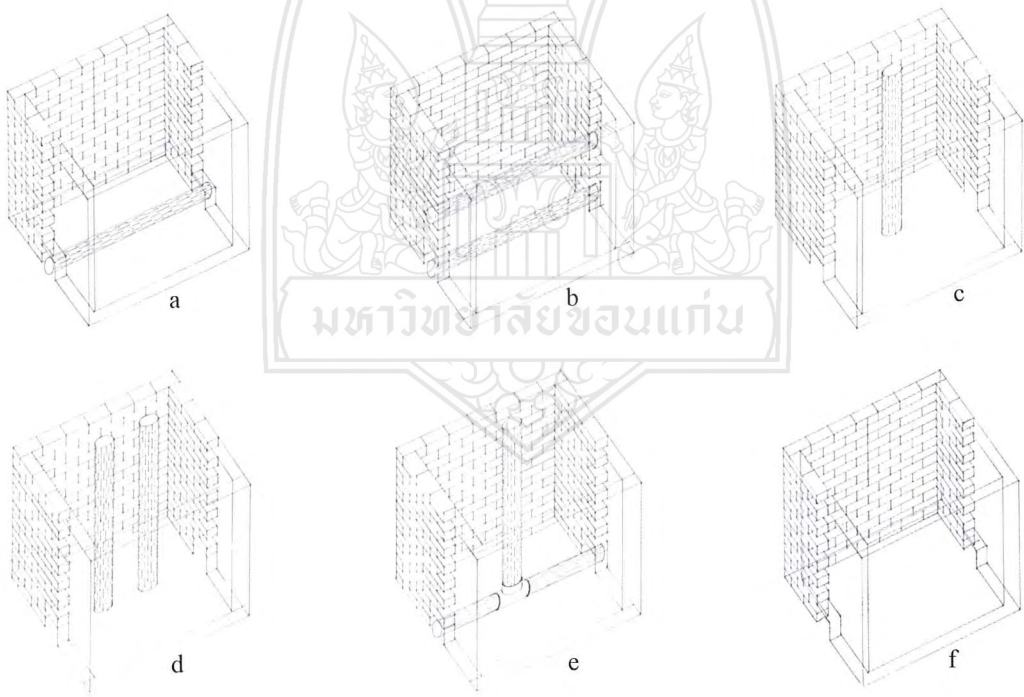


Figure 2. Model of compost bin with different placements of perforate pipes [a (T_1), b (T_2), c (T_3), d (T_4), e (T_5), f (T_6 and T_7)]

The top layer of each treatment was covered with 15 cm. layer of mature compost to minimize moisture and temperature losses. Temperatures within each compost bin were recorded every 3 days in 3 different locations i.e. 20 cm from the top, at the centre of the bins and 20 cm from the bottom. Ambient temperature was employed as an indicator of the completion of composting.

Samples of compost were taken weekly for the evaluation of moisture content. Chemical properties of compost samples taken at the start of the study and at 4 and 8 weeks were investigated by means of the changes in pH (1:5), electrical conductivity (EC, 1:5), organic matter (OM), organic carbon (OC), C:N ratio, total nitrogen (N) and total phosphorus (P). The analytical methods of chemical properties are as shown in table 1

Table 1. Analytical Methods of Chemical properties

Chemical properties	Analytical methods
pH	pH meter (sample:water, 1:5)
Electrical conductivity (EC)	EC meter (sample:water, 1:5)
Organic matter (OM)	Walkley and Black Titration (Walkley and Black, 1934)
Organic carbon : total nitrogen ratio (C:N ratio)	Organic C (OM/1.7241):total N
Total nitrogen	Micro-kjedahl method (Bremner, 1996)
Total phosphorus	Vanadomolybdate yellow color, spectrophotometer (Attanandana and chanchareonsook, 1999)

Results and Discussion

Temperature

Study on temperature profiles in 3 locations within compost piles showed relatively similar patterns of temperature during the course of composting process in all treatments (Figure 3, 4 and 5). Temperature in the compost piles increased and reached maximum level within 7 to 10 days, then after declined and reached ambient temperature within 72 to 125 days (Table1).

There was a marked decrease in temperature in treatments T2, T4 and T5 in which 2 perforate pipes were inserted in different pattern. These responses may indicate increasing aeration in the treatments with twin perforate pipes resulting in shorter period for the completion of composting. In order to develop appropriate composting processing technologies, further study on the effect of various number of perforate pipes and patterns of insertion are needed.

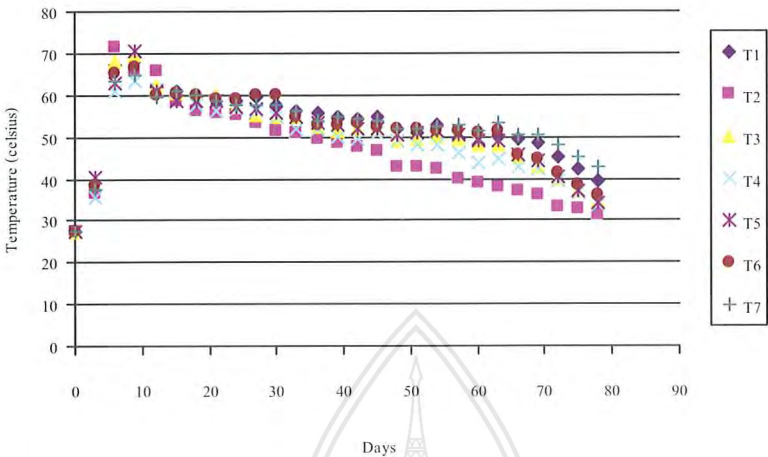


Figure 3. Temperature profiles at 20 cm. from the top of compost pile

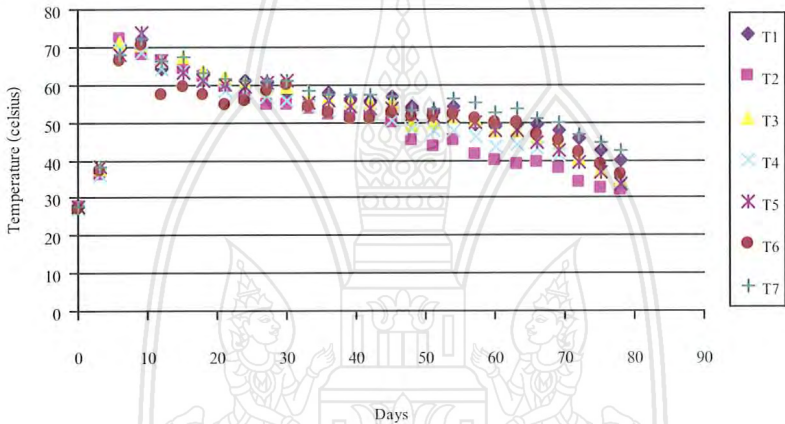


Figure 4. Temperature profiles at centre of the bins of compost pile

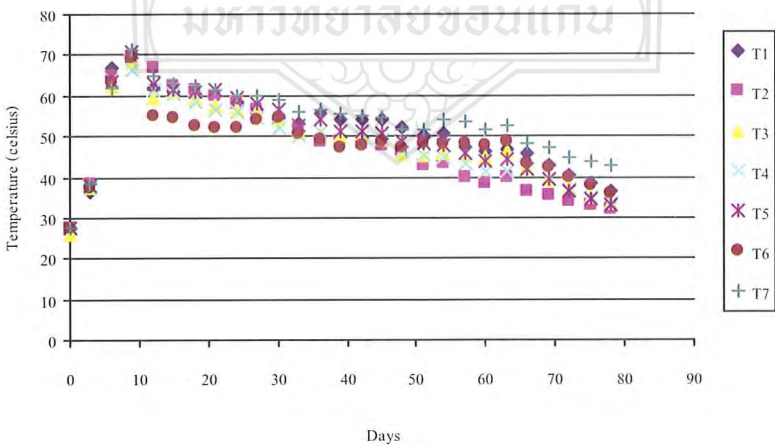


Figure 5. Temperature profiles at 20 cm. from the bottom of compost pile

Table 2. Analysis of variance of temperature profile

Treatment	Peak Temp. °C	Days to reach peak temperature	Total Duration > 55 °C (days)	Increasing rate to reach peak temperature(°C/day)	Decreasing rate to reach ambient temperature (°C/day)	Days to reach 32 °C
T ₁	70.50	9.00	28.50	15.954	-0.9549	114.00 ^{ab}
T ₂	69.50	7.50	24.00	18.475	-1.4571	72.00 ^c
T ₃	71.00	9.00	34.50	15.979	-1.1749	91.50 ^b
T ₄	66.00	9.00	22.50	14.428	-1.1509	85.50 ^{bc}
T ₅	71.50	9.00	28.50	15.813	-1.1673	89.50 ^{bc}
T ₆	69.00	9.00	27.00	15.212	-0.8295	111.00 ^{ab}
T ₇	69.00	9.00	40.50	15.208	-0.8118	124.50 ^a
Prop.>F	0.5757	0.4918	0.1312	0.4855	0.1076	0.0465
F- Test	ns	ns	ns	ns	ns	*
CV (%)	4.05	9.13	19.11	11.30	18.18	13.39

Remark : Mean in a column followed by the same letter are not significantly different according to DMRT (p=0.05)

There were no significant difference in terms of increasing rate, decreasing rate and time to reach peak temperature. However, treatments with twin horizontal perforate pipes showed shorter time to reach peak temperature as well as higher increasing and decreasing rates than other treatments.

The results show significant difference in number of days to completion of composting process (Table 1). Regarding temperature profile, composting process was the most effective in treatment with twin horizontal perforate pipes indicating higher oxygen supply than other treatments. The fastest duration was 72 days which recorded in twin horizontal perforate pipes. Whereas the slowest composting process was 124.5 days which recorded in static pile. Since dur-

ing the initial days of composting of static pile was insufficient oxygen then the pile become anaerobic which anaerobic processes are generally slower and less efficient than aerobic process (Buckley, 2001)

A minimum period of 22.5 days with temperature above 55 °C was recorded in treatment 4 with twin vertical perforate pipes and a maximum of 40.5 days was recorded in treatment 7 in static pile. There was no significant difference in terms of peak temperature and duration above 55 °C indicating sufficient temperature and length of time for sanitation in all treatments.

Chemical properties of raw materials

No significant difference was found among

composting treatments in organic matter content, organic carbon content, C:N ratio and total phosphorus content. Total nitrogen content in twin horizontal perforate pipes (T_2) was significantly higher comparing to other treatments (Table 3). Better aeration in compost pile with twin horizontal perforate pipes may suppress ammonification resulting in less nitrogen loss during composting.

A formation of organic acids resulted in a decrease of pH during composting and an increase of

pH occurred at the completion of composting process (Simandi et al., 2006). This might explain higher pH recorded in treatments with twin horizontal perforate pipes and turning pile, probably indicating higher or better air supply.

One vertical perforate pipe was significantly higher than to other treatments (Table 3). The soluble ions from material such as sodium, chloride, ammonium-nitrogen, sulfates, potassium, calcium and magnesium influence on EC increasing (Thompson, 2005).

Table 3. Chemical properties of Dairy cow manure mixed with rice straw at the beginning of the experiment

	OM (%)	OC (%)	Total N (%)	C:N ratio	pH	EC (dS/m)	Total P (%)
Dairy cow manure + rice straw	58.80	34.11	1.58	21.76	7.79	5.44	1.36

Table 4. Chemical properties of compost at 8 weeks

Treatment	OM (%)	OC (%)	Total N (%)	C:N ratio	pH	EC (dS/m)	Total P (%)
T_1	33.45	19.40	1.26 ^c	15.23	7.83 ^{ab}	4.09 ^{bc}	0.93
T_2	42.01	24.39	1.82 ^a	13.44	7.79 ^{ab}	4.55 ^a	0.86
T_3	36.96	21.45	1.52 ^b	14.07	7.55 ^{bc}	4.81 ^a	0.82
T_4	41.74	24.21	1.49 ^b	16.39	7.13 ^d	4.45 ^{ab}	0.76
T_5	40.14	23.28	1.39 ^{bc}	17.01	7.29 ^{cd}	4.41 ^{ab}	0.82
T_6	32.70	18.97	1.37 ^{bc}	13.95	8.10 ^a	3.90 ^c	0.81
T_7	40.75	23.63	1.22 ^c	19.29	7.66 ^b	4.61 ^a	0.71
Prop.>F	0.3256	0.3257	0.0001	0.0818	0.0001	0.0005	0.2175
F- Test	ns	ns	**	ns	**	**	ns
CV (%)	18.36	18.37	8.39	17.91	2.93	5.53	13.64

Remark : Mean in a column followed by the same letter are not significantly different according to DMRT (p=0.05)

Organic matter

The change in organic content during composting in general is a good indicator of effective composting processes. There are no significant difference among 7 treatments in terms of organic matter content at the end of this study (Table 4).

Table 5. Organic matter content at the beginning of the experiment and at 8 weeks

Treatment	OM at beginning (%)	OM at week 8 (%)	Difference in OM at 0 weeks and at week 8 (%)
T ₁	58.80	33.45	25.35
T ₂	58.80	42.01	16.75
T ₃	58.80	36.96	21.83
T ₄	58.80	41.74	17.06
T ₅	58.80	40.14	18.67
T ₆	58.80	32.70	26.10
T ₇	58.80	40.75	18.05
Prop>F	-	0.3256	0.3256
F- Test	-	ns	ns
CV (%)	-	18.36	34.20

Remark : Mean in a column followed by the same letter are not significantly different according to DMRT (P=0.05)

C/N ratio

There are no significant difference of C/N ratio at the start of this study as well as at week 8. The twin horizontal perforate pipes showed the trend of decreasing of C:N ratio faster than other treatments. Meanwhile static pile (T₇) which represent anaerobic decomposition showed the slowest in term of decreasing rate of C:N ratio. (Table 5)

Table 6. Analysis of variance of C/N ratio

Treatment	C/N at beginning	C/N at week 8	Difference in C/N at the beginning and at week 8
T ₁	21.76	15.23	6.51
T ₂	21.76	13.44	8.29
T ₃	21.76	14.07	7.66
T ₄	21.76	16.39	5.35
T ₅	21.76	17.01	4.72
T ₆	21.76	13.95	7.79
T ₇	21.76	19.29	2.45
Prop.>F	-	0.0818	0.0818
F- Test	-	ns	ns
CV (%)	-	17.91	45.83

Remark : Mean in a column followed by the same letter are not significantly different according to DMRT (p=0.05)

Conclusions

Results of this study indicates that perforate pipes can effectively promote passive aeration in compost piles. It is obvious that passive aeration can be employed in the production of organic fertilizer. However, further study is needed to compare composting efficiency between passive and active aeration. Study of passive aeration for different raw materials as well as improvement of passive aeration are also needed.

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Applied Mathematical Model for Napkin Planning in Restaurant

Sirirat Wongprakornkul¹

Abstract

The objectives of this study were to present the mathematical model for solving the optimum solution which meets the lowest cost of napkin plan and to perform the action plan of napkin-management problem. For generating the mathematical model of napkin-planning problem, network flow analysis was applied to display all flows occurred in the problem which eases to formulate a mathematical model or an integer linear programming model. The objective function of this model was to find out the minimum cost for napkin management with in constrains of known number of customers per day and known number of ways of napkin laundry. The solutions were shown the amount of all napkins and how to manage napkins in each day. In this study, one week napkin planning problem was solved. We found that there are 973 needed napkins per week. First 500 napkins had been bought, so now 473 new napkins were bought more for this week. The minimum napkin cost was 24,465.75 Baht. To comparison with solving with manual by using regular mathematics, the numbers of bought napkins and new napkins were the same, 973 napkins. Furthermore, the napkin cost was 25,231.75 Baht. Obviously, the napkin cost solving with Operations research technique was lower to 766 Baht.

Keywords : Network Flow Analysis, Integer linear Programming, Napkin Planning



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Introduction

System perspective is one of critical values to be high performance organizations. The systematic operating begins with action planning for performing efficiently and effectively. Linear programming and integer linear programming are well known techniques lead into mathematical model formulation of real world problems; such as production planning problem, network flow problem, transportation problem, assignment problem, plant layout problem and etc. These techniques could solve problems within limited resources or constraints like raw materials, men, labors, machines, times, and/or energy. The quest for optimal solution, mathematical model is created with all relations constraints and objective function.

In this paper raised mathematical tools; network flow analysis and integer linear programming, which were applied to do action planning in napkin-planning problem. The purposes of this action plan were to reach the minimum napkin-management cost, to meet customer needs every day, and to know the plan to manage napkins to laundry. There were four steps for solving the problem as mentioned before. First, network flow diagram was created. This step was useful to understand how napkins move in each day. Second, the mathematical model was presented by imitating the network flow diagram in step 1. Third, the model was solved for the optimal solution. And the last one, the solution was managed the napkin plan.

Objectives

- 1) To present the mathematical model for solving the optimum solution or meeting the least of napkin-management cost.
- 2) To perform the action plan of napkin-management problem.

Limitation

This research composes of 2 processes as following:

- 1) Collecting napkin consumption in each day by observing number of customers in 3 periods for seven days then calculating all customers by conducting probability weighted average of number of customer in each period.

- 2) Applying integer linear programming to find out quantity of sent napkin for cleaning in each day and quantity of purchasing for fulfill service by the least of napkin-management cost.

Literature reviews

Nonlinear programming problems with linear constraints were formulated for planning an advertising campaign of goods and services. In this paper said that a generalization of the mathematical model and operations research problems were presented as the framework of an approach for planning (Belenky and Belenkii, 2002).

A multicommodity circulation minimum cost network flow model with side constraints was claimed to increase productivity. It has long been recognized that productivity in manufacturing plants can often be increased by producing similar products in cells. This involves (i) assigning parts to individual machines, and (ii) forming machines into cells. These two activities have traditionally been carried out separately. However, most solution procedures for (i) above utilize a solution to (ii), and vice versa. Here we present a unified approach that deals with (i) and (ii) simultaneously (Foulds and Neumann, 2003).

Dynamic programming techniques were proposed that problems of efficiently responding to a situation in a Command, Control, Communications, and Intelligence (C3I) network can be investigated. An initial model in the form of an integer linear program was decomposed to reveal a recurrence relation, which could then be solved using a method of successive approximations. Because the recurrence relation could not be directly realized as an algorithm, a modified problem was proposed that considers

only those candidate solutions having at most some prescribed number of transmission and transform operations. Solving this for successively increasing numbers of acceptable transforms eventually yields the optimum for the original model. With the understanding that the set of all possible allocations of information to nodes of the network may be infinite, some way of ensuring eventual termination of the algorithm is required. Two possibilities for limiting the state space were explored in this paper; the modified problem can be solved for some suitably large maximal number of operations, or a known upper bound on the optimal cost can be assumed (Reid, 1999).

Mathematical modeling was presented as a theoretical investigation of the influence of blood flow through a tumour-induced capillary network, whereby the vascular architecture adapts as it grows to the associated haemodynamic forces resulting in what we describe as adaptive tumour-induced angiogenesis (ATIA). The network is generated in response to tumour angiogenic factors (TAFs), which are released from hypoxic cells within a solid tumour. First, a refined model was described for tumour-induced angiogenesis, which aims to describe the capillary growth process at the cellular level by explicitly taking into account the effects of matrix degrading enzymes and the local properties of the host tissue during endothelial cell migration. Then, blood rheological properties were incorporated into the formulation and investigate the influence of wall shear stress induced by the blood flow during dynamic vascular growth. After that a number of feedback mechanisms affecting vascular resistance and network architecture were gone on to examine. The results clearly demonstrate that the combined effects of network architecture and vessel compliance should be included in future models of angiogenesis if therapy protocols and treatment efficacy are to be adequately assessed (Stéphanou et al., 2006).

The optimization based heuristic approaches that suggested for solving an integrated one-dimensional

cutting stock-transportation problem (1DCSP&TP) was computed by decomposition principle. A 1DCSP&TP was formed into a mathematical model. Due to a one-dimensional cutting stock problem is a classical NP-hard problem which cannot be solved with the polynomial computation time. Therefore, 1DCSP&TP is an application of large scale problem. Column-Generation Technique and Bender decomposition were applied for creating all possible essential cutting patterns. After that, the proposed heuristic method was raised to solve the problem. The solution of 1D-CSP&TP is necessary for planning production and transportation strategies in the lowest cost (Wongprakornkul and Charnsethikul, 2007).

Results

In this study, one restaurant was a case study for napkin planning. It was found that network analysis could conduct the napkin-management problem as a network flow diagram. The relationship and movement of napkins were shown in the diagram. Furthermore, network and flow of napkin management could be analyzed for specifying parameters to perform integer linear programming. The parameters are detailed below:

1. Daily napkin consumption
2. Napkin laundry method
3. Costs consist of napkin price and cleaning cost.

Data Collecting

Napkin consumption was assumed by number of customers in each day. Data were allowed by the restaurant manager to observe in every 30 minutes for the whole operating period of day, 10:30 to 20:30, totally 10 hours as shown in table 1. On November 28, 2007, there were four periods of the large number of customers in 12:01-12:30, 12:31-13:00, 17:01-17:30, and 17:31-18:00. Other periods, numbers of customers were in low and medium as shown in Figure 1.

Table 1. Number of customers in every 30 minutes

Period	Customers	Period	Customers
10:30-11:00	5	15:31-16:00	5
11:01-11:30	6	16:01-16:30	13
11:31-12:00	14	16:31-17:00	9
12:01-12:30	26	17:01-17:30	18
12:31-13:00	15	17:31-18:00	17
13:01-13:30	6	18:01-18:30	11
13:31-14:00	7	18:31-19:00	14
14:01-14:30	12	19:01-19:30	6
14:31-15:00	6	19:31-20:00	9
15:01-15:30	11	20:01-20:30	4

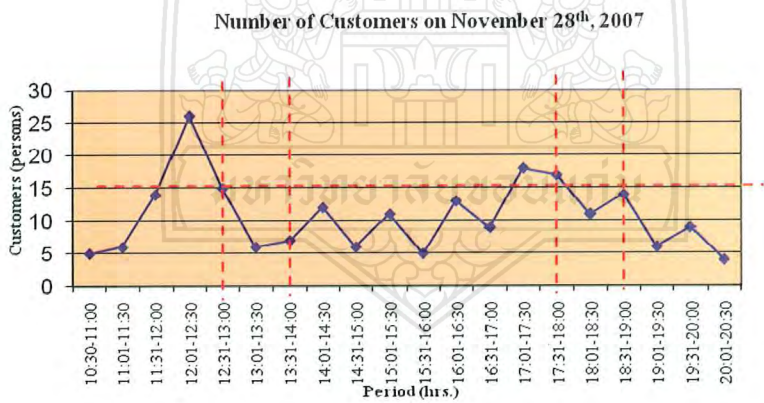


Figure 1. Number of customers

Figure 2 shows periods that are high and low to medium for number of customers. Because of the observations was undertaken in every 30 minutes, there were 20 durations from 10:30 to 20:30. Hence, dividing duration of each period (either high or low to medium) by 20 yielded probability of number of customers for each period as shown in Figure 3.

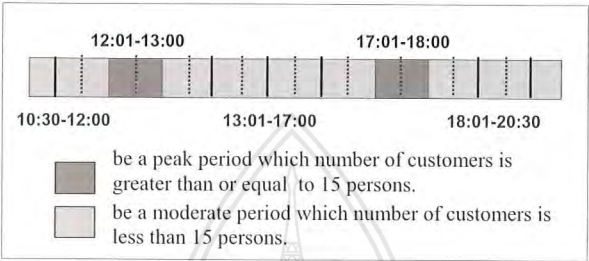


Figure 2. Number of customers in each period

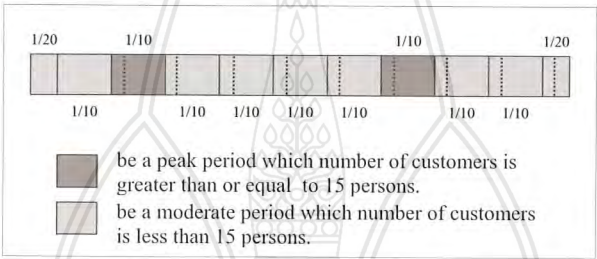


Figure 3. Empirical distribution of number of customers

The probability of high number of customers was 0.2. Whereas, the probability of low to medium number of customers was 0.8. Therefore, number of total customers in each day was calculated by multiplying the probability with the observed number of customers in each period. In this study, the collected data for 7 days from November 30 to December 6, 2007 were divided into 6 periods; 12:01-12:30, 12:31-13:00, 14:31-15:00, 15:01-15:30, 17:01-17-30, and 17:31-18:00 as shown in table 2.

Table 2. Number of customers categorized into 6 periods

Date	Number of customers						Total
	12:01-12:30	12:31-13:00	13:31-15:00	15:01-15:30	17:01-17.30	17:31-18:00	
Nov.30	17	10	8	5	15	8	63
Dec. 1	21	12	4	2	10	16	65
Dec. 2	38	24	27	18	13	24	144
Dec. 3	30	36	16	19	19	27	147
Dec. 4	15	14	10	7	9	15	70
Dec. 5	42	54	31	48	23	33	231
Dec. 6	13	7	6	9	5	9	49

Calculating the number of total customers in each day was conducted by the weighted average technique. The sample of calculation of total customers on November 30, 2007 was shown below:

Total customers =
$$\left[\left(\frac{17+10+15+8}{4} \times 0.2 \right) + \left(\frac{8+5}{2} \times 0.8 \right) \right] \times 20 = 154$$

Total customers from November 30 to December 6 calculated as above were shown in table 3.

Table 3. Number of customers per day

Date	Total customers
Nov. 30	154
Dec. 1	107
Dec. 2	459
Dec. 3	392
Dec. 4	189
Dec. 5*	784
Dec. 6	154

Note: *The Father’s Day (Dec. 5)

To show how efficient of the mathematical model for napkin planning, any kind of data can be employed as the input to the model. Thus, the unusual data of the customers on the Father’s day, which were obviously higher than the other days, could be used. In addition, the input data were used to evaluate the cost of napkin planning by comparison between the mathematical model and manual calculation.

Integer Linear Programming

For easy generating integer linear programming model, network flow diagram was done to see the network and flow of napkin as shown in figure 4.

For example, napkins used for the first and second day were come from 2 sources; the existing and purchasing. Napkins used in the third day were come from the existing, purchasing and fast cleaning of the first day used napkin and the fourth day were the existing, purchasing, fast cleaning of the second day and normal cleaning from the first day.

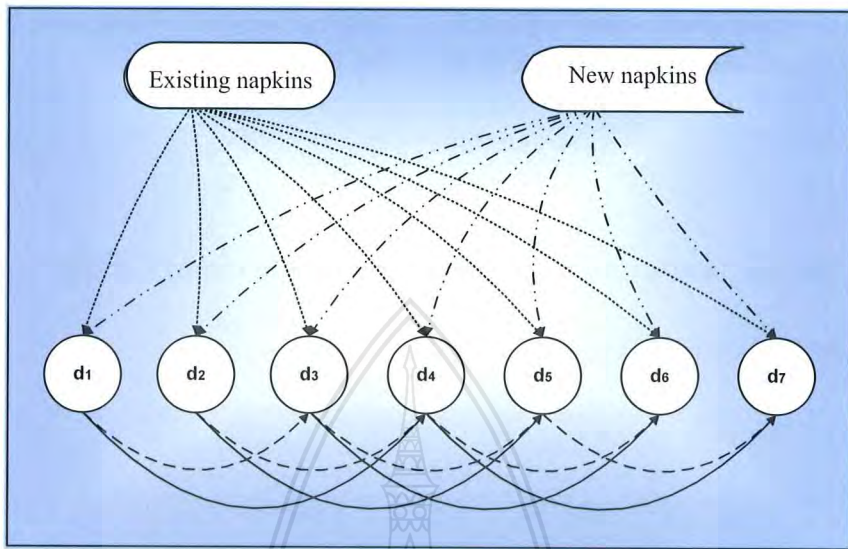


Figure 4. Network flow diagram of napkin planning

Parameters of integer linear programming model for this study were specified by the help of the network flow diagram as the following steps:

1. **Specify decision variables:**

x_i Number of existing napkins used in each day i ; $i = 1, 2, \dots, 7$

y_i Number of purchased napkins used in each day i ; $i = 1, 2, \dots, 7$

r_i Number of fast cleaning napkins (within one day return) used in each day i ; $i = 1, 2, \dots, 5$

p_i Number of normal cleaning napkins (within two day return) used in each day i ; $i = 1, 2, \dots, 4$

2. **Establish objective function:**

Z Cost of napkin management (Baht per week)

3. **Constraints:**

d_i Napkin demand or number of customers in each day i ; $i = 1, 2, \dots, 7$

k Total number of existing napkins

4. **Coefficients of decision variables:**

a Fast cleaning cost (Baht/piece)

b Normal cleaning cost (Baht/piece)

c Napkin price (Baht/piece)

Model of integer linear programming, as shown below, was generated by the network flow diagram and all gathered data which was specified the existing napkin as 500 pieces.

$$\text{Min } Z = 35.75 \sum_{i=1}^7 y_i + 7 \sum_{i=1}^5 r_i + 5 \sum_{i=1}^4 p_i$$

Subject to

$$x_1 + y_1 \geq 154$$

$$x_2 + y_2 \geq 107$$

$$x_3 + y_3 + r_1 \geq 459$$

$$x_4 + y_4 + r_2 + p_1 \geq 392$$

$$x_5 + y_5 + r_3 + p_2 \geq 189$$

$$x_6 + y_6 + r_4 + p_3 \geq 784$$

$$x_7 + y_7 + r_5 + p_3 \geq 154$$

$$x_1 + y_1 - r_1 - p_1 \geq 0$$

$$x_2 + y_2 - r_2 - p_2 \geq 0$$

$$x_3 + y_3 + r_1 - r_3 - p_3 \geq 0$$

$$x_4 + y_4 + r_2 + p_1 - r_4 - p_4 \geq 0$$

$$x_5 + y_5 + r_3 + p_2 - r_5 \geq 0$$

$$x_1 + x_2 + \dots + x_7 = 500$$

$$x_i, y_i, r_i, p_i \geq 0 \text{ are integer; } i = 1, 2, \dots, 7$$

5.3 Processing

By Excel Solver of Microsoft Excel, data for assessing were prepared as shown in figure 5.

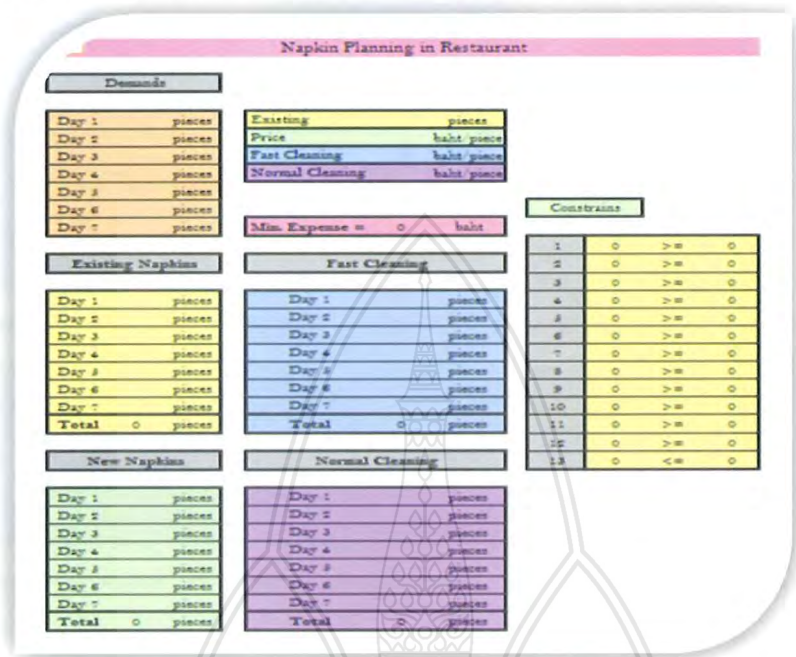


Figure 5. Input window for processing

The lowest cost of the management was 24,465.75 Baht per week as shown in table 3 and its flow diagram was demonstrated in figure 6.

Table 4. Results of the integer linear programming for napkin planning.

Results			
Variable	amount (napkins)	Variable	amount (napkins)
x_1	0	y_6	0
x_2	0	y_7	0
x_3	247	r_1	0
x_4	238	r_2	0
x_5	15	r_3	67
x_6	0	r_4	392
x_7	0	r_5	154
y_1	154	p_1	154
y_2	107	p_2	107
y_3	212	p_3	392
y_4	0	p_4	0
y_5	0	Total cost = 24,465.75 Baht	

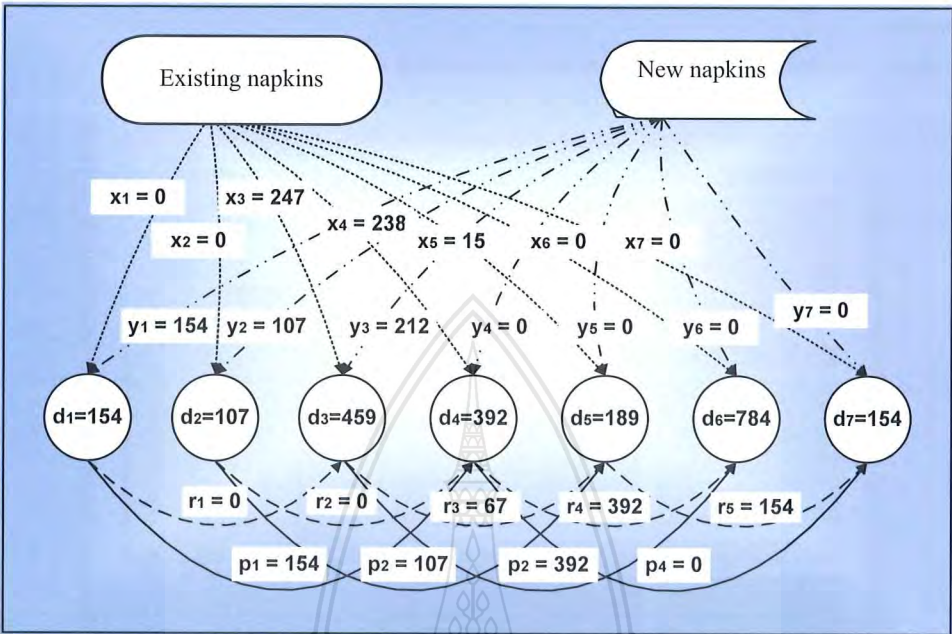


Figure 6. Network flow diagram of napkin-management plan for one week with 500 existing napkins.

After the model was computed, results could clearly interpret to napkin-management plan for one week as shown in table 5. Moreover, the efficiency comparison between processing with integer linear programming model and computing by calculator, under existed napkins need to use first, were shown in table 5 and 6. It was found that the cost of napkin planning with applied mathematical model was cheaper than one with calculator computing.

Table 5. Napkin planning for one week processing through integer linear programming model

Napkins	Exist	New	Extra Laundry	Normal Laundry	Total cost (Baht)
Cost (Baht)	0	35.75	7	5	
Nov. 30	0	154	0	154	6,275.50
Dec. 1	0	107	0	107	4,360.25
Dec. 2	247	212	67	392	10,008.00
Dec. 3	238	0	392	0	2,744.00
Dec. 4	15	0	154	0	1,078.00
Dec. 5	0	0	0	0	0
Dec. 6	0	0	0	0	0
Total	500	473	613	653	24,465.75

Table 6. Napkin planning for one week computing by calculator

Napkins	Exist	New	Extra Laundry	Normal Laundry	Total cost (Baht)
Cost (Baht)	0	35.75	7	5	
Nov. 30	154	0	154	0	1,078
Dec. 1	107	0	107	0	749
Dec. 2	239	66	189	270	5,032.5
Dec. 3	0	285	392	0	12,932.75
Dec. 4	0	0	154	0	1,078
Dec. 5	0	122	0	0	4,361.5
Dec. 6	0	0	0	0	0
Total	500	473	996	270	25,231.75

Conclusions

This study could find out the optimum of napkin management for restaurant by creating network flow diagram to facilitate generating linear programming model. Excel Solver was then used for optimum calculation.

The optimum point that delivers the minimum cost of napkin management is 24,465.75 Baht per week. The current expense is 25,231.75 Baht per week that is 766 Baht per week higher than the application. Linear programming could be applied to increase effectiveness and efficiency of the napkin management.

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Central Quasi Median Estimator when the Sample Size is Odd

Sauwanit Ratanarumkarn¹

Abstract

It is a well known fact that the sample median based on a random sample of size $2k$ is almost as accurate as the one based on the odd size larger by 1 ($2k+1$). Quasi medians, the averages of two symmetrically placed order statistics, were introduced by Hodges and Lehmann (1967) as alternative estimators of the location parameter partially to solve this particular problem. Our paper explores the analogous idea for the data on the circle. We propose CQM - a circular mean of the two most central order statistics as the median estimator that improves the well known Mardia median - a median estimator on a circle introduced by Mardia (1972).

Keywords : Circular median, Mardia median, Quasi median.

Introduction

Circular random variables assume values within $[\alpha, \alpha + 2\pi)$ (with convenient, most common, α choices being 0 and $+\pi$). Because of the importance of rotational invariance most of both distributional and sample concepts are defined differently from the linear data. One of such concept is the median. Because of the no “beginning” and no “end” on the circle, the median was introduced as any ξ_0 such that

$$\int_{\xi_0}^{\xi_0 + \pi} f(\theta) d\theta = \int_{\xi_0 + \pi}^{\xi_0 + 2\pi} f(\theta) d\theta = 1/2 \quad (1)$$

with $f(\xi_0) > f(\xi_0 + \pi)$, where $f(\theta)$ is a density of a continuous distribution on a circle (see Mardia, 1972).

A sample median was introduced by Mardia (1972) as a data point (or a midpoint of two neighboring observations in the case of an even sample size), P , such that:

1. Half of the sample points $\theta_1, \dots, \theta_n$ are on each side of the diameter PQ through P,
2. The majority of the sample points are nearer to P than to Q,
3. The circular mean deviation of $\theta_1, \dots, \theta_n$ from $\theta(\text{CMD}(\theta))$, where θ is either one of the data points, or one of the midpoints in the case of an even data size) is minimized at P.

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The condition 3 was added to practically eliminate cases when the sample median is not unique. $CMD(\theta)$, a circular mean deviation of $\theta_1, \dots, \theta_n$ from any given point θ , is defined as

$$CMD(\theta) = \pi - \frac{1}{n} \sum_{i=1}^n |\pi - |\theta_i - \theta||, \tag{2}$$

and it is one of possible measure of spread on the circle. Other possible measures are circular variance or circular median deviation, and they will be introduced in Section 2.

Minimizing CMD (condition 3) in the case of a large data set can be time consuming and since occasionally there may be more than one point that satisfies conditions 1 and 2, Otieno and Anderson-Cook (2003) proposed as an alternative -the median estimator called New Median (NM) where instead of condition 3 a circular mean of all points θ_p that satisfy conditions 1 and 2 is taken as a point estimate.

Mardia median, if it exists¹, is either a data point, or a midpoint -similarly as on the line. One of similar properties is that its variability is almost not decreasing when the even sample size (2k) is increased by 1 (2k + 1). This is illustrated in Figure 1.

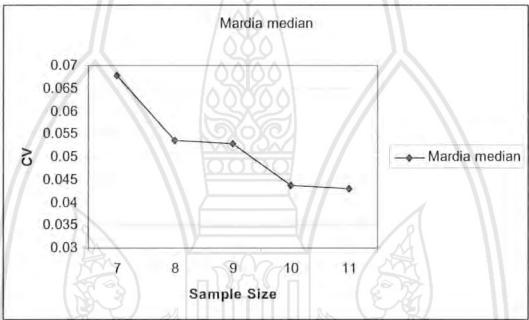


Figure 1. $vM(0, 2)$ based on 10,000 repetitions

Hodges and Lehmann (1967) introduced quasi medians, M_r , as averages of two order statistics of the same “order”, r , when counted from both ends:

$$M_r = \begin{cases} (Y_{m+1-r} + Y_{m+1+r}) / 2, & \text{if } n = 2m + 1 \\ (Y_{m-r} + Y_{m+1+r}) / 2, & \text{if } n = 2m, \end{cases}$$

where $y_1 < y_2 < \dots < y_n$ are the ordered observations, r is fixed, and $2r < n$.

In this paper we propose a new estimator of a circular median, CQM-central quasi median, a circular mean of two observations closest to the Mardia median θ_p : one on its left (θ_{p-1}) and one on its right (θ_{p+1}).

$$\hat{\theta}_{CQM} = circ.mean(\theta_{p-1}, \theta_{p+1}) \tag{3}$$

$$= \arctan \left(\frac{\sin \theta_{p-1} + \sin \theta_{p+1}}{\cos \theta_{p-1} + \cos \theta_{p+1}} \right) \tag{4}$$

¹ There are cases, especially for small data sets, when none of the data points (midpoints) satisfies conditions 1 and/or 2 (see Ratanaruamkarn (2006) for examples)

Such estimator coincides with Mardia median in samples of even size, and has less variability than Mardia median when the sample size is odd.

Properties of estimators on a circle are difficult to study theoretically because of the circular distance defined as

$$\text{dist}(\theta, \gamma) = \min(|\theta - \gamma|, 2\pi - |\theta - \gamma|) \quad (5)$$

$$= \pi - |\pi - |\theta - \gamma||. \quad (6)$$

where $0 \leq \text{dist}(\theta, \gamma) \leq \pi$, and subsequently defined measures of variation. The exception here is the case of von Mises distribution $\text{vM}(\mu, \kappa)$ on a circle that can be approximated by a normal distribution $N(\mu, \frac{1}{\kappa})$ when the concentration

parameter κ is high enough. The statistical analysis can then be, to a large extent, reduced to that on the line. Variance for measurements on the line can be used as a measure of variation and consequently performance of quasi medians is analogous to that described in Hodges and Lehmann (1967).

In our paper we focus on cases when normal approximation can not be used: Small, moderate, and large samples in low or moderate concentration (high variability) cases. In the simulation study we will use data from von Mises (symmetric) distribution also contaminated on location and variation, and skewed distributions -wrapped Laplace and wrapped exponential.

To compare the proposed estimator CQM with MM (Mardia Median) and the NM (New Median) we will be using following measures of variation: circular variance (CV), circular mean deviation (CMD), circular median deviation (CMED), and circular mean error (CME). These measures are defined as follow:

$$CV = 1 - \bar{R} \quad (7)$$

where $\bar{R} = \frac{R}{n}$, $\vec{R} = (\sum_{i=1}^n \cos \alpha_i, \sum_{i=1}^n \sin \alpha_i)$, and

$$R = \|\vec{R}\| = \sqrt{(\sum_{i=1}^n \cos \alpha_i)^2 + (\sum_{i=1}^n \sin \alpha_i)^2},$$

$$CMD(\hat{\theta}_p) = \pi - \frac{1}{n} \sum_{i=1}^n |\pi - |\theta_i - \hat{\theta}_p||, \quad (8)$$

similarly to CMD, we define the circular median deviation as:

$$CMED(\hat{\theta}_p) = \text{median}\{\pi - |\pi - |\theta_i - \hat{\theta}_p||\} \quad (9)$$

and

$$CME(\xi_0) = \pi - \frac{1}{n} \sum_{i=1}^n |\pi - |\theta_i - \xi_0|| \quad (10)$$

where $\theta_i = (\cos \alpha_i, \sin \alpha_i)$, $-\pi \leq \theta_i \leq \pi$, $i = 1, \dots, n$ are data points, $\hat{\theta}_p$ is a point estimate of ξ_0 a true population median.

Simulation Study

In the simulation study data were generated from following circular distributions:

1. von Mises distribution, with probability density function

$$f(\theta) = [2\pi I_0(\kappa)]^{-1} \exp[\kappa \cos(\theta - \mu)], \quad 0 \leq \theta \leq 2\pi \quad (11)$$

where $0 \leq \mu < 2\pi$, $0 \leq \kappa < \infty$ are mean and concentration parameters, respectively, and

$I_0(\kappa)$ is the modified Bessel function of the first kind and of order zero (see Jammalamadaka and SenGupta, 2001).

2. Uniform distribution, with probability density function:

$$f(\theta) = \frac{1}{2\pi}, \quad 0 \leq \theta \leq 2\pi \quad (12)$$

(to be used for contamination).

3. Wrapped exponential distribution with probability density function

$$f(\theta) = \frac{\lambda e^{-\lambda\theta}}{1 - e^{-2\pi\lambda}}, \quad 0 \leq \theta \leq 2\pi. \quad (13)$$

The distribution is not symmetric and the population median direction ($\bar{\theta}$) is

$$\bar{\theta} = \frac{1}{\lambda} \ln \frac{2}{1 + e^{-\lambda\pi}} + I^*(\lambda) \quad (14)$$

$$\text{where } I^*(\lambda) = \begin{cases} 0, & \lambda > 0 \\ \pi, & \lambda < 0. \end{cases}$$

For $\lambda = 0$ wrapped exponential distribution is reduced to uniform distribution in which median can not be determined (see Jammalamadaka and Kozubowski, 2004a).

4. Wrapped generalized Laplace distribution (see Jammalamadaka and Kozubowski, 2004b), with probability density function

$$f(\theta) = \frac{\lambda\kappa}{1 + \kappa^2} \left(\frac{e^{-\lambda\kappa\theta}}{1 + e^{-2\pi\lambda\kappa}} + \frac{e^{(\lambda/\kappa)\theta}}{e^{2\pi\lambda/\kappa} - 1} \right), \quad 0 \leq \theta \leq 2\pi. \quad (15)$$

The population median direction is

$$\xi_0 = \begin{cases} \xi^*, & \lambda > 0, 0 < \kappa < 1 \\ \xi^* + \pi, & \lambda > 0, \kappa > 1 \\ 0, & \kappa = 1, \end{cases} \quad (16)$$

where $\xi^* \in [0, \pi]$ satisfies

$$\frac{1}{1 + \kappa^2} \left(\frac{e^{-\lambda\kappa\xi^*}}{1 + e^{-\lambda\kappa\pi}} + \kappa^2 \frac{e^{\lambda\xi^*/\kappa}}{1 + e^{\lambda\pi/\kappa}} \right) = \frac{1}{2}. \quad (17)$$

We generated samples for consecutive small sizes ($n = 7, 8, 9, 10, 11$), moderate sizes ($n = 19, 20, 21, 22, 23$) and then large sizes ($n = 29, 30, 31, 32, 33$) in the following distributions: symmetric distribution $vM(0, 0.5)$, $vM(0, 2.0)$, distribution contaminated on spread $(1 - \varepsilon)vM(0, \kappa) + \varepsilon U(-\pi, \pi)$, contaminated on location $(1 - \varepsilon)vM(0, \kappa) + \varepsilon vM(\frac{\pi}{4}, \kappa)$, where $\varepsilon = 0.1$, wrapped exponential, and wrapped Laplace. Each sample was generated 10,000 times.

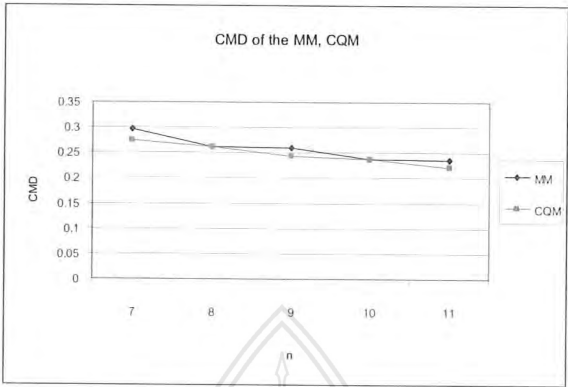


Figure 2. CMD of the MM and CQM for $vM(0, 2.0)$, small sample sizes

Figure 2 illustrates the trend of CMD for Mardia median (MM) and for CQM in the case of small sample sizes. We can see that when we add one more observation from 8 to 9 or 10 to 11, MM's CMD does not decrease as n increases, but the CMD of CQM decreases as n increases.

It can clearly be seen that the variability of CQM estimator is less than that of MM when the sample size is odd. Similar results were obtained for moderate sample sizes, the graph for $n = 19, \dots, 23$ can be seen in Figure 3. For large sample sizes, the graph for $n = 29, \dots, 33$ can be seen in Figure 4.

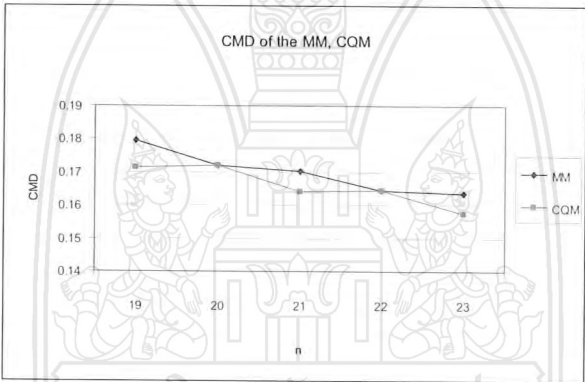


Figure 3. CMD of the MM and CQM for $vM(0, 2.0)$, moderate sample sizes

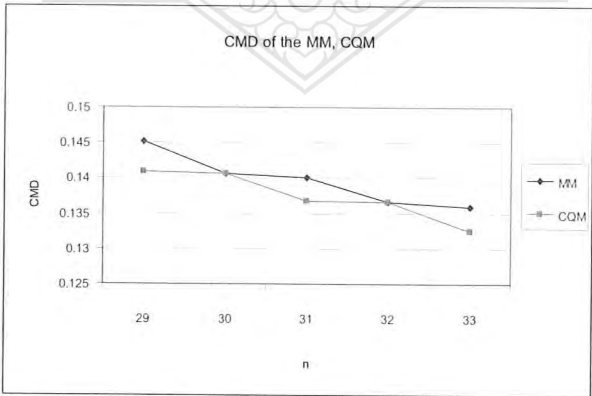


Figure 4. CMD of the MM and CQM for $vM(0, 2.0)$, large sample sizes

Conclusion

Results of the simulation study are given in Tables 1 - 6 in the Appendix. For samples randomly generated from various distributions (symmetric with and without contamination and skewed) we obtained the circular means for each of three point estimators being compared (MM, CQM, and NM), 95% CI for the population median (2.5th and 97.5th percentile of the empirical distribution) and four different measures of variation (circular variance (CV), circular mean deviation (CMD), circular median deviation (CMED), and circular mean error (CME)). The last column in each table provides the number of cases (out of 10,000) in which generated samples satisfied necessary conditions 1 and 2 for the Mardia median to exist. The comparison of the CQM - proposed estimator of a population median, with the Mardia Median was based on three series of sample sizes $n=7, 8, 9, 10, 11$ (small), $n=19, 20, 21, 22, 23$ (moderate) and $n=29, 30, 31, 32, 33$ (large). The performance of the CQM is analogous in all cases although the difference between CQM and Mardia median decreases when n increases. This result was expected since with the increasing size of a sample the expected differences between observations (spacing) decrease. Also it is consistent with the results of Hodges and Lehmann (1967) which imply that the asymptotic efficiency of Mardia Median and CQM equals 1 in the cases when the von Mises distribution on the circle can be approximated by the normal distribution, so when the concentration coefficient K in $vM(\mu, K)$ is high (practically $K > 10$, see Otieno and Anderson-Cook (2003)). It coincides with MM in the case of samples of an even size, and it has smaller variability when the sample size is odd. This trend can be seen in Figures 2 - 4 obtained from three selected sample sizes.

Similarly to quasi medians proposed by Hodges and Lehmann (1967), the CQM is a variability reducing adjustment to Mardia median - estimator of a population median on the circle.

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Appendix

Table 1. MM, CQM, and NM for vM(0,0.5), based on 10,000 random samples.

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	0.006216	(-2.536, 2.531)	0.4826	0.9070	0.7116	0.9070	9654
	CQM	0.006783	(-2.510, 2.575)	0.4761	0.8980	0.6980	0.8980	9654
8	MM	0.013634	(-2.508, 2.461)	0.4630	0.8786	0.6696	0.8787	9992
	CQM	0.013634	(-2.508, 2.461)	0.4630	0.8786	0.6696	0.8787	9992
9	MM	-0.016040	(-2.401, 2.356)	0.4317	0.8398	0.6499	0.8398	9716
	CQM	-0.018190	(-2.380, 2.366)	0.4229	0.8285	0.6415	0.8285	9716
10	MM	0.002027	(-2.331, 2.370)	0.4133	0.8139	0.6170	0.8139	9985
	CQM	0.002027	(-2.331, 2.370)	0.4133	0.8139	0.6170	0.8139	9985
11	MM	0.002084	(-2.160, 2.360)	0.3953	0.7920	0.6100	0.7920	9781
	CQM	-0.002268	(-2.154, 2.271)	0.3864	0.7789	0.5905	0.7789	9781
19	MM	-0.005732	(-1.787, 1.789)	0.2769	0.6281	0.4779	0.6281	9930
	CQM	-0.006596	(-1.768, 1.728)	0.2708	0.6187	0.4659	0.6187	9930
20	MM	-0.001662	(-1.739, 1.673)	0.2641	0.6100	0.4637	0.6100	9982
	CQM	-0.001662	(-1.739, 1.673)	0.2641	0.6100	0.4637	0.6100	9982
21	MM	-0.003810	(-1.673, 1.678)	0.2542	0.5961	0.4550	0.5961	9940
	CQM	-0.004276	(-1.650, 1.699)	0.2506	0.5900	0.4466	0.5901	9940
22	MM	-0.001463	(-1.657, 1.652)	0.2513	0.5910	0.4469	0.5910	9983
	CQM	-0.001463	(-1.657, 1.652)	0.2513	0.5910	0.4469	0.5910	9983
23	MM	-0.007240	(-1.641, 1.573)	0.2358	0.5665	0.4219	0.5666	9948
	CQM	-0.006223	(-1.639, 1.552)	0.2319	0.5605	0.4206	0.5605	9948
29	MM	-0.007584	(-1.365, 1.425)	0.1938	0.5056	0.3849	0.5056	9964
	CQM	-0.007665	(-1.374, 1.402)	0.1919	0.5022	0.3783	0.5022	9964
30	MM	-0.004921	(-1.340, 1.338)	0.1881	0.4970	0.3800	0.4971	9987
	CQM	-0.004921	(-1.340, 1.338)}	0.1881	0.4970	0.3800	0.4971	9987
31	MM	-0.005284	(-1.362, 1.296)	0.1854	0.4948	0.3743	0.4948	9970
	CQM	-0.005378	(-1.357, 1.292)	0.1829	0.4904	0.3747	0.4904	9970
32	MM	0.000209	(-1.275, 1.294)	0.1767	0.4823	0.3783	0.4823	9995
	CQM	0.000209	(-1.275, 1.294)	0.1767	0.4823	0.3783	0.4823	9995
33	MM	0.004152	(-1.242, 1.261)	0.1691	0.4699	0.3662	0.4699	9971
	CQM	0.005068	(-1.224, 1.254)	0.1667	0.4653	0.3610	0.4654	9971

