Species Diversity of Mosquito Larvae (Diptera: Culicidae) in the wild Pitcher Plants *Nepenthes* spp. in Surat Thani, Trang and Songkhla provinces

Vutthy Vong

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in Biology (International Program)

Prince of Songkla University

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Major Program	Biology (International Program)

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ABSTRACT

This study was investigation of larval mosquito species in pitcher plants *Nepenthes* species; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* in three sites; Surat Thani, Trang, and Songkhla province Southern Thailand. 50 pitchers, 25 upper pitchers and 25 lower pitchers, in each *Nepenthes* species or each study site were collected two times during rainy and dry season. Mosquito larvae 5 species following 3 genus and 1,155 individuals were identified. The higher abundance of larval mosquitoes was *Tripteroides tenax* (85%, 991 individuals) and following *Uranotaenia demeillori* (13.33%, 154 individuals), *Tripteroides* species.1 (4.59%, 53 individuals), *Toxorhynchites albipes* (1.65%, 19 individuals), and *Uranotaenia bicolor* (1.3%, 15 individuals).

Both principal component analysis (PCA), which used for reducing variables of pitchers, and multiple linear regression used to find out significant correlation between pitcher's variables and abundance of larval mosquitoes. The results of PCA showed pitcher size, active zone of pitchers, actual fluids in pitchers, dry detritus, and electrical conductivity in pitchers were positively correlated with abundance of larval mosquitoes. Whereas, pH, protozoa abundant, and rotifer abundant correlated negatively with abundance of larval mosquitoes. Multiple linear regression showed 21% influence of pitcher variables to predict on mosquito larvae abundant.

Spearman rank analysis for influence pitcher environmental conditions on larval mosquito species indicated pitcher environmental conditions seemed to have less impacting on larval mosquito species. Actual fluid in pitchers was positive correlated slightly with larval mosquito *Tripteroides tenax* (r = 0.33,

P = 0.003) and electrical conductivity correlated slightly positive with *Tripteroides* species.1 (r = 0.12, *P* = 0.03) but electrical conductivity correlated negative with *Uranotaenia demeillori* (r = -0.12, *P* = 0.02).

Mean larval mosquito abundant were compared between seasons by using one-way ANOVA and mean larval mosquito abundant were found significant difference between rainy and dry season (one-way ANOVA, $P < 0.05$).

One-way ANOVA analysis compares mean of abundance larval mosquito between lower pitchers and upper pitchers and the significant difference were not detected between larval mosquito abundant in lower pitchers and upper pitchers (one-way ANOVA, $P > 0.05$). However, all explanations for those finding are discussed.

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Vutthy Vong

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Chapter 1

Introduction

Mosquitoes were classified in order Diptera and family Culicidae (Harbach and Kitching, 2005). Adult mosquito body morphological was distinguished into three main parts such as head, thorax, and abdomen (Rueda, 2008, Rattanarithikul et al., 2005). Mosquitoes 3,500 species had been recognized and some genera were vector human diseases such as *Anopheles* (malaria, filariasis), *Aedes* (Yellow fever, dengue, Chikungunya) and *Culex* (West Nile, Japanese encephalitis, Filariasis) (Tolle, 2009).

The life cycle of mosquitoes were divided into four main stages such as eggs, larvae, pupa, and adults that adult females obtained blood meals from mammals and other creatures whereas adult males consumed nectar flowers. Adult female mosquitoes deposited their eggs on surface water including water pool, artificial container, and water storage by some special structure plants (Rueda, 2008). Mosquito *Aedes aegypti*, *Culex quinquefasciatus, Anopheles culicifacies* and *Anopheles varuna* had been realized to lay their eggs on surface water flowing habitat while other larval mosquitoes preferred dirty water and water constantly movement (Piyaratne et al., 2005). Habitats of mosquitoes divided two parts; container habitats, which comprised natural and artificial container, and ground waters (Rattanarithikul et al., 2005). Pitcher plants were considered as natural habitats that had their leaves for containing fluid, small ecological system, which allowed various arthropod species existence. Pitcher plants played an important role as insect traps and digested insect preys in order to absorb inorganic minerals such as N, P, and K (Schulze et al., 1997).

The fluids of pitcher plants *Nepenthes* genus were realized to provide habitats for arthropods including different larval mosquito species. Especially, vector mosquito species, which were *Aedes aegyti* and *Aedes albopictus,* used to utilize fluids container pitchers of *Nepenthes* as oviposition sites for laying their eggs and larvae survival (Chou et al., 2016). The information of pitchers genus *Nepenthes* was recognized that had ability to yield habitats for gravid female mosquito oviposition and larvae survival were investigated. However, there is surprisingly about limited information of pitcher *Nepenthes* species and how both environmental factors inside and around pitchers related to mosquito population and communities. Understanding how environmental factors influence both gravid female oviposition and survival larvae mosquitoes in pitchers *Nepenthes* spp. are a fundamental element for utilization of accommodated vector management strategies and understand the risk of diseