UNIVERSITI PUTRA MALAYSIA

BIOLOGY OF Limnocharis flava (L.) BUCHENAU AND neptunia oleracea LOUR. AND THEIR STATUS AS VEGETABLE CROPS IN SARAWAK, MALAYSIA

NOORASMAH SAUPI

FPSM 2014 2

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for Degree of Doctor Philosophy of Science

February 2014

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Abstract of thesis presented to Senate of the Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

BIOLOGY OF *Limnocharis flava* (L.) BUCHENAU AND *Neptunia oleracea* LOUR. AND THEIR STATUS AS VEGETABLE CROPS IN SARAWAK, MALAYSIA

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Chairman : Assoc. Prof. Dr. Muta Harah Zakaria @ Ya, PhD
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The invasion and fast growth of aquatic macrophytes, *Limnocharis flava* (L.) Buchenau and *Neptunia oleracea* Lour. caused problems in rice fields and drainage systems. However, they are also offered for sale in native markets and being consumed as leafy vegetables among local urban peoples mainly in central region of Sarawak, Malaysia. These aquatic weeds are harvested from the wild, and there has been no attempt to cultivate them. Hence, this research evaluates their distribution and determine their ecological and morphological characteristics, i.e., habitats and plant adaptations to environments. These information were used for observational and detailed studies on developmental stages, crop and yield patterns, and the availability of these vegetables in native markets and further evaluates their nutritive status.

Limnocharis flava were recorded growing in various habitats of 32 locations, i.e., township and residential ditches, oil palm plantation irrigation and roadside drainage systems. Based on Principal Component Analysis (PCA) of the various environmental parameters, three distinct environments formed the growing sites of *L. flava*, i.e., group A – areas with pH 5.01 – 5.50 and high water temperature, 33.01 – 34.00°C, group B – areas with low water temperature, 27.01 – 28.00°C that flow from roadside drainage system and group C – areas with slow moving water in wide drainage system, 2.0 – 2.5 m with water depth of 1.0 – 1.5 m. Plants grew densely in ditch which had comparatively high concentration of dissolved PO_4^{3-}, NH_3^- and NO_2^- and total N and C, available P, K, Na, Mg and Mn in substrate, e.g., at Public Library Mukah and possessed relatively longer petiole and sheath length, and also bigger blade, floral structures, fruit and seed. There are three types of plant life forms, i.e., submerged, emergent and semi aquatic or marginally. The submerged life form was observed from seed germination to the juvenile plant stage and rarely occurred in the adult mature plant. The submerged juvenile plants' leaves lacked stomata on both surfaces. The plant propagated through seed and plantlet. The plant developed from seed to reproductive plant within 20 to 28 days that involved seven developmental stages, i.e., swollen seed, germinating seed, seedling, juvenile plant with plumular leaves, juvenile plant with petiolate leaves, mature vegetative and reproductive plants. New plantlet emerges in the

middle of the umbel inflorescence of the reproductive plant which then grew into new vegetative and reproductive plants.

For *N. oleracea,* the plants showed two life forms as adaption to the environments, i.e., terrestrial (at Kg Medong and Kg Kekan) and floating (at Kg Penipah). However, the terrestrial life form is less common. The terrestrial plant had woodier stem than the floating plant. The stems were enveloped by thick white aerhenchyma tissues formed when stem is in contact with water within 6 to 8 days. It grew densely in the ditch which had comparatively high concentration of dissolved NO_2^-, NO_3^- and NH_3^- and total N and available Na in substrate, e.g., at Kg Penipah. The plant propagated through seed and stem cutting. Six developmental stages were observed during development from seed to reproductive plant which took 24 to 26 days, i.e., swollen seed, germinating seed, seedling, juvenile plant, mature vegetative and reproductive plants. Auxiliary buds were also observed at the stem of *N. oleracea* which then developed into new vegetative and reproductive plants.

Limnocharis flava and *N. oleracea* can be propagated in created environment, e.g., in tank. NO_3^- or a combination of nutrient (NO_2^-, NO_3^- and NH_3^-) responsible in the increased in number of leaf and inflorescence, blade length and width, and petiole diameter of *L. flava* propagated from seed and plantlet. As for propagation of *N. oleracea,* only NO_3^- was responsible in the increased in length of plant grown from seed and stem cutting. Seven harvesting activities at two weeks interval performed after five weeks of transplanting showed there were no differences in the yield of *L. flava* shoots. For *N. oleracea* propagation from seed and stem cutting, allowed eight harvesting activities at one week interval after five weeks transplanting also showed there were no differences in the yield of shoots.

In the evaluation of plant availability in native markets, *L. flava* were available in the months of January to April, June to July and October in Sibu central market and in February to March in Bintulu tamu. As for *N. oleracea,* it was available only in April and October in Sibu central market. The periodic availability of these plants in native markets was attributed to the preference of other high profitable commodities, e.g., *Durio zibethinus* and endemic fruit, *Canarium odontophyllum*. Tender shoots comprising leaves and inflorescences were consumed raw or blanched or stir-fried. The proximate composition and mineral content analysis revealed that, both *L. flava* and *N. oleracea* possessed high moisture content of 83.75 – 94.59%. *Limnocharis flava* was also high in crude fat content (0.12 – 0.39%), while *N. oleracea* was identified as having high in protein content (3.01 – 3.23%). The tank culture is the favorable method for continuously production instead of gathering the plants from the wild. However, it is necessary to evaluate the annual production of these species at bigger-scale experiments. Other analyses on vitamins, anti-oxidants, anti-nutritional and toxicological are important to evaluate other usefulness of the plant properties.

Abstrak tesis dipersembahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOLOGI *Limnocharis flava* (L.) BUCHENAU DAN *Neptunia oleracea* LOUR. DAN STATUS MEREKA SEBAGAI TANAMAN SAYURAN DI SARAWAK, MALAYSIA

Oleh

NOORASMAH BINTI SAUPI

Pengerusi : Prof. Madya Dr. Muta Harah Zakaria @ Ya
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Penaklukan dan pertumbuhan pantas dua makrofit akuatik, *Limnocharis flava* (L.) Buchenau dan *Neptunia oleracea* Lour. menyebabkan masalah di sawah padi dan sistem perparitan. Walau bagaimanapun, ia juga dijual di pasar tempatan (tamu Sarawak) dan dimakan sebagai sayuran berdaun di kalangan penduduk bandar terutamanya di kawasan tengah Sarawak, Malaysia. Rumpai akuatik ini dituai dari kawasan alamiah, dan belum ada lagi percubaan untuk menanam mereka. Oleh itu, kajian ini menilai taburan dan menentukan ciri-ciri ekologi dan morfologi iaitu habitat dan adaptasi tumbuhan kepada persekitaran. Maklumat ini digunakan untuk pemerhatian dan kajian terperinci pada peringkat perkembangan, corak penanaman dan hasilan, dan keterdapatan sayur-sayuran ini di pasar tamu dan seterusnya menilai status khasiatnya.

Limnocharis flava direkodkan tumbuh di pelbagai habitat di 32 lokasi iaitu parit perbandaran dan perumahan, sistem saliran ladang kelapa sawit dan perparitan sisi jalan. Berdasarkan kepada Analisis Komponen Utama (PCA) pelbagai parameter persekitaran, tiga persekitaran berbeza membentuk tempat pertumbuhan *L. flava* iaitu kumpulan A – kawasan dengan pH 5.01 – 5.50 dan suhu air yang tinggi, 33.01 – 34.00°C, kumpulan B – kawasan dengan suhu air yang rendah 27.01 – 28.00°C yang mengalir dari sistem perparitan sisi jalan dan kumpulan C – kawasan dengan air yang mengalir perlahan dalam sistem perparitan yang lebar, 2.0 – 2.5 m dengan kedalaman 1.0 – 1.5 m. Tumbuhan tumbuh dengan padat dalam parit yang mempunyai secara perbandingannya kepekatan tinggi PO_4^{3-}, NH_3^- and NO_2^- terlarut dan jumlah N dan C, P, K, Na, Mg dan Mn tersedia dalam substrat contohnya di Perpustakaan Awam Mukah dan tumbuhan mempunyai tangkai daun dan upih daun yang panjang, dan juga helai daun, struktur bunga, buah dan biji yang lebih besar. Terdapat tiga jenis bentuk kehidupan iaitu tenggelam, termuncul dan separa akuatik atau marginal. Bentuk kehidupan tenggelam telah diperhatikan daripada peringkat percambahan biji ke tumbuhan juvenile dan jarang berlaku dalam tumbuhan dewasa yang matang. Daun tumbuhan juvenil tidak mempunyai stomata di kedua-dua permukaan. Tumbuhan dibiak melalui biji dan plantlet tumbuhan. Tumbuhan berkembang daripada biji kepada tumbuhan reproduktif dalam tempoh 20 hingga 28 hari yang melibatkan tujuh peringkat perkembangan iaitu

biji membengkak, biji bercambah, anak benih, tumbuhan juvenil dengan daun plumular, tumbuhan juvenil dengan daun bertangkai, tumbuhan vegetatif matang dan tumbuhan reproduktif. Plantlet baharu muncul di tengah jambak bunga umbel tumbuhan reproduktif yang kemudian berkembang menjadi tumbuhan vegetatif.

Bagi *N. oleracea,* tumbuhan menunjukkan dua bentuk kehidupan sebagai adaptasi kepada persekitaran iaitu daratan (di Kg Medong dan Kg Kekan) dan terapung (di Kg Penipah). Walau bagaimanapun, bentuk kehidupan daratan adalah jarang ditemui. Tumbuhan daratan mempunyai batang lebih berkayu berbanding dengan tumbuhan terapung. Batang tumbuhan kedua-dua bentuk kehidupan diselaputi oleh tisu arenkima putih tebal yang terbentuk apabila tersentuh dengan air dalam jangkamasa 6 hingga 8 hari. Ia tumbuh dengan padat dalam parit yang mempunyai secara perbandingannya kepekatan NO_2^-, NO_3^- dan NH_3^- terlarut yang tinggi dan jumlah N dan Na tersedia dalam substrat contohnya di Kg Penipah. Tumbuhan membiak melalui biji dan keratan batang. Enam peringkat perkembangan diperhatikan semasa tumbesaran daripada biji kepada tumbuhan reproduktif yang mengambil masa 24 hingga 26 hari iaitu biji membengkak, biji bercambah, anak benih, tumbuhan juvenil, tumbuhan vegetatif matang dan tumbuhan reproduktif. Tunas sisi juga diperhatikan pada batang *N. oleracea* yang kemudiannya berkembang menjadi tumbuhan vegetatif baharu.

Limnocharis flava dan *N. oleracea* dapat dibiak dalam persekitaran yang diolah contohnya dalam tangki. NO_3^-, atau kombinasi nutrien (NO_2^-, NO_3^- dan NH_3^-) bertanggunjawab dalam peningkatan bagi bilangan daun dan jambak bunga, panjang dan lebar helai daun, dan diameter tangkai daun *L. flava* yang ditanam daripada biji dan plantlet. Untuk penanaman *N. oleracea,* hanya NO_3^- bertindak dalam peningkatan panjang tumbuhan yang tumbuh daripada biji dan keratan batang. Tujuh aktiviti penuaian selang dua minggu dilakukan selepas lima minggu pemindahan tanaman menunjukkan tidak terdapat perbezaan dalam hasilan pucuk *L. flava*. Untuk penanaman *N. oleracea* dari biji dan keratan batang, lapan aktiviti penuaian selang dua minggu dilakukan selepas lima minggu pemindahan tanaman telah juga menunjukkan tidak terdapat perbezaan dalam hasilan pucuk.

Dalam penilaian keterdapatan tumbuhan di pasar tamu, *L. flava* terdapat pada bulan Januari hingga April, Jun hingga Julai dan Oktober di pasar sentral Sibu dan Februari hingga Mac di tamu Bintulu. Bagi *N. oleracea,* ia terdapat hanya dalam bulan April dan Oktober di pasar sentral Sibu. Keterdapatan berkala tumbuhan ini di pasar tamu adalah disebabkan oleh keutamaan diberi kepada komoditi yang memberi keuntungan tinggi, contohnya *Durio zibethinus* dan buah endemik, *Canarium odontophyllum*. Pucuk lembut yang mengandungi daun dan jambak bunga dimakan secara mentah atau dicelur atau digoreng kering. Analisis komposisi proksimat dan kandungan mineral menunjukkan bahawa kedua-dua *L. flava* dan *N. oleracea* mempunyai kandungan kelembapan

yang tinggi iaitu 83.75 – 94.59%. *Limnocharis flava* juga tinggi dalam kandungan lemak (0.12 – 0.39%), manakala *N. oleracea* dikenalpasti mempunyai kandungan protein yang tinggi (3.01 – 3.23%). Kaedah penanaman menggunakan tangki adalah sesuai untuk pengeluaran kedua-dua spesies secara berterusan menggantikan kaedah pemungutan tumbuhan dari kawasan alamiah. Walau bagaimanapun, adalah perlu juga untuk menilai pengeluaran tahunan spesies ini dalam eksperimen berskala yang besar. Analisis lain terhadap kandungan vitamin, anti-oksida, anti-nutrisi dan toksikologi adalah penting untuk menilai kandungan khasiat tumbuhan.

ACKNOWLEDGMENTS

Alhamdulillah, Praise to Allah with his bestowed guidance, I was able to submit this thesis.

My deepest and sincere gratitude toward my supervisor, Assoc. Prof. Dr. Muta Harah Binti Zakaria @ Ya and and co supervisor Prof. Dr. Japar Sidik Bin Bujang who gave much of their valuable experiences in theirs work, assisting with the ground truth and not forgetting their co-operation, support, encouragement, their constructive comments and suggestions from an early draft to the end which improved the manuscript. Not forgetting the help and support from Prof. Dr. Aziz Bin Arshad as my co supervisor.

This study is part of a larger study on Ethnobotanical Study on Aquatic Macrophytes Used by Indigenous Peoples under the Science Fund, Ministry of Science and Technology 04-01-04-SF0864: Vote No. 5450405. Many thanks also to the Malaysian Ministry of Higher Education (MOHE) and Universiti Putra Malaysia (UPM) for awarding me the scholarship and financial support for my study. I am also grateful to the Faculty of Agriculture and Food Sciences for providing facilities and assistance for this study.

Foremost, I would also like to show appreciation towards my family especially my father Saupi Bin Amit, my husband Abd Karim Bin Mohamad and my daughter Nurin Faqihah Binti Abd Karim; who supported, constantly counseled and persistently helped me throughout this study. Thanks to all sisters and brother, my friends for their tremendous effort, assistance and also their suggestions and comments to enable the report to be completed suggestions.

I certify that a Thesis Examination Committee has met on 21 February 2014 to conduct the final examination of Noorasmah Binti Saupi on her thesis entitled "Biology of *Limnocharis flava* (L.) Buchenau and *Neptunia oleracea* Lour. and their status as vegetable crops in Sarawak, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor Philosophy of Science in Agronomy.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor Philosophy. The members of the Supervisory Committee are as follows:

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LIST OF ABBREVIATIONS AND SYMBOLS

e.g.	for example
i.e.	that is
No.	number
Jln	Jalan
Kg	Kampung
Bt	Batu
Sg	Sungai
Tg	Tanjung
LAKU	Lembaga Air Kawasan Utara
SJK	Sekolah Jenis Kebangsaan
SMK	Sekolah Menengah Kebangsaan
SPAD	Sarawak Plantation Agriculture Sdn. Bhd.
UPMKB	Universiti Putra Malaysia Campus Bintulu Sarawak

CHAPTER 1

GENERAL INTRODUCTION

Aquatic macrophytes are considered as those large plants which grow in excessive amounts in a continuous supply of water or at least present in soils which are covered with water during a major part of the growing season and interfered with the intended usage of particular area (Weldon *et al.,* 1973; Edwards, 1980). They are divided into four categories based on their life form, namely floating, submerged, emergent and marginal (Edwards, 1980; Mashhor, 1988; Said *et al.*, 1991; Muta Harah *et al.,* 2005; Closs *et al.*, 2006; Singh, 2008).

The high density and population of aquatic macrophytes and their ability for vegetative growth in drainage systems, ponds, reservoirs and paddy fields can cause problems to the humans in terms of in agriculture, health, hydroelectricity dams, reduced water quality and recreational purposes (Table 1.1). For that reason, many researchers considered aquatic macrophytes as invasive or unwanted and refer to them as "aquatic weeds" (Weldon *et al.,* 1973; Edwards, 1980; Said *et al.,* 1991; Mashhor, 1994). One of the best controls of weeds is by utilizing them (Mashhor, 1988). Various reports have been documented on their uses mainly in waste treatment management, agriculture, foods and paper pulps (Table 1.2). As for example, in West Malaysia, 56 species had been used as medicinal plant, 32 species for feed plants, 27 species for vegetables, 16 species for aquarium and 14 species for green manure (Said *et al.,* 1991).

The major pathways involving aquatic macrophytes for food production in South East Asia is shown in Figure 1.1. Basically, aquatic macrophytes provided three types of foods to human being, the foliage for green vegetables, grain or seeds for protein, starch and oil and swollen fleshy roots for starch (National Academy of Sciences, 1976).

Previous studies on the uses of aquatic macrophytes in Sarawak showed that 31 species are edibles, 11 species are use for medicinal, 12 species for fodder, 19 species for ornamental, 2 species for food wrapper, 3 species for paper and mat and 2 species for bio-filter (Muta Harah *et al.,* 2005). Twenty three of freshwater macrophytes are reported utilized for food (Suzalina Akma, 2008; Dayangku Alifah, 2009). Local peoples consumed them either as raw or cooked vegetables. However, most of these aquatic macrophytes in Sarawak are harvested from the wild, and there has been no cultivation for these edible species on a commercial scale (Muta Harah *et al.,* 2005; Suzalina Akma, 2008; Mohd Syahrul, 2009; Dayangku Alifah, 2009). From observations in Sarawak, at least in several places, e.g., Sibu and Bintulu, the common aquatic macrophytes, *Limnocharis flava* (L.) Buchenau and *Neptunia oleracea* Lour. are consumed locally as leafy vegetables. Peoples gathered these plants from several places e.g., irrigation and drainage systems for own consumption and also offered for sale in native markets.

Table 1.1: A summary compiled on problems caused by noxious growth of aquatic macrophytes.

Location	Problems caused by aquatic weeds	References
	Agriculture	
South East Asia	Competition with main crop, *Oryza sativa.*	Pancho and Soerjani (1978)
Thailand	Competition with main crop, *O. sativa.*	Edwards (1980)
Kalasin Province	Blocking the irrigation system.	Cruz-Garcia and Price (2011)
Pa Mong Dam	A large of floating mat plants provides habitats for rats, clog the pumps and block the gateway.	Chomchalow and Pongpangan (1976)
India		
Kumarakom	Competition with main crop, *O. sativa.*	Abhilash *et al.* (2008)
Kerala	Blocking the irrigation system.	Nayar and Sworupanandan (1978)
Kerala	Heavy reduction on the growth of cultivated plants by *Salvinia* sp.	Varshney and Singh (1976)
Andaman Island	Blocking the irrigation system.	Karthigeyan *et al.* (2004)
Indonesia	Competition with main crop, *O. sativa,* clog the pumps and block the gateway.	Koesterman *et al.* (1987)
West Malaysia	Competition with main crop, *O. sativa.*	Haynes and Les (2004); Samy *et al.* (2005)
Muda, Kedah	Competition with main crop, *O. sativa.*	Mashhor (1988); Begum *et al.* (2005); Mohd Adly *et al.* (2009)
Seberang Prai, Penang	Competition with main crop, *O. sativa.*	Abdul Shukor *et al.* (2009)
Kg Sg Bakau, Perlis	Competition with main crop, *O. sativa.*	Ain Nihla *et al.* (2011)
East Malaysia		
Kota Samarahan, Sarawak	Competition with main crop, *O. sativa.*	Baki (1993)
	Aquaculture	
India	Hindrance to fisheries.	Varshney and Singh (1976)
Indonesia		
Irian Jaya	Hindrance to fisheries.	Sukarwo (1991)
Thailand	Reduce the population of fish.	Chomchalow and Pongpangan (1976)

Continued Table 1.1

	Health	
Namibia		
Olushandja Dam	Floating mats of *Ludwigia stolonifera* provide a home for bilharzia-carrying snails.	Burke (2000)
Thailand	*Eichhornia crassipes* provides ideal conditions for growth and multiplication of mollusks.	Chomchalow and Pongpangan (1976)
India	Provide condusive habitat for vectors of malaria.	Varshney and Singh (1976)
Kerala	*Salvinia* generate foul gases which affect the health.	George (1976)
West Malaysia		
Titiwangsa Lake, Perdana Lake and Jaya Lake	Harbored disease vectors.	Mohd Fauzi (1991)
	Residential, irrigation and water reservoirs	
Australia		
Northern Australia	Blocking man made drainage systems.	Waterhouse (2003); DPI (2009)
India	Chocking the flowing waters.	Varshney and Singh (1976)
Rajasthan and Madhya	Submerged weeds reduced the water flow by about 50-70% in Right Main Canal Rajashtan.	Mehta and Sharma (1976)
Sri Lanka	Reduce the flow of water in drainage systems.	Dassanayake (1976)
West Malaysia	Water loss due to *E. crassipes* transpiration.	Mashhor (1994)
East Malaysia		
Miri and Bintulu, Sarawak	Blocking man-made drainage systems.	Suzalina Akma (2008)
	Hydroelectric power	
Thailand		
Pa Mong Dam	Reducing of power generation.	Chomchalow and Pongpangan (1976)
Sri Lanka	Reducing of power generation.	Dassanayake (1976)
Burkina-Paso		
Sahelian lakes	Reducing of power generation.	Müller (2005)
Nigeria	Reducing of power generation.	Abulude (2005)
Indonesia		
Kalimantan	Loss of water through evapotranspiration of *Polygonium barbatum.*	Djasmani (1991)
West Malaysia	*Eichhornia crassipess, Pistia* spp. and *Salvinia* sp. blocking water outlets.	Mohd Fauzi (1991)

Continued Table 1.1

	Recreation and navigation	
Netherland	Dense stands of aquatic vegetation often cause nuisance for boating, swimming and by obstruction of water flow.	van Nes *et al.* (2002)
Thailand		
Nam Pong and Lam Pao	*Hydrilla verticillata* entangling the boat engine.	Chomchalow and Pongpangan (1976)
Pa Mong Dam	The dense mats of *E. crassipess* covering the lake.	Chomchalow and Pongpangan (1976)
India		
Kerala	Larges masses of *Salvinia* brought by large rivers to cochin port area causing hindrance to ships.	George (1976)
West Malaysia		
Titiwangsa Lake, Perdana Lake and Jaya Lake	Destroy the aesthetic values of lakes.	Mohd Fauzi (1991)

Table 1.2: A summary compiled on the uses of some aquatic weeds.

Location	Uses of aquatic weeds	References
	Waste water treatments	
Denmark	*Phragmites australis* has been used in wastewater treatment.	Brix and Schierup (1989)
West Malaysia		
Kg Sg Bakau, Perlis	*Limnocharis flava* have been identified suitable for use in constructed wetland to treat landfill leachate.	Ain Nihla *et al.* (2011)
India	Combination of *Eichhornia crassipes-Lemna minor* and *E. crassipes-Pistia stratiotes* were found the best possible for removal N and P from dairy industry.	Upadhay (2004)
Thailand		
Prachinburi Province	*Neptunia oleracea* was used to treat the effluent from shrimp effluent tank.	Suppadit *et al.* (2005)
Colombia		
Ayapel, Betanci and Lorica wetlands, Córdoba	*Eichhornia crassipes*, *Ludwigia helminthorriza,* and *Polygonum punctatum* could be proposed as Cu and Zn phytoremediators.	Núñez *et al.* (2011)
Namibia		
Olushandja Dam	Sedges and reedbeds naturally filtering domestic pollutants in Olushandja dam.	Burke (2000)
	Agriculture	
Australia		
Griffith, New South Wales	*Azolla pinnata* has been used as fertilizer, supplying a substantial amount of organic N for the crop mainly rice, and also to improve soil structure.	Cary and Bowmer (1991)
South East Asia	*A. pinnata, Salvinia* sp., *Pistia stratiotes, Lemna minor*, *Hydrilla verticullata, L. flava, Typha angustifolia* and *Sagittaria latifolia* can be used as a live stock fodder.	Edwards (1980)
	Foods	
America		
Northern America	*Alisma subcordatum*, *Commelina communis*, *Nastartium officinale*, *Nelumbo lutea, Nuphar luteum, Nymphae odorata, Portulaca oleracea, S. latifolia* and *T. angustifolia* are edibles.	Duke (2001)
India		
Gorakhpur	*Veronica anagallis-aquatica, E. crassipes, Ipomoea reptans, Polygonum hydropiper, Allmania nodiflora* and *Cassia sophora* have been reported as potential source of leaf proteins.	Pandey and Srivastava (1991)
South East Asia	*Trapa bispinosa, N. oleracea, L. flava, Monochoria vaginalis, Ipomoea aquatica, Colocasia esculenta* have been reported as foods.	Edwards (1980)

Continued Table 1.2

West Malaysia	*Limnocharis flava, M. vaginalis* and *I. aquatica* are recorded as leafy vegetables.	Samy *et al.* (2005)
	Paper and pulp	
Indonesia		
Krawang	*Eichhornia crassipes* that had long fiber and low lignin have been reported a potential source of pulps.	Joedodibroto *et al.* (1983)
India		
Yamuna Nagar	*Salvinia molesta* with high holocellulose and low amount of lignin has been indentified for pulp and paper production.	Bhardwaj (2005)

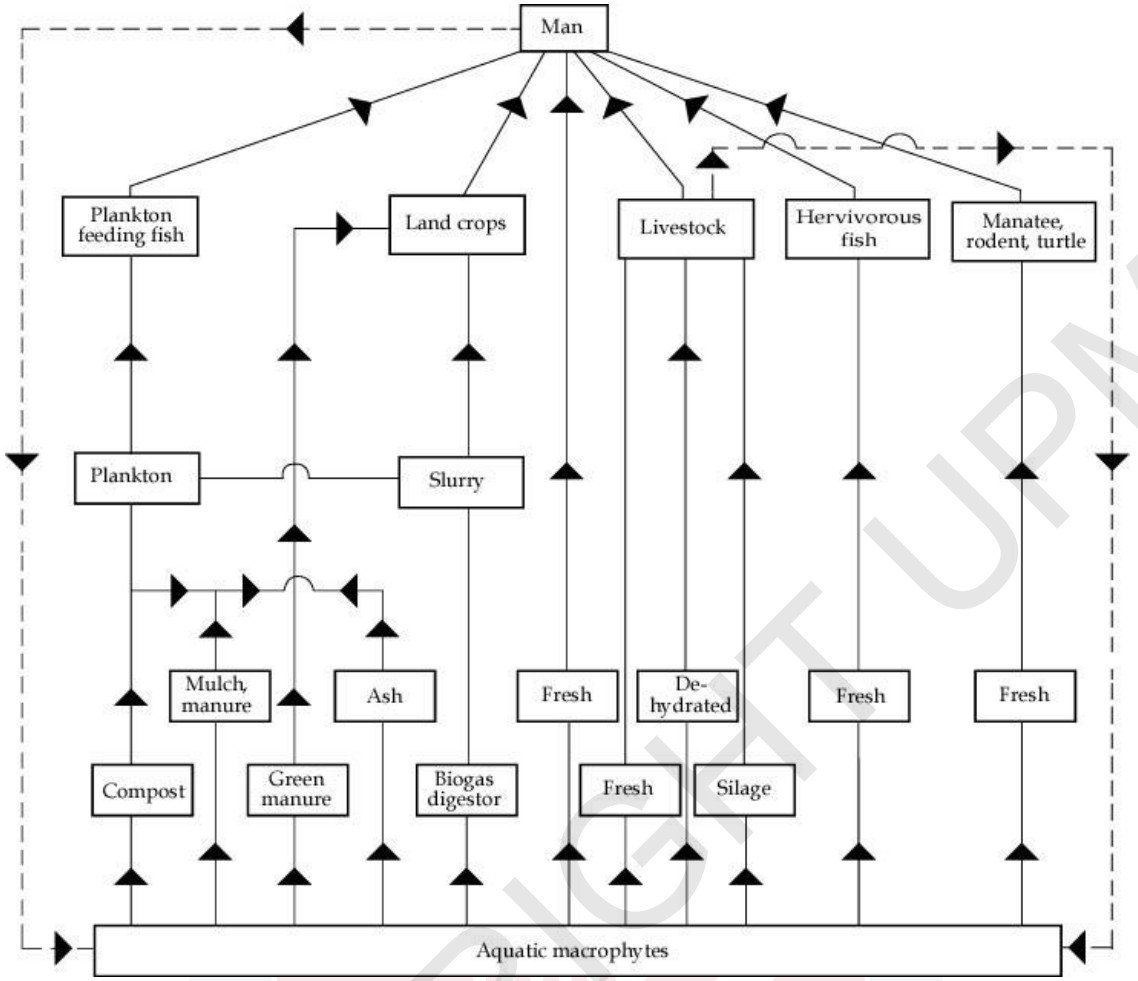

Figure 1.1: The major pathways involving aquatic macrophytes in food productions in South East Asia (Source: Edwards, 1980).

Limnocharis flava is synonym to *Alisma flava, L. emarginata* Kunth and *L. plumieri* Richard (van den Bergh, 1994). It is belonging to Butomaceae or Limnocharitaceae (Kaul, 1978; van den Bergh, 1994). *Limnocharis flava* is known locally by various names as paku rawan, keladi itik, jinjir (West Malaysia) and emparuk (Sarawak) (Voon *et al.,* 1990; van den Bergh, 1994; Rukayah, 2002; Samy *et al.,* 2005; Mohd Syahrul, 2009; Dayangku Alifah, 2009). It was introduced into tropical Asia before 1870 and become thoroughly naturalized throughout Malaysia (Edwards, 1980; Karim *et al.,* 2004) and becomes serious tropical weeds invading irrigated rice fields, channels, wetland areas of Southeast Asian countries (Cook *et al.,* 1974; Waterhouse, 2003; Begum *et al.,* 2005; Abhilash *et al.,* 2008). This species can multiply by bulbils developed on the inflorescence stalk and through seed dispersal (Nayar and Sworupanandan, 1978; Waterhouse, 2003). A single fruit produces about 1000 seeds and a single plant may produce as many as 1 million seeds per year (Kotawala, 1976; Abhilash *et al.,* 2008). Several reports concerning on the description and propagation of *L. flava* are published i.e., vegetative and reproductive morphology (van Steenis, 1958; Kaul, 1967; 1976; Henderson, 1974; Kostermans *et al.,* 1987; Keng, 1983; Jones, 1993; van den Bergh, 1994; Lim *et al.,* 1998; Karthigeyan *et al.*, 2004), habitat (Begum *et al.*, 2005; Abhilash *et al.*, 2008; Abdul Shukor *et al.,* 2009) and plant development and propagation (Wilder, 1974; Kaul, 1978; Nayar and Sworupanandan, 1978; Song *et al.,* 2000; Brooks *et al.,* 2008).

In Java, Indonesia, young plants are much-esteemed vegetable although not appreciated elsewhere (Kostermans *et al.,* 1987). A young shoot comprising leaves and petioles and flower cluster or unopened inflorescence are collected, consumed either raw or grilled or cooked for a short time before being eaten (Cook *et al.,* 1974; Edwards, 1980; Kostermans *et al.,* 1987; Voon *et al.,* 1990; van den Bergh, 1994; Rukayah, 2002; Haynes and Les, 2004; Muta Harah *et al.*, 2005; Samy *et al.*, 2005). It is commonly cultivated in fertile soil in West Java and Thailand and harvested after 2 – 3 months before being marketed as secondary crop (van den Bergh, 1994; Haynes and Les, 2004). Each bunch contained 20 sprouts and 1000 bunches can be harvested in an area of 1 ha (van den Bergh, 1994). This lesser known vegetable is also commonly sold in native markets in Sarawak, East Malaysia at RM 1.00 to 2.00 per bunch (Muta Harah *et al.*, 2005; Mohd Syahrul 2009; Dayangku Alifah, 2009).

Other aquatic macrophytes commonly consumed as green leafy vegetable is water mimosa, *N. oleracea.* It is synonym to *N. prostrata* (Lamk) Baillon and *N. natans* (L.f) Druce. It is known by various names as keman air, keman gajah, kangkung puteri in West Malaysia and daun tangki in Sarawak (Rukayah, 2002; Halimatul Saadiah, 2003). A plant has bipinnate leaves and stems made buoyant by their spongy white covering. The young shoot comprising of leaves, spongy stems and young seedpods can be eaten raw and cooked as green vegetables (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994; Rukayah, 2002; Halimatul Saadiah, 2003; Muta Harah

et al., 2005; Samy *et al*., 2005; Mohd Syahrul, 2009, Dayangku Alifah, 2009; Jain *et al.,* 2011). Subjects pertaining to *N. oleracea* include its description and morphology (Windler, 1966; Ridley, 1967; Shah and James, 1968; Holtum, 1969; Henderson, 1974; Pancho and Soerjani, 1978; Paisooksantivatana, 1994; Kamarudin and Latiff, 2002; Holtum and Ivan, 2002), distribution and ecology (Windler, 1966; Ridley, 1967) and propagations (Paisooksantivatana, 1994).

It can be found floating or prostrate near the edge of water reservoir, water channel and ditches with stagnant to slow moving water of pH 5.4 – 6.0 (Cook *et al.,* 1974; Paisooksantivatana, 1994). In Thailand, it is commonly used as a vegetable and cultivated in inundated fields or in canals (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994). *Neptunia oleracea* can be harvested after 3 – 4 weeks after planting and 250 shoots are gathered into bunch then traded in local market. About 30,000 – 50,000 shoots can be harvested for each harvest in one hectare area (Paisooksantivatana, 1994). In Sarawak *N. oleracea* is sold in Sibu central market and Tamu Nyelong Sarikei depending on the availability at RM 1.00 – 2.00 per bunch (Muta Harah *et al*., 2005).

Generally in Malaysia, most of the studies of wild or indigenous fruits and vegetables are recorded in West Malaysia e.g., research on wild fruit plants (Chung *et al.,* 2004), salad and vegetables (Rukayah, 2002), and herbs (Samy *et al.,* 2005). In Sarawak, reports pertaining on utilization and marketable of indigenous fruits and vegetables were documented by Voon *et al.* (1990) and Mohd Syahrul (2009). Several studies were focusing on aquatic macrophytes distribution and utilization (Muta Harah *et al.,* 2005; Suzalina Akma, 2008) and marketable species (Dayangku Alifah, 2009). Those studies reported on the occurrences of *L. flava* and *N. oleracea* in Sarawak native markets with data observation on their availability throughout the year.

Despite the uses of *L. flava* and *N. oleracea* as food by the local people, the plants have not been given due attention in terms of their propagation modes, availability and nutritional content. For this reason, this present study was conducted to complement those studies mentioned above focusing on the plants' biology, availability in native markets, proximate and minerals compositions, and also their propagation modes. It is hope that the information would be used for advocating their increased utilization as vegetable crops. The objectives of this study are:

(i) to determine the distribution, habitat and environmental conditions of *L. flava* and *N. oleracea* in central region of Sarawak;
(ii) to evaluate the morphological characteristics of *L. flava* and *N. oleracea*;
(iii) to determine the developmental stages, propagation methods and productions of *L. flava* and *N. oleracea*;

(iv) to assess the availability and nutrient contents of *L. flava* and *N. oleracea* in local markets.

The findings are reported in the different chapters. Chapter 3 is on the distribution, types of habitat and environmental conditions. Chapter 4 and Chapter 5 present the vegetative and reproductive morphology of *L. flava* and *N. oleracea* respectively. The propagation and production of these plants is given in Chapter 6. Chapter 7 reported on the availability and nutrient status of both plant species. The summary, general conclusion and recommendation for future research are discussed in Chapter 8.

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BIODATA OF STUDENT

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CANDIDATE OF Ph.D OF AGRONOMY

Noorasmah Binti Saupi was born at Kampung Jemoreng, Matu in 1981. She obtained Sijil Pelajaran Malaysia (SPM) from Sekolah Menengah Sains Miri in 1998. In 1999 she graduated with Sijil Matrikulasi and subsequently in 2003, Bachelor of Science (Hons.) in Plant Resource Science and Management from Universiti Malaysia Sarawak. In 2007, she obtained Master of Science in Plant Ecology from Universiti Malaysia Sarawak and the M.Sc. Thesis entitled "Ecology of *Cryptocoryne cordata* var. *zonata* in Sarawak, Malaysia". From July 2008 until present day she is doing her post-graduate study.

Previous academic and other relevant appointments:

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LIST OF PUBLICATIONS

Journal:

1. **Saupi, N.,** Zakaria, M and Bujang, J.S. 2009. Analytic chemical composition and mineral content of yellow velvetleaf (*Limnocharis flava* L. Buchenau)'s Edible parts. *Journal of Applied Sciences.* 9 (16): 2969-2974.
2. **Noorasmah Saupi**, Muta Harah Zakaria, Japar Sidik Bujang and Aziz Arshad. 2014. Proximate composition and mineral contents of *Neptunia oleracea* Lourerio, an aquatic plant from Malaysia. Emirates Journal of Food and Agriculture. Accepted.

Poster presented:

1. **Noorasmah Saupi**, Muta Harah Zakaria, Japar Sidik Bujang and Aziz Arshad. 2010. Proximate composition of *Neptunia oleracea* Loureiro edible parts. *International Conference on Food Research (ICFR2010): Sustainable and Quality Food for All*. 22-24th November 2010. JW Marriott Putrajaya, Malaysia.

LIST OF CORRECTION

NAME	**:**	NOORASMAH BINTI SAUPI
MATRIC NO.	**:**	GS22324
PROGRAM	**:**	PhD
APPROVED FIELD OF STUDY	**:**	AGRONOMY
FACULTY	**:**	AGRICULTURE AND FOOD SCIENCES (UPMKB)
SUPERVISOR	**:**	PROF. MADYA DR. MUTA HARAH ZAKARIA
SUGGESTED THESIS TITLE	**:**	BIOLOGY OF *Limnocharis flava* (L.) BUCHENAU AND *Neptunia oleracea* LOURERIO AND THEIR STATUS AS VEGETABLE CROPS IN SARAWAK, MALAYSIA

NO.	COMMENTS FROM THE EXAMINATION COMMITTEE	CORRECTIONS MADE	PAGE NO.
1.	**Title:** The title of the thesis should be "Biology of *Limnocharis flava* (L.) Buchenau and their Status as Vegetable Crops in Sarawak"	Biology of *Limnocharis flava* (L.) Buchenau and their Status as Vegetable Crops in Sarawak, Malaysia	
2.	**Abstract:** Abstract is too long and it should be shortened. It should also not have too many speculation	Abstract was shortened.	ii-iii
3.	**List of Abbreviation and Symbols:** Abbreviation for specific terms should be in respective Table/Figure/Plate.	Deleted and specified in Table/Figure/Plate.	
4.	**Chapter 1:** **Grammatical and spelling errors:** a. Aquatic macrophytes are considered as those large plants which grow in excessive amounts in a continuous supply of water or at least present in soils which are covered with water during a major part of the growing season and interfered the intended usage of particular area (Weldon *et al.,* 1973; Edwards, 1980).	a. Aquatic macrophytes are considered as those large plants which grow in excessive amounts in a continuous supply of water or at least present in soils which are covered with water during a major part of the growing season and interfered with the intended usage of particular area (Weldon *et al.,* 1973; Edwards, 1980).	1
	b. The high density and population of aquatic macrophytes and their ability for vegetative growth in drainage systems, ponds, reservoirs and paddy fields can cause problems to the	b. The high density and population of aquatic macrophytes and their ability for vegetative growth in drainage systems, ponds, reservoirs and paddy fields can cause problems to the	1

	human in term of in agriculture sector, human health, hydroelectricity dams, reduced water quality and recreational purposes (Table 1.1).	humans in terms of in agriculture, health, hydroelectricity dams, reduced water quality and recreational purposes (Table 1.1).	
	c. For that reason, many researchers referred aquatic macrophytes as invasive or unwanted to the “aquatic weeds” (Weldon *et al.,* 1973; Edwards, 1980; Said *et al.,* 1991; Mashhor, 1994). One of the best controls of weeds is by utilizing them (Mashhor, 1988).	c. For that reason, many researchers considered aquatic macrophytes as invasive or unwanted and refer to them as “aquatic weeds” (Weldon *et al.,* 1973; Edwards, 1980; Said *et al.,* 1991; Mashhor, 1994).	1
	d. Various reports have been documented on the use of them mainly in waste treatment management, agriculture, foods and paper pulps (Table 1.2).	d. Various reports have been documented on their uses mainly in waste treatment management, agriculture, foods and paper pulps (Table 1.2).	1
	e. A young shoot comprise leaves and petioles and flower cluster or unopened inflorescence are collected, consumed either raw or grilled or cooked for a short time before being eaten (Cook *et al.,* 1974; Edwards, 1980; Kostermans *et al.,* 1987; Voon *et al.,* 1990; van den Bergh, 1994; Rukayah, 2002; Haynes and Les, 2004; Muta Harah *et al.*, 2005; Samy *et al.*, 2005).	e. A young shoot comprising of leaves and petioles and flower cluster or unopened inflorescence are collected, consumed either raw or grilled or cooked for a short time before being eaten (Cook *et al.,* 1974; Edwards, 1980; Kostermans *et al.,* 1987; Voon *et al.,* 1990; van den Bergh, 1994; Rukayah, 2002; Haynes and Les, 2004; Muta Harah *et al.*, 2005; Samy *et al.*, 2005).	8
	f. It is by various names as keman air, keman gajah, kangkung puteri in Peninsular Malaysia and daun tangki in Sarawak (Rukayah, 2002; Halimatul Saadiah, 2003). A plant with bipinate leaves and stems made buoyant by their spongy white covering. The young shoot comprising leaves, spongy stems and young seedpods can be eaten raw and cooked as	f. It is known by various names as keman air, keman gajah, kangkung puteri in Peninsular Malaysia and daun tangki in Sarawak (Rukayah, 2002; Halimatul Saadiah, 2003). A plant has bipinnate leaves and stems made buoyant by their spongy white covering. The young shoot comprising of leaves, spongy stems and young seedpods can be eaten raw	8

	green vegetables (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994; Rukayah, 2002; Halimatul Saadiah, 2003; Muta Harah *et al*., 2005; Samy *et al*., 2005; Mohd Syahrul, 2009, Dayangku Alifah, 2009; Jain *et al.,* 2011).	and cooked as green vegetables (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994; Rukayah, 2002; Halimatul Saadiah, 2003; Muta Harah *et al*., 2005; Samy *et al*., 2005; Mohd Syahrul, 2009, Dayangku Alifah, 2009; Jain *et al.,* 2011).	
	g. *Neptunia oleracea* can be harvested after 3 – 4 weeks after planting and 250 shoots are gathered into bunch then traded in local market.	g. *Neptunia oleracea* can be harvested after 3–4 weeks after planting and 250 shoots are gathered into bunch then traded in local market.	9
5.	**Chapter 3:** **3.2 Materials and methods:** Water and soil sample size : "Water from these sites were collected in the bottle and placed into ice chest before being transported to the laboratory to determine the concentration of ortho-phosphate, ammonia, nitrate and nitrite. Soil samples were collected from rooting depth (up to 30 cm) of *L. flava* and *N. oleracea* using plastic auger following the method by Abhilash *et al.* (2008). The samples were then placed in labeled plastic bags for transportation back to the laboratory to determine the concentration of total organic nitrogen and carbon, available macro and micro nutrients."	Five hundred milliliter of water from these sites were collected in the bottle and placed in an ice chest before being transported to the laboratory to determine the concentration of ortho-phosphate, ammonia, nitrate and nitrite. Three replications of water sample were collected from each site. Two kilogram of soil samples were collected from rooting depth (up to 30 cm) of *L. flava* and *N. oleracea* using plastic auger following the method by Abhilash *et al.* (2008). The samples were then placed in labeled plastic bags for transportation back to the laboratory to determine the concentration of total organic nitrogen and carbon, available macro and micro nutrients. Three replications of soil sample were also collected	38