Table 2. MM, CQM, and NM for $vM(0,2,0)$, based on 10,000 random samples

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	-0.004099	(-0.737, 0.742)	0.0678	0.2965	0.2439	0.2965	9992
	CQM	-0.007975	(-0.691, 0.681)	0.0588	0.2737	0.2287	0.2737	9992
8	MM	0.003226	(-0.651, 0.654)	0.0535	0.2625	0.2180	0.2625	10000
	CQM	0.003226	(-0.651, 0.654)	0.0535	0.2625	0.2180	0.2625	10000
9	MM	-0.003647	(-0.646, 0.639)	0.0528	0.2605	0.2167	0.2605	10000
	CQM	-0.001777	(-0.608, 0.586)	0.0463	0.2436	0.2021	0.2436	10000
10	MM	0.004304	(-0.595, 0.593)	0.0437	0.2369	0.1993	0.2369	10000
	CQM	0.004304	(-0.595, 0.593)	0.0437	0.2369	0.1993	0.2369	10000
11	MM	0.002576	(-0.582, 0.590)	0.0431	0.2356	0.1966	0.2356	10000
	CQM	0.000696	(-0.549, 0.553)	0.0384	0.2211	0.1861	0.2211	10000
19	MM	0.003892	(-0.439, 0.436)	0.0248	0.1794	0.1539	0.1794	10000
	CQM	0.002548	(-0.420, 0.415)	0.0227	0.1714	0.1459	0.1714	10000
20	MM	0.001262	(-0.425, 0.427)	0.0232	0.1720	0.1451	0.1720	10000
	CQM	0.001262	(-0.425, 0.427)	0.0232	0.1720	0.1451	0.1720	10000
21	MM	-0.002338	(-0.418, 0.421)	0.0228	0.1702	0.1422	0.1702	10000
	CQM	-0.002920	(-0.407, 0.407)	0.0211	0.1641	0.1372	0.1641	10000
22	MM	-0.000482	(-0.404, 0.402)	0.0209	0.1643	0.1389	0.1643	10000
	CQM	-0.000482	(-0.404, 0.402)	0.0209	0.1643	0.1389	0.1643	10000
23	MM	-0.002854	(-0.412, 0.409)	0.0210	0.1636	0.1377	0.1636	10000
	CQM	-0.002026	(-0.395, 0.397)	0.0195	0.1575	0.1306	0.1575	10000
29	MM	-0.002156	(-0.363, 0.366)	0.0165	0.1452	0.1196	0.1452	10000
	CQM	-0.002535	(-0.350, 0.354)	0.0156	0.1409	0.1181	0.1409	10000
30	MM	-0.003222	(-0.352, 0.341)	0.0155	0.1406	0.1183	0.1406	10000
	CQM	-0.003222	(-0.352, 0.341)	0.0155	0.1406	0.1183	0.1406	10000
31	MM	0.001219	(-0.341, 0.358)	0.0155	0.1400	0.1168	0.1400	10000
	CQM	0.001760	(-0.335, 0.344)	0.0147	0.1367	0.1143	0.1367	10000
32	MM	0.000325	(-0.342, 0.342)	0.0147	0.1366	0.1154	0.1366	10000
	CQM	0.000325	(-0.342, 0.342)	0.0147	0.1366	0.1154	0.1366	10000
33	MM	-0.001295	(-0.341, 0.334)	0.0145	0.1359	0.1127	0.1359	10000
	CQM	-0.001739	(-0.333, 0.326)	0.0138	0.1325	0.1111	0.1325	10000

Table 3. MM, CQM, and NM for contamination on spread: $(1-\epsilon)vM(0, K) + \epsilon U(-\pi, \pi)$, where $\epsilon = 0.1$, $K = 2.0$, based on 10,000 random samples.

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	-0.003283	(-0.850, 0.860)	0.0878	0.3334	0.2708	0.3334	9982
	CQM	-0.003703	(-0.806, 0.824)	0.0806	0.3173	0.2565	0.3174	9982
8	MM	0.002709	(-0.773, 0.773)	0.0726	0.3017	0.2448	0.3017	10000
	CQM	0.002709	(-0.773, 0.773)	0.0726	0.3017	0.2448	0.3017	10000
9	MM	0.000509	(-0.742, 0.752)	0.0683	0.2931	0.2386	0.2931	9989
	CQM	0.000017	(-0.709, 0.712)	0.0626	0.2802	0.2304	0.2802	9989
10	MM	0.003476	(-0.685, 0.675)	0.0582	0.2705	0.2203	0.2705	9999
	CQM	0.003476	(-0.685, 0.675)	0.0582	0.2705	0.2203	0.2705	9999
11	MM	0.000020	(-0.681, 0.651)	0.0544	0.2631	0.2180	0.2631	9999
	CQM	-0.000274	(-0.649, 0.620)	0.0492	0.2497	0.2077	0.2497	9999
19	MM	0.001765	(-0.508, 0.493)	0.0318	0.2013	0.1665	0.2013	10000
	CQM	0.000817	(-0.486, 0.480)	0.0297	0.1945	0.1611	0.1945	10000
20	MM	0.000537	(-0.483, 0.471)	0.0290	0.1920	0.1606	0.1920	10000
	CQM	0.000537	(-0.483, 0.471)	0.0290	0.1920	0.1606	0.1920	10000
21	MM	0.001423	(-0.476, 0.476)	0.0286	0.1907	0.1599	0.1908	10000
	CQM	0.000812	(-0.459, 0.460)	0.0264	0.1830	0.1531	0.1830	10000
22	MM	-0.001914	(-0.456, 0.450)	0.0261	0.1824	0.1520	0.1824	10000
	CQM	-0.001914	(-0.456, 0.450)	0.0261	0.1824	0.1520	0.1824	10000
23	MM	-0.002257	(-0.450, 0.455)	0.0264	0.1830	0.1540	0.1831	10000
	CQM	-0.001408	(-0.442, 0.445)	0.0247	0.1773	0.1477	0.1773	10000
29	MM	-0.000461	(-0.407, 0.405)	0.0207	0.1623	0.1339	0.1623	10000
	CQM	-0.000271	(-0.399, 0.397)	0.0197	0.1577	0.1293	0.1577	10000
30	MM	-0.000901	(-0.394, 0.386)	0.0193	0.1566	0.1313	0.1566	10000
	CQM	-0.000901	(-0.394, 0.386)	0.0193	0.1566	0.1313	0.1566	10000
31	MM	-0.002679	(-0.388, 0.383)	0.0191	0.1561	0.1309	0.1561	10000
	CQM	-0.002525	(-0.382, 0.371)	0.0182	0.1525	0.1274	0.1525	10000
32	MM	-0.001151	(-0.383, 0.382)	0.0181	0.1518	0.1261	0.1518	10000
	CQM	-0.001151	(-0.383, 0.382)	0.0181	0.1518	0.1261	0.1518	10000
33	MM	-0.003893	(-0.375, 0.367)	0.0179	0.1511	0.1269	0.1511	10000
	CQM	-0.003946	(-0.364, 0.360)	0.0171	0.1477	0.1242	0.1478	10000

Table 4. MM, CQM, and NM for contamination on location: $(1-\epsilon)vM(0, K) + \epsilon vM(\frac{\pi}{4}, K)$, here $\epsilon = 0.1, K = 2.0$, based on 10,000 random samples.

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	0.075150	(-0.672, 0.848)	0.0730	0.3078	0.2570	0.3132	9992
	CQM	0.074384	(-0.633, 0.798)	0.0646	0.2881	0.2407	0.2936	9992
8	MM	0.070276	(-0.610, 0.769)	0.0599	0.2774	0.2319	0.2831	10000
	CQM	0.070276	(-0.610, 0.769)	0.0599	0.2774	0.2319	0.2831	10000
9	MM	0.068940	(-0.620, 0.771)	0.0595	0.2770	0.2333	0.2825	9998
	CQM	0.069120	(-0.564, 0.718)	0.0526	0.2600	0.2145	0.2654	9998
10	MM	0.066120	(-0.546, 0.710)	0.0486	0.2485	0.2051	0.2532	10000
	CQM	0.066120	(-0.546, 0.710)	0.0486	0.2485	0.2051	0.2532	10000
11	MM	0.069140	(-0.534, 0.679)	0.0471	0.2464	0.2065	0.2525	10000
	CQM	0.069780	(-0.499, 0.654)	0.0420	0.2327	0.1934	0.2388	10000
19	MM	0.067927	(-0.394, 0.539)	0.0284	0.1900	0.1590	0.1973	10000
	CQM	0.068135	(-0.380, 0.514)	0.0260	0.1822	0.1530	0.1899	10000
20	MM	0.070235	(-0.378, 0.519)	0.0257	0.1813	0.1511	0.1900	10000
	CQM	0.070235	(-0.378, 0.519)	0.0257	0.1813	0.1511	0.1900	10000
21	MM	0.069230	(-0.378, 0.520)	0.0254	0.1801	0.1511	0.1881	10000
	CQM	0.068970	(-0.359, 0.505)	0.0235	0.1733	0.1469	0.1820	10000
22	MM	0.070300	(-0.349, 0.495)	0.0229	0.1712	0.1441	0.1801	10000
	CQM	0.070300	(-0.349, 0.495)	0.0229	0.1712	0.1441	0.1801	10000
23	MM	0.063240	(-0.359, 0.478)	0.0226	0.1699	0.1417	0.1778	10000
	CQM	0.062380	(-0.340, 0.467)	0.0213	0.1651	0.1394	0.1729	10000
29	MM	0.065830	(-0.308, 0.446)	0.0186	0.1542	0.1307	0.1627	10000
	CQM	0.065920	(-0.305, 0.432)	0.0176	0.1498	0.1265	0.1586	10000
30	MM	0.062980	(-0.298, 0.431)	0.0170	0.1472	0.1246	0.1551	10000
	CQM	0.062980	(-0.298, 0.431)	0.0170	0.1472	0.1246	0.1551	10000
31	MM	0.070580	(-0.295, 0.439)	0.0168	0.1466	0.1227	0.1572	10000
	CQM	0.070570	(-0.282, 0.423)	0.0159	0.1422	0.1189	0.1532	10000
32	MM	0.067230	(-0.281, 0.431)	0.0161	0.1438	0.1203	0.1536	10000
	CQM	0.067230	(-0.281, 0.431)	0.0161	0.1438	0.1203	0.1536	10000
33	MM	0.066450	(-0.297, 0.427)	0.0165	0.1448	0.1215	0.1543	10000
	CQM	0.065860	(-0.280, 0.417)	0.0155	0.1407	0.1191	0.1503	10000

Table 5. MM, CQM, and NM for wrapped exponential distribution ($\lambda_1 = \lambda_2$), median = 0.6508 (see equation (14)), based on 10,000 random samples.

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	0.711993	(0.168, 1.648)	0.0708	0.2992	0.2514	0.2958	9993
	CQM	0.747486	(0.218, 1.583)	0.0611	0.2798	0.2369	0.2799	9993
8	MM	0.711102	(0.201, 1.537)	0.0568	0.2686	0.2265	0.2666	10000
	CQM	0.711102	(0.201, 1.537)	0.0568	0.2686	0.2265	0.2666	10000
9	MM	0.699991	(0.197, 1.504)	0.0548	0.2626	0.2213	0.2603	9996
	CQM	0.723654	(0.237, 1.461)	0.0486	0.2483	0.2102	0.2480	9996
10	MM	0.700423	(0.229, 1.437)	0.0467	0.2431	0.2066	0.2411	10000
	CQM	0.700423	(0.229, 1.437)	0.0467	0.2431	0.2066	0.2411	10000
11	MM	0.689688	(0.226, 1.414)	0.0453	0.2399	0.2034	0.2378	10000
	CQM	0.705629	(0.260, 1.381)	0.0408	0.2286	0.1959	0.2277	10000
19	MM	0.671900	(0.304, 1.191)	0.0261	0.1822	0.1561	0.1812	10000
	CQM	0.676500	(0.318, 1.165)	0.0239	0.1743	0.1491	0.1735	10000
20	MM	0.671300	(0.314, 1.154)	0.0236	0.1727	0.1455	0.1717	10000
	CQM	0.671300	(0.314, 1.154)	0.0236	0.1727	0.1455	0.1717	10000
21	MM	0.669600	(0.310, 1.154)	0.0236	0.1724	0.1461	0.1716	10000
	CQM	0.673500	(0.324, 1.134)	0.0220	0.1660	0.1415	0.1655	10000
22	MM	0.674700	(0.328, 1.162)	0.0225	0.1687	0.1420	0.1677	10000
	CQM	0.674700	(0.328, 1.162)	0.0225	0.1687	0.1420	0.1677	10000
23	MM	0.670400	(0.325, 1.148)	0.0218	0.1659	0.1387	0.1651	10000
	CQM	0.673700	(0.337, 1.130)	0.0205	0.1609	0.1347	0.1601	10000
29	MM	0.671100	(0.355, 1.084)	0.0174	0.1481	0.1250	0.1476	10000
	CQM	0.672700	(0.363, 1.063)	0.0164	0.1441	0.1237	0.1437	10000
30	MM	0.669600	(0.360, 1.070)	0.0163	0.1439	0.1221	0.1435	10000
	CQM	0.669600	(0.360, 1.070)	0.0163	0.1439	0.1221	0.1435	10000
31	MM	0.667700	(0.358, 1.059)	0.0158	0.1421	0.1205	0.1418	10000
	CQM	0.670300	(0.367, 1.057)	0.0151	0.1385	0.1171	0.1382	10000
32	MM	0.667700	(0.364, 1.045)	0.0151	0.1395	0.1200	0.1390	10000
	CQM	0.667700	(0.364, 1.045)	0.0151	0.1395	0.1200	0.1390	10000
33	MM	0.666800	(0.365, 1.038)	0.0150	0.1384	0.1168	0.1380	10000
	CQM	0.668100	(0.374, 1.036)	0.0143	0.1354	0.1151	0.1350	10000

Table 6. CQM, and NM for wrapped Laplace distribution, $\lambda_1 = 2.0$, $\lambda_2 = 0.5$, $p = 0.2$, median = -0.6147 (see equation (16)), based on 10,000 random samples.

Sample Size	Measure	Point Est	95% CL	CV	CMD	CMED	CME	n
7	MM	-0.625056	(-2.300, 1.130)	0.2539	0.5981	0.4606	0.5976	9823
	CQM	-0.651496	(-2.238, 1.087)	0.2440	0.5809	0.4381	0.5802	9823
8	MM	-0.630077	(-2.245, 1.064)	0.2289	0.5604	0.4297	0.5598	9995
	CQM	-0.630077	(-2.245, 1.064)	0.2289	0.5604	0.4297	0.5598	9995
9	MM	-0.611741	(-2.151, 0.729)	0.2035	0.5239	0.4094	0.5241	9877
	CQM	-0.629788	(-2.090, 0.766)	0.1974	0.5124	0.3915	0.5116	9877
10	MM	-0.610140	(-2.102, 0.618)	0.1904	0.5032	0.3866	0.5035	9994
	CQM	-0.610140	(-2.102, 0.618)	0.1904	0.5032	0.3866	0.5035	9994
11	MM	-0.603219	(-1.971, 0.474)	0.1687	0.4741	0.3775	0.4749	9937
	CQM	-0.618973	(-1.910, 0.493)	0.1618	0.4613	0.3637	0.4611	9937
19	MM	-0.610100	(-1.664, 0.145)	0.1047	0.3670	0.3021	0.3673	9987
	CQM	-0.613500	(-1.656, 0.142)	0.0999	0.3577	0.2911	0.3578	9987
20	MM	-0.603800	(-1.579, 0.151)	0.0953	0.3502	0.2901	0.3508	10000
	CQM	-0.603800	(-1.579, 0.151)	0.0953	0.3502	0.2901	0.3508	10000
21	MM	-0.604300	(-1.601, 0.118)	0.0955	0.3509	0.2908	0.3517	9990
	CQM	-0.608200	(-1.592, 0.113)	0.0920	0.3436	0.2846	0.3441	9990
22	MM	-0.604200	(-1.584, 0.101)	0.0915	0.3433	0.2857	0.3442	9997
	CQM	-0.604200	(-1.584, 0.101)	0.0915	0.3433	0.2857	0.3442	9997
23	MM	-0.611600	(-1.578, 0.091)	0.0871	0.3350	0.2796	0.3353	9987
	CQM	-0.614300	(-1.555, 0.081)	0.0839	0.3278	0.2737	0.3279	9987
29	MM	-0.601300	(-1.454, 0.024)	0.0704	0.3011	0.2525	0.3021	9996
	CQM	-0.603100	(-1.424, 0.020)	0.0679	0.2956	0.2485	0.2965	9996
30	MM	-0.603200	(-1.427, 0.007)	0.0653	0.2898	0.2431	0.2906	9999
	CQM	-0.603200	(-1.427, 0.007)	0.0653	0.2898	0.2431	0.2906	9999
31	MM	-0.595100	(-1.403, 0.022)	0.0639	0.2867	0.2404	0.2883	9998
	CQM	-0.597300	(-1.383, 0.004)	0.0620	0.2823	0.2382	0.2837	9998
32	MM	-0.602500	(-1.400, 0.008)	0.0627	0.2842	0.2378	0.2852	9999
	CQM	-0.602500	(-1.400, 0.008)	0.0627	0.2842	0.2378	0.2852	9999
33	MM	-0.598900	(-1.356, -0.008)	0.0597	0.2789	0.2373	0.2801	10000
	CQM	-0.600900	(-1.342, -0.017)	0.0581	0.2753	0.2349	0.2763	10000

Simple Proof of Determining All Nonisomorphic Monoids of Order 3

Somchit Chotchaisthit¹

Abstract

In this paper, we use only elementary basic concepts to find all nonisomorphic monoids of order 3. It turns out that there are only 7 nonisomorphic monoids of order 3.

Keywords : Monoids

To test whether a given Cayley table is associative is not an easy task. As a consequence, it is difficult to find all nonisomorphic semigroups (or monoids) of a given order. One way to solve the above problem is using a computer. In this paper, we use only elementary basic concepts to find all nonisomorphic monoids of order 3.

It is well-known (Hebisch and Weinert, 1998) that there are only 5 nonisomorphic semigroups $(\{a,b\},*)$ of order 2 as follows :

*	a	b
a	a	a
b	a	a

S_1

Zero semigroup

*	a	b
a	a	b
b	b	a

S_2

group of residues modulo 2

*	a	b
a	a	b
b	b	b

S_3

semilattice semigroup

*	a	b
a	a	a
b	b	b

S_4

Left zero semigroup

*	a	b
a	a	b
b	a	b

S_5

Right zero semigroup

From the above tables, S_2 and S_3 are the only monoids of order 2. Note that both S_2 and S_3 are commutative. If we add an identity element 1 to S_1, S_2, S_3, S_4, S_5 , then we obtain nonisomorphic monoids M_1, M_2, M_3, M_4, M_5 :

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<table><tr><td>*</td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>l</i></td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td></tr><tr><td><i>b</i></td><td><i>b</i></td><td><i>a</i></td><td><i>a</i></td></tr></table> M_1	*	<i>l</i>	<i>a</i>	<i>b</i>	<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<table><tr><td>*</td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>l</i></td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>b</i></td><td><i>b</i></td><td><i>b</i></td><td><i>a</i></td></tr></table> M_2	*	<i>l</i>	<i>a</i>	<i>b</i>	<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>a</i>	<table><tr><td>*</td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>l</i></td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td></tr><tr><td><i>b</i></td><td><i>b</i></td><td><i>b</i></td><td><i>b</i></td></tr></table> M_3	*	<i>l</i>	<i>a</i>	<i>b</i>	<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<table><tr><td>*</td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>l</i></td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td></tr><tr><td><i>b</i></td><td><i>b</i></td><td><i>b</i></td><td><i>b</i></td></tr></table> M_4	*	<i>l</i>	<i>a</i>	<i>b</i>	<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<table><tr><td>*</td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>l</i></td><td><i>l</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>a</i></td><td><i>a</i></td><td><i>a</i></td><td><i>b</i></td></tr><tr><td><i>b</i></td><td><i>b</i></td><td><i>a</i></td><td><i>b</i></td></tr></table> M_5	*	<i>l</i>	<i>a</i>	<i>b</i>	<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>
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Consider the following Cayley tables M_6 and M_7 .

*	<i>l</i>	<i>a</i>	<i>b</i>
<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>
<i>a</i>	<i>a</i>	<i>b</i>	<i>l</i>
<i>b</i>	<i>b</i>	<i>l</i>	<i>a</i>

M_6

*	<i>l</i>	<i>a</i>	<i>b</i>
<i>l</i>	<i>l</i>	<i>a</i>	<i>b</i>
<i>a</i>	<i>a</i>	<i>l</i>	<i>b</i>
<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>

M_7

Evidently, M_6 is a group isomorphic to Z_3 (the group of residues modulo 3). Moreover, it is not isomorphic to M_1, M_2, M_3, M_4, M_5 . However, M_7 has an identity 1 and a zero element b. To show that M_7 is associative, it is enough to check that $a * (a * a) = (a * a) * a$, which is obviously true, so M_7 is a monoid.

Note that if two tables of two monoids of order 3 are different, then the only isomorphism between these monoids must map a to b and b to a .

Suppose that M_7 is isomorphic to one of $M_i (i=1, 2, \dots, 6)$. Then $l = f(1) = f(a * a) = f(a) * f(a) = b * b$ and $b * b \neq l$ in $M_i (i=1, 2, \dots, 6)$. Therefore M_7 is not isomorphic to $M_i (i=1, 2, \dots, 6)$.

Now we show that any monoid of order 3 is isomorphic to one of $M_i (i=1, 2, \dots, 7)$.

Let $M = \{1, a, b\}$ and assume that $(M, *)$ is a monoid with identity 1. So we obtain a table A. We consider the following 3 cases according to the assignments of $a * a$.

<table><tr><td>*</td><td>l</td><td>a</td><td>b</td></tr><tr><td>l</td><td>l</td><td>a</td><td>b</td></tr><tr><td>a</td><td>a</td><td></td><td></td></tr><tr><td>b</td><td>b</td><td></td><td></td></tr></table> A	*	l	a	b	l	l	a	b	a	a			b	b			<table><tr><td>*</td><td>l</td><td>a</td><td>b</td></tr><tr><td>l</td><td>l</td><td>a</td><td>b</td></tr><tr><td>a</td><td>a</td><td>l</td><td></td></tr><tr><td>b</td><td>b</td><td></td><td></td></tr></table> B_1	*	l	a	b	l	l	a	b	a	a	l		b	b			<table><tr><td>*</td><td>l</td><td>a</td><td>b</td></tr><tr><td>l</td><td>l</td><td>a</td><td>b</td></tr><tr><td>a</td><td>a</td><td>a</td><td></td></tr><tr><td>b</td><td>b</td><td></td><td></td></tr></table> B_2	*	l	a	b	l	l	a	b	a	a	a		b	b			<table><tr><td>*</td><td>l</td><td>a</td><td>b</td></tr><tr><td>l</td><td>l</td><td>a</td><td>b</td></tr><tr><td>a</td><td>a</td><td>b</td><td></td></tr><tr><td>b</td><td>b</td><td></td><td></td></tr></table> B_3	*	l	a	b	l	l	a	b	a	a	b		b	b		
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Case 1: $a * a = 1$ (table B_1).

Note that $a * (a * b) = (a * a) * b = 1 * b = b$, so $a * b$ cannot be equal to 1 or a . Thus $a * b = b$. Since $a * (b * a) = (a * b) * a = b * a$, it follows that $b * a = b$. Hence $b * b = (a * b) * b = a * (b * b)$, $b * b$ cannot equal 1 or a . Thus $b * b = b$. Now B_1 is fulfilled as follows:

*	1	a	b
1	1	a	b
a	a	1	b
b	b	b	b

B_1

Case 2: $a * a = a$ (table B_2).

Note that $a * (a * b) = (a * a) * b = a * b$. If $a * b = 1$, then $a * 1 = 1$. This is impossible. Thus $a * b = a$ or $a * b = b$. So we consider 2 subcases.

Subcase 2.1: $a * b = a$. We have $(b * a) * a = b * (a * a) = b * a$. In case $b * a = 1$, then $1 * a = 1$. This is impossible. Thus $b * a = a$ or $b * a = b$.

If $b * a = a$, then $b * b$ can be 1 or a or b (table B_{21}, B_{22}, B_{23}).

If $b * a = b$, then $b * b = (b * a) * b = b * (a * b) = b * a = b$ (table B_{24}).

Subcase 2.2: $a * b = b$. We have $b * a = b * (a * a) = (b * a) * a$. In the case that $b * a = 1$, we have $1 = 1 * a$. This is impossible. Thus $b * a = a$ or $b * a = b$.

If $b * a = a$, then $b * b = b * (a * b) = (b * a) * b = a * b = b$ (table B_{25}).

If $b * a = b$, then $b * b = (a * b) * b = a * (b * b)$. Moreover $b * b \neq 1$ otherwise $1 = a * 1$. Thus $b * b = a$ or $b * b = b$ (table B_{26} and table B_{27}).

*	1	a	b
1	1	a	b
a	a	a	a
b	b	a	1

B_{21}

*	1	a	b
1	1	a	b
a	a	a	a
b	b	a	a

B_{22}

*	1	a	b
1	1	a	b
a	a	a	a
b	b	a	b

B_{23}

*	1	a	b
1	1	a	b
a	a	a	a
b	b	b	b

B_{24}

*	1	a	b
1	1	a	b
a	a	a	b
b	b	a	b

B_{25}

*	1	a	b
1	1	a	b
a	a	a	b
b	b	b	a

B_{26}

*	1	a	b
1	1	a	b
a	a	a	b
b	b	b	b

B_{27}

Case 3: $a * a = b$ (table B_3).

Note that $a * b = a * (a * a) = (a * a) * a = b * a$ and $b * b = (a * a) * b = a * (a * b)$. So we consider 3 subcases.

Subcase 3.1: $a * b = b * a = 1$. Then $b * b = a * 1 = a$ (table B_{31}).

Subcase 3.2: $a * b = b * a = a$. Then $b * b = a * a = b$ (table B_{32}).

Subcase 3.3: $a * b = b * a = b$. Then $b * b = a * b = b$ (table B_{33}).

*	1	a	b
1	1	a	b
a	a	b	1
b	b	1	a

B_{31}

*	1	a	b
1	1	a	b
a	a	b	a
b	b	a	b

B_{32}

*	1	a	b
1	1	a	b
a	a	b	b
b	b	b	b

B_{33}

Finally, we can prove $B_{21} \cong B_1, B_{23} \cong B_{27}, B_{32} \cong B_{26}, B_{33} \cong B_{22}$. Moreover $B_1 = M_7, B_{27} = M_3, B_{26} = M_2, B_{22} = M_1, B_{24} = M_4, B_{25} = M_5$ and $B_{31} = M_6$. Now we have the following theorem.

Theorem Up to isomorphisms, $M_1, M_2, M_3, M_4, M_5, M_6$ and M_7 are the only monoids of order 3.

From the theorem, the following consequence is directly obtained.

Corollary Up to isomorphisms, M_1, M_2, M_3, M_6 and M_7 are the only commutative monoids of order 3.