		from each site.”	
	3.3 Results and discussion: Symbol of sample number, N.	n	44, 57, 59, 62,
	Use of LSD for mean comparison must be based on LSD value (p≤0.05), and this LSD values must be inserted in each tables for each parameter / variables.	All mean comparison based on LSD values and inserted in each tables.	44, 57, 59, 62
	Alphabetical order a>b>c on water pH of *N. oleracea* habitat in Table 3.7: b, c, a	b>a> c	59
	Grammatical error: most	highest	55
	Each experimental chapter should have a conclusion section.	**3.4 Conclusion**	63
6.	**Chapter 4** **4.3 Results and discussion:** Symbol of sample size, N	n	69, 70
	Use of LSD for mean comparison must be based on LSD value (p≤0.05), and this LSD values must be inserted in each tables for each parameter / variables.	All mean comparison based on LSD values and inserted in each tables.	69, 70
	Each experimental chapter should have a conclusion section.	**4.4 Conclusion**	78, 80

7.	**Chapter 5** **5.3 Results and discussion:** Symbol of sample size, N	n	84, 85
	Use of LSD for mean comparison must be based on LSD value (p≤0.05), and this LSD values must be inserted in each tables for each parameter / variables.	All mean comparison based on LSD values and inserted in each tables.	84, 85
	Each experimental chapter should have a conclusion section.	**5.4 Conclusion**	94
	Number to word: 31 to 58	Thirty one to fifty eight	89
	10	Ten	92
	3 to 4	Three to four	92
	Rephrase: 10 exserted stamens with 4.01 to 6.54 mm long and 0.11 to 0.24 mm width white slender flattened filaments and 0.50 to 1.56 mm long and 0.19 to 0.83 mm width bilocular yellow anther that, lacking a terminal stalked gland (Plate 5.3d).	Ten exserted stamens with 4.01 to 6.54 mm long and 0.11 to 0.24 mm width white slender flattened filaments and 0.50 to 1.56 mm long and 0.19 to 0.83 mm width bilocular yellow anther lacking a terminal stalked gland (Plate 5.3d).	92
8.	**Chapter 6** **6.3 Results and discussion:** Symbol of sample size, N.	n	112, 114, 115, 116, 117, 119,

			121, 122, 125, 126, 128
	The results are sufficiently discussed, but the candidate need to offer some reasons for the results recorded: a. For *N. oleracea* grown from the seed and stem cutting, only NO_3^- is responsible for the increased in plant length with *p* value 0.657 and 0.878 respectively. b. The high growth performance on vegetative part was also demonstrated for *N. oleracea* by stem cutting materials. Besides, stem cutting produce high shoot formation and has a tendency to gain more yields. In Thailand, both stem cutting and seed are practiced for cultivation (Paisooksantivatana, 1994). This present study showed the yield is almost ten times higher than in field culture as reported by Paisooksantivatana (1994).	a. For *N. oleracea* grown from the seed and stem cutting, only NO_3^- is responsible for the increased in plant length with *p* value 0.657 and 0.878 respectively. The rate of NO_3^- uptake increases during vegetative growth in legume crops (Imsande and Touraine, 1994). b. The high growth performance on vegetative part was also demonstrated for *N. oleracea* by stem cutting materials. Besides, stem cutting produce high shoot formation and which has a tendency to gain more yields. The plant that frequently cut stimulates development of side shoots (Palada and Crossman, 1999). In Thailand, both stem cutting and seed are practiced for cultivation (Paisooksantivatana, 1994). This present study showed the yield is almost ten times higher than in field culture as reported by Paisooksantivatana (1994) may be attributed to the competition when the plant grow in limited space of tank.	120 129
	Each experimental chapter should have a conclusion section.	**6.4 Conclusion**	132

9.	**Chapter 7** **6.3 Results and discussion:** Symbol of sample size, N.	n	142, 144, 147, 149, 152, 155, 157
	Use of LSD for mean comparison must be based on LSD value (p≤0.05), and this LSD values must be inserted in each tables for each parameter/variables.	All mean comparison based on LSD values and inserted in each tables.	142,144, 146, 149
	Each experimental chapter should have a conclusion section.	**7.4 Conclusion**	155
10.	**References:** All of the references cited must be correctly taken care as suggested by the examiners. Please refer to their reports:		
	a. Duke (2001) not listed in References	a. Duke, J. A. (2001). *Handbook of edible weeds.* USA: Herbal Reference Library.	168
	b. Shardendu and Ambasht (1991) not listed in References	b. Present but not in sequence after the reference of "Shah, J. J. and James, M. R. (1968). Sieve tube elements in the stem of *Neptunia oleracea* Lour. *Australian Journal of Botany, 16*, 433-444."	177

	c. Reference of Abilash *et al.* (2008) or Abhilash *et al.* (2008)	c. Abhilash *et al.* (2008)	2, 8, 13, 14, 16, 36, 38, 52, 59, 63, 62, 64, 95
	d. Reference of Edwards (1980) or Edward (1980)	d. Edwards (1980)	5, 13, 36, 38
	e. Reference of Koestermans *et al.* (1987) or Kostermans *et al.* (1987)	e. Kostermans *et al.* (1987)	8, 13, 17, 52
	f. Reference of Subba-Rao *et al.* (1995) or Subha-Rao *et al.* (1995)	f. Subba-Rao *et al.* (1995)	17, 87
	g. Reference of Closs *et al.,* 2006 or Closs *et al.*, 2004	g. Closs *et al.*, 2006	1
	h. Reference of Windler (1966) or Windler (1996)	h. Windler (1966)	30
	i. Correction of first author surname of Yi-Ju *et al.* (2007)	i. Chou, Y.J., Elliot, G. N., James, E. K., Lin, K. Y., Chou, J. S., Sheu, S. Y., Sheu, D. S., Sprent, J. I. and Chen, W. M. (2007). *Labrys neptuniae* sp. Nov., isolated from root nodules of the aquatic legume *Neptunia oleracea. International Journal of Systematic and Evolutionary Microbiology, 57,* 577-581.	12, 17, 26, 30, 37, 58, 87
11.	**Spelling of incorrect biological terms:**		
	gymnoecium	gynoecium	77, 78, 92,
	androceium	androecium	92

	hermaphoridite	hermaphrodite	92
	aerhenchyma	aerenchyma	87, 160
	euthropication	eutropication	177
	subratum	substratum	181
	auxiliary	axillary	110, 160
	orthpohosphate	orthophosphate	181
	sysnonym	synonym	80