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Screening of Monocot Lectins in Vietnam and Using Them in Bacteria Typing

Bui Phuong Thuan^{1*}, Le Quy Thuong¹

Abstract

In our studies, 102 different plant extractions belong to 13 widely distributed monocot families in Vietnam were used to screening lectin activity. 54 species of them (53 %) have shown hemagglutination activity towards rabbit red blood cells (but not human ABO erythrocytes). These species belong to 8 families: Amaryllidaceae, Alliaceae, Orchidaceae, Arecaceae, Araceae, Commelinaceae, Zingiberaceae and Poaceae (the monocot lectins isolated world-wide until now have been from only 4 first families).

The lectin extractions were being investigated in ability to type bacteria which are food- poisoning and harmful to human.

Namely, they were used to differentiate 4 bacterial species belong to *Staphylococcus* (*Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus* and *Staph. xylosus*), as well as to distinguish them from other bacteria such as *Salmonella*, *Shigella* or *Escherichia coli*. The results obtained showed that certain lectins can recognise selectively each bacteria under study. For example, 2 lectins (from *Zephyranthes rosea* (Spreng) Lindl. and *Typhonium trilbatum* Schott) can recognise *Staph. aureus*, meanwhile 4 others (from *Narcissus tazetta* var. *chinesis*, *Crinum asiaticum*, *Cymbidium aloifolium* (L.) Sw. and *Allium schoenoprasum* L.) can agglutinate only *Staph. Epidermidis* Moreover, the combination of lectins with different characterizations could enable the typing of these bacteria.

We were also able to build a classification scheme for bacteria typing and use these key lectins to distinguish different bacteria by a simple test with quite rapid results.

Keywords : Lectins, Agglutination, Bacteria typing.

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Introduction

Are defined as carbohydrate-binding proteins, lectins can recognise and bind specifically and reversibly to certain mono- or oligosaccharides, or also glycans of different glycoconjugates. Because of this unique and exclusive characteristics, lectins have become very useful tools in glycoconjugate research as well in histochemistry, histopathology (Etsler, 1986).

Lectin-cell binding can elicit a wide variety of biological phenomena. Lectins are significant reagents for investigating cell surface receptors, and have been used to fractionate different cell kinds. The interactions of plant lectins with microorganisms have been applied for the typing of bacteria, fungi, and protozoa (Bui Phuong Thuan et al., 2000; Calderon et al., 1997).

Microbial surfaces bear many of the sugar residues capable of interacting with lectins. Lectins are attractive reagents for the clinical diagnostic laboratory because of their diverse specificity, commercial availability, high specific activities, wide range of molecular weights and stability in standard buffers (Calderon et al., 1997; Munoz-Crego et al., 1999). The specificity of lectin binding to bacterial surface carbohydrates has been reported and exploited as a tool for typing microorganisms such as *Neisseria*, *Legionella*, *Bacillus*, *Campylobacter*, *Helicobacter* (Khin et al., 2000; Malcolm and Doyle, 1990). The construction of lectin biosensor could be an advantage method for detection of pathogenic bacteria (Masarova et al., 2004). Lectins are distributed ubiquitously in nature ranging from microorganisms to the plants and animals (Etsler, 1986). At present there is a wide interest in the monocot lectins because of their valuable specificities such as antimicrobial, antiviral activities (van Damme et al., 1995; van Damme et al., 1996; Naval et al., 1992). Unlike all other plant lectins studied

thus far, the monocot mannose-binding lectins are encoded by large families of closely related genes, and the expression of different lectin genes from species to species can explain the occurrence of multiple lectin isoforms at the protein level (van Damme et al., 1998).

In the study, we have focused on the screening of monocot lectin source from our rich tropical flora and on using these lectins for typing of some foodborne harmful bacteria in quite a simple but useful method.

Material and methods

Materials

Plants (including stems, leaves, seeds, roots, tubers, shoot) were collected from different regions in the North of Vietnam. Rabbit (species *Oryctolagus cuniculus*) erythrocytes were washed thoroughly before use. Bacteria used in the study were obtained from the Vietnam Type Culture Collection, National Institute of Hygiene and Epidemiology, some were isolated from food (including raw materials and cooked food) obtained from Hanoi markets. They are harmful and cause food-borne diseases for human (Olsen et al., 1993): *Salmonella typhimurium*; *Shigella flexneri*; *E. coli*; *Bacillus subtilis*; *Staphylococcus* (*Staph. aureus*; *Staph. epidermidis*; *Staph. saprophyticus*; *Staph. xylosus*).

Methods

Crude extracts of lectins were prepared from collected monocotplant materials. **Hemagglutination assay:** Hemagglutination assays were carried out in microtitre plates. A total volume of 100 μL was used in each well: 50 μL aliquots of serial twofold dilutions of the lectin in 150 mM NaCl and 50 μL of 4% rabbit erythrocyte suspension. The microtitre plate with the 100 μL erythrocyte suspension, containing the serial

double dilutions of the sample lectin, was incubated for 1 h at room temperature. The lowest concentration required to completely agglutinate the red blood cells was determined visually. *Bacteria agglutination assay*: Bacteria were grown on propriated medium, harvested, treated and diluted in PBS up to the density of 1.0 at 540 nm. Bacteria solution was mixed with lectin solution on the glass slide, incubation at room temperature for 2 to 3 h, examination visually of the wells for agglutination (or precipitation)

Results and discussion

Investigation of lectin activity in monocot plants

In the study, 102 different plant extractions from species belong to 13 families were used to investigate the hemagglutinating activity. These families including: Amaryllidaceae, Alliaceae, Agavaceae, Iridaceae, Iridaceae, Dioscoreaceae, Zinggiberaceae, Cannaceae, Musaceae, Orchidacea, Poaceae, Arecaceae and Araceae. They are widely distributed monocot families in Vietnam.

Table 1. The monocot plants with highest lectin activity

No	Families	Species	Common names	Parts studied
1	Amaryllidaceae	<i>Narcissus tazetta</i> var. <i>chinensis</i> Roem.	French daffodil	Stem
2		<i>Haemanthus multiflorus</i> (Tratt.) Martyn ex Willd	Bunga Desember (or Blood Lily)	Leaf
3		<i>Zephyranthes rosea</i> (Spreng) Lindl.	Cuban zephyrlily	Tuber
4		<i>Zephyranthes ajax</i> Hort.		Tuber
5	Alliaceae	<i>Allium fistulosum</i> L.	Welsh onion	Tuber
6		<i>Allium schoenoprasum</i> L.	Wild chives	Tuber
7	Commelinaceae	<i>Tradescantia spathacea</i> Sw.	Oyster Plant	Tuber
8	Zinggiberaceae	<i>Alpinia galanga</i> (L.) Sw.	Greater galangal, Chewing John	Rhizome
9		<i>Alpinia purpurata</i> (Vieill.) K. Schum	Red ginger	Rhizome
10		<i>Zingiber rubens</i> Roxb.	Red ginger	Rhizome
11		<i>Kaempferia galanga</i>	Aromatic ginger	Rhizome

12	Orchidaceae	<i>Dendrobium thyrsiflorum</i> Reichb. f.	The Pine Raceme Dendrobium	Stem
13		<i>Coelogyne lawrenceana</i> Rolfe		Stem
14		<i>Coelogyne prolifera</i> Lindl	Schultes' Coelogyne	Stem
15		<i>Dendrobium lindleyi</i>	Dendrobium	Stem
16	Poaceae	<i>Bambusa stenostachya</i> Hack.	Spiny bamboo	Shoot
17		<i>Bambusa blumeana</i> J. H. Schultes	Bamboo	Shoot
18		<i>Nehouzeana dulloa</i>		Shoot
19	Arecaceae	<i>Livistona chinensis</i> (Jacq.)	Fountain palm	Seed
20	Araceae	<i>Alocasia indica</i> Schott		Tuber
21		<i>Colocasia esculenta</i> (L.) Schott	Elephant's ears,	Tuber
22		<i>Colocasia india</i> Harsk.	Taro	Tuber
23		<i>Alocasia odora</i> C.Koch.		Tuber
24		<i>Typhonium trilobatum</i> (L.) Schott		Tuber
25		<i>Typhonium flagelliforme</i> (Lodd.) Bl.		Tuber
26		<i>Colocasia gigantea</i> (Bl.) Hook. f		Tuber

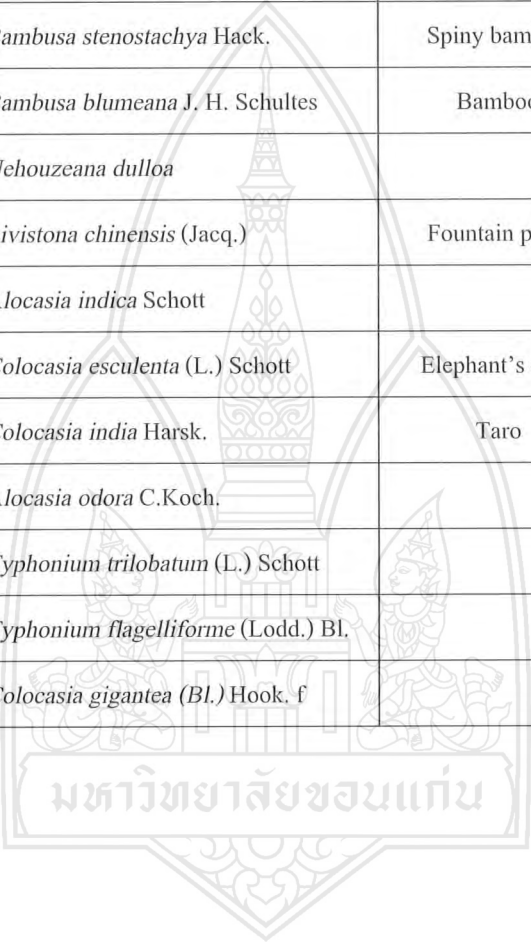


Table 2. Lectin interations with different bacteria belonging to various genus

No	Lectins	Salmonella typhimurium					Shigella flexneri				Escherichia coli				Bacillus cereus				
		St1	St2	St3	St4	St5	St3	St5	St6	St7	Ec 1	Ec 2	Ec 3	Ec 4	Bc 1	Bc 2	Bc 3	Bc 4	Bc 5
1	Crinum amabile Don	-	-	-	-	-	+	2+	2+	+	+	+	+	+	-	-	-	-	-
2	Crinum asiaticum	-	-	-	-	-	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-
3	Polianthes tuberosa	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-
4	Zephyranthes rosea	2+	2+	2+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Allium cepa L.	-	-	-	-	-	-	-	-	-	+	+	2+	2+	-	-	-	-	-
6	Allium sativum L.	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-
7	A. schoenoprasum	+	2+	2+	-	-	+	+	+	+	-	-	-	-	+	+	+	2+	2+
8	Belamcanda chinensis	-	-	-	-	-	+	-	2+	2+	-	-	+	+	-	-	-	-	-
9	Amomum aromaticum	-	-	-	-	-	+	+	2+	+	-	-	-	-	-	-	-	-	-
10	Kaempferia galanga	-	-	-	-	-	2+	2+	2+	+	+	+	+	-	-	-	-	-	-
11	Zingiber rubens	-	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
12	Coelegyne psestrantha	-	-	-	-	-	+	+	+	+	-	+	2+	2+	2+	2+	2+	2+	2+
13	Cymbidium aloifolium	+	2+	2+	2+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Dendrobium faulhaberianum	-	-	-	-	-	2+	2+	2+	+	+	2+	2+	2+	2+	2+	2+	2+	2+
15	Dendrobium aloifolium	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Oryza sativa L.	-	-	-	-	-	2+	2+	2+	2+	2+	2+	2+	2+	-	-	-	-	-
17	Bambusa blumeana	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	2+
18	Livistona saribus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2+	2+	+
19	Amorphophallus konjac	-	-	-	-	-	+	+	2+	2+	+	2+	2+	2+	-	-	-	-	-
20	Colocasia esculenta	-	-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	2+	2+	+
21	C. esculenta var. antiquorum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	2+	2+
22	Caladium bicolor	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
23	Colocasia india	-	-	-	-	-	-	-	-	-	3+	3+	3+	3+	-	-	-	-	-
24	Typhonium trilbatum	-	-	-	-	-	+	2+	2+	2+	-	+	+	2+	-	-	-	-	-
25	Typhonium blumei	+	+	+	+	2+	2+	2+	2+	2+	+	+	+	+	-	-	-	-	-
26	Typhonium flagelliorne	+	+	2+	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-
27	Alocasia odora	-	-	-	-	-	3+	3+	3+	3+	3+	2+	+	2+	-	-	-	-	-
28	Epipremnum pinnatum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	2+	2+	2+	2+
29	Haemanthus multiflorus	-	-	-	-	-	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-
30	Zephyranthes ajax	-	-	-	-	-	2+	2+	2+	2+	2+	2+	2+	2+	-	-	-	-	-

No	Lectins	Salmonella typhimurium					Shigella flexneri				Escherichia coli				Bacillus cereus				
		St1	St2	St3	St4	St5	Sf3	Sf5	Sf6	Sf7	Ec 1	Ec 2	Ec 3	Ec 4	Bc 1	Bc 2	Bc 3	Bc 4	Bc 5
31	Zingiber zerumbet	-	-	-	-	-	2+	2+	2+	2+	+	+	+	+	-	-	-	-	-
32	Languas galanga	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: +, 2+, 3+: agglutination degrees; - : non- agglutination
St1 - St5- *Samonella typhimurium* strains; Sf3, Sf5, Sf6, Sf7- *Shigella flexneri* strains
Ec1 - Ec4- *Escherichia coli* strains; Bc1 - Bc5- *Bacillus cereus* strains

The obtained results show that 54/102 species (53 %) have shown hemagglutination activity towards rabbit red blood cells (but not human ABO erythrocytes). These species belong to 8 families: Amaryllidaceae, Alliaceae, Orchidaceae, Arecaceae, Araceae, Commelinaceae, Zinggiberaceae and Poaceae. Plants with highest lectin activity are presented on the Table 1.

The agglutination activity is especially high in two families: Amaryllidaceae with 8/10 (80 %) and Araceae with 13/17 (76,5 %) studied species contain lectin. This finding is in concordance with those of other authors, maybe because of rather high protein content of these plants (about 40 % - lectins are the most prominent proteins in the tuber tissue) (van Damme et al., 1995, Naval et al., 1992). Evidence was also presented that a previously described major storage protein of Colocasia tubers corresponds to the lectin (van Damme, 1996)

Among the 48 species which have no hemagglutinating activity, all the Iridaceae species caused haemolysis.

One of the important applications of lectins in microbiology is direct aggregation of suspended microorganisms.

In the study, the agglutinating activity of various crude lectin extractions on bacteria has been tested. Based on the fact that lectins can agglutinate selectively bacteria species or even strains within one species (depending on the oligosaccharide components and glycoprotein structures on their surfaces), the combination of certain lectins can enable to type these bacteria.

The results obtained on the Table 2 and Table 3

showed that lectins from differents plants have different effect on bacteria. Based on these differences, an identification key was built for the investigated bacteria in order to distinguish them.

The identification key on Figure 1 is used to differentiate 5 bacteria species which belong to various genus: *Salmonella typhimurium*, *Shigella flexneri*, *E. coli*, *Bacillus cereus*, *Staphylococcus* (with 4 species *Staph. aureus*; *Staph. epidermidis*; *Staph. saprophyticus*; *Staph. xylosus*).

As shown on the identification key schema: *Zingiber zerumbet* lectin can divide 5 tested bacteria into 2 groups: agglutinated (+) (Ist group, composed of Sf, Ec) and unagglutinated (-) (IInd group, composed of Sta, St, Bs).

In the Ist group, owinging to the difference in agglutinating activity, *Haemanthus multiflorus* lectin can help to distinguish *Shigella flexneri* (Sf) (positive reaction, (+) from *E. coli* (Ec) (negative reaction, (-)

For the IInd group, *L. galanga* lectin agglutinates only *Salmonella typhimurium* (St), while *E. pinnatum* lectin can distinguish *Bacillus cereus* (Bc) (+) from *Staphylococcus* (Sta) (-).

Further, among 4 Shtaphylococus species, *K. galanga* can differentiate *Staph. aureus* (Sa), *Staph. xylosus* (Sx) (Ist group, +) from *Staph. epidermidis* (Se), *Staph. saprophyticus* (Ss) (IInd group, -), and *Z. rosea* types *Staph. aureus* (+) from *Staph. xylosus* (-), whenever *N. tazetta* recognises only *Staph. epidermidis* (+) but not *Staph. saprophyticus* (-).

Agglutination effect of lectins on bacteria belonging to various genus

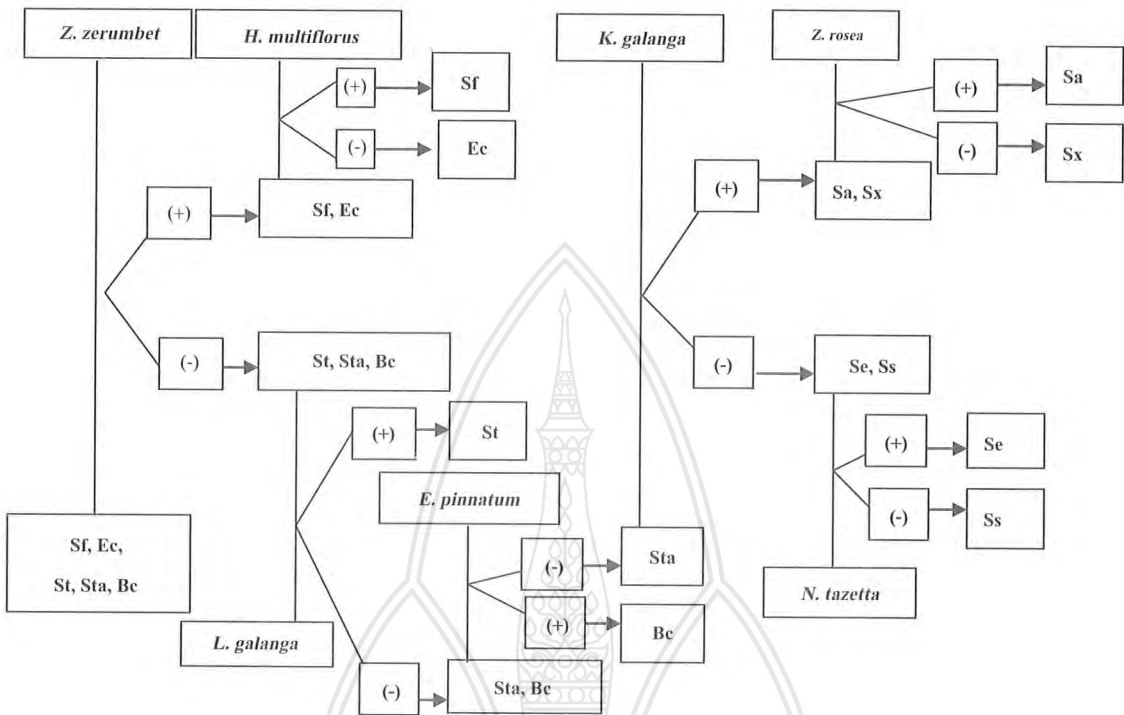


Figure 1. Overall classification key of investigated bacteria

Note- 1) The bacteria abbreviations: *St*: *Salmonella typhimurium*; *Sf*: *Shigella flexneri*; *Ec*: *E. coli*; *Bc*: *Bacillus cereus*; *Sta*: *Staphylococcus*; *Sa*: *Staph. aureus*; *Se*: *Staph. epidermidis*; *Ss*: *Staph. saprophyticus*; *Sx*: *Staph. xylosus*
2) The lectin abbreviations: *Z. zerumbet*: *Zingiber zerumbet*; *H. multiflorus*: *Haemanthus multiflorus* (Tratt.) Martyn ex Willd; *L. galanga*: *Languas galanga*; *E. pinnatum*: *Epipremnum pinnatum* (L.) Engl.; *K. galanga*: *Kaempferia galanga*; *Z. rosea*: *Zephyranthes rosea* (Spreng) Lindl.; *N. tazetta*: *Narcissus tazetta* var. *chinesis*.
3) (+) : agglutination ; (-) : non- agglutination

By this way, the tested bacteria were typed with the help of a set of 7 lectins: *Zingiber zerumbet*, *Haemanthus multiflorus* (Tratt.) Martyn ex Willd, *Languas galanga*, *Epipremnum pinnatum* (L.) Engl., *Kaempferia galanga*, *Zephyranthes rosea* (Spreng) Lindl., *Narcissus tazetta* var. *chinesis*.

Agglutination effect of lectins on Staphylococcus species

The results on the Table 3 showed that one set of specific lectins can be used to differentiate

the *Staphylococcus* species under study. Namely, *Zephyranthes rosea* and *Typhonium trilbatum* lectin can recognise *Staph. aureus*. 4 lectins from *Narcissus tazetta*, *Crinum asiaticum*, *Allium schoenoprasum* L. and *Cymbidium aloifolium* can be combined to distinguish *Staph. epidermidis*. *C. esculenta* var. *antiquorum* lectin can be used to recognise *Staph. saprophyticus*, while 3 lectins (*Polianthes tuberosa*, *Allium cepa* L., *Coelegyne psestrantha*) can recognise specifically *Staph. xylosus*.

Table 3. Agglutination effect of lectins on different *Staphylococcus* species

No	Lectins	Staphylococcus																	
		S. aureus					S. epidermidis					S.saprophyticus				S. xylosus			
		a1	a2	a3	a4	a5	e1	e2	e3	e4	e5	s1	s2	s3	s4	11	13	14	15
1	Narcissus tazetta	-	-	-	-	-	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	-
2	Crinum amabile	-	-	-	-	-	2+	2+	2+	2+	2+	2+	2+	2+	2+	+	+	+	2+
3	Crinum asiaticum	-	-	-	-	-	2+	+	+	2+	-	-	-	-	-	-	-	-	-
4	Polianthes tuberosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	2+	3+
5	Zephyranthes rosea	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Allium cepa L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	+	2+
7	Allium tuberosum	-	-	+	+	+	-	-	2+	+	-	+	+	+	+	+	2+	2+	2+
8	Allium schoenoprasum	-	-	-	-	-	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	-
9	Allium sativum	-	-	-	-	-	-	2+	-	+	-	-	-	-	-	-	-	-	-
10	Amomum aromaticum	-	+	+	+	-	-	-	3+	-	+	-	-	-	-	2+	2+	2+	2+
11	Kaempferia galanga	+	2+	2+	2+	2+	-	-	-	-	-	-	-	-	-	+	+	2+	2+
12	Zingiber rubens	-	-	-	-	-	2+	2+	2+	3+	+	+	+	2+	-	+	2+	2+	2+
13	Coelegyne psestrantha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	2+	2+
14	Cymbidium aloifolium	-	-	-	-	-	2+	2+	2+	+	+	-	-	-	-	-	-	-	-
15	Spathoglottis plicata	+	+	+	+	+	2+	2+	2+	2+	2+	+	+	+	+	-	-	-	-
16	Oryza sativa L.	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	2+	2+
17	Bambusa stenostachya	-	-	-	-	-	+	2+	2+	2+	+	-	-	-	-	+	+	+	+
18	Bambusa blumeana	-	-	-	-	-	2+	2+	2+	+	-	-	-	-	-	2+	2+	2+	2+
19	Livistona chinensis	-	-	-	-	-	-	-	-	-	3+	-	-	-	-	2+	2+	2+	2+
20	Amorphophallus konjac	2+	+	+	-	-	-	-	-	-	-	+	+	+	-	2+	2+	-	-
21	Colocasia esculenta	2+	2+	2+	+	-	-	-	-	-	-	-	-	-	-	2+	2+	+	+
22	Caladium bicolor	+	+	-	+	+	-	-	-	+	+	+	+	+	+	-	-	+	+
23	Typhonium trilbatum	+	2+	2+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
24	Typhonium flagelliorme	-	-	-	-	-	-	-	2+	-	-	+	+	+	+	-	-	-	-
25	Alocasia odora	+	+	+	-	-	-	-	-	-	-	+	+	2+	2+	-	-	+	+

Note: +, 2+, 3+: agglutination degrees; - : non- agglutination
a1, a2, a3, a4, a5 : *Staphylococcus aureus*; e1, e2, e3, e4, e5 : *Staphylococcus epidermidis*
s1, s2, s3, s4 : *Staphylococcus saprophyticus* ; 11, 13, 14, 15 : *Staphylococcus xylosus*

The identification of *Staphylococcus epidermidis*

Based on the differences in ability to be agglutinated by lectins, an identification key was also built in order to distinguish 5 studied *Staphylococcus epidermidis* strains.

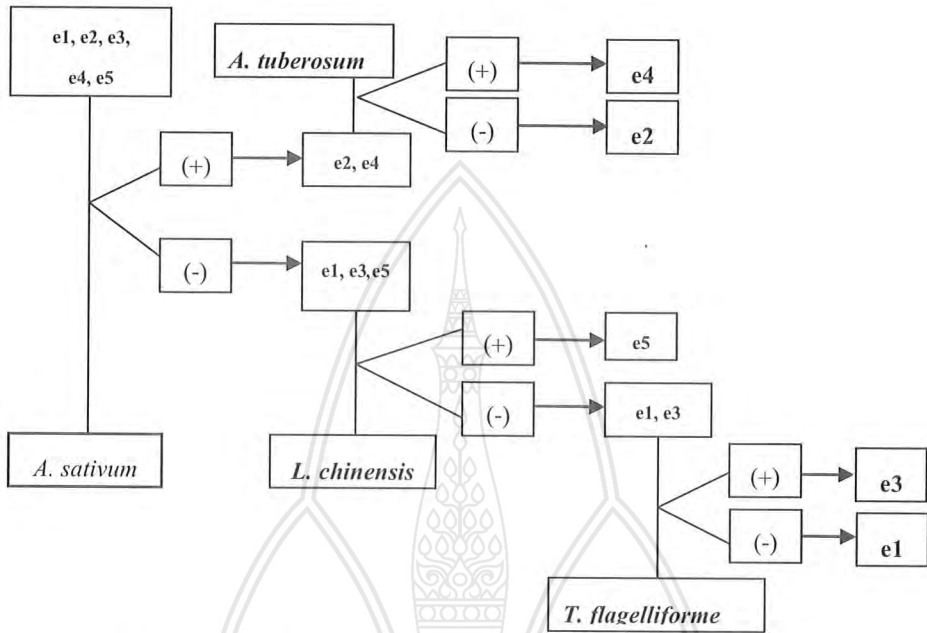


Figure 2. Classification key for *Staphylococcus epidermidis* strains

Note: e1; e2; e3; e4; e5- *Staph. epidermidis* strain symbols

As shown on the identification key schema on the Figure 2 *A. sativum* lectin can divide 5 tested *Staphylococcus epidermidis* strains into 2 groups: agglutinated (+) (Ist group, composed of strains e2 and e4) and unagglutinated (-) (IInd group, composed of strains e1, e3 and e5).

In the Ist group, owing to the difference in agglutinating activity, *A. tuberosum* lectin can help to distinguish strains e4 (+) from strains e2 (-).

For the IInd group, *L. chinensis* lectin agglutinates only strains e5, whenever *T. flagelliforme* lectin can distinguish strains e3 (+) from e1 (-)

By this way, a set of 4 lectins (obtained from *A. sativum*, *A. tuberosum*, *L. chinensis* and *T. flagelliforme*) need to be used to distinguish all 5 tested *Staphylococcus epidermidis* strains.

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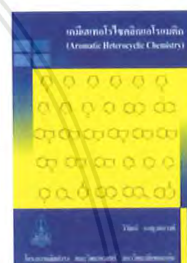
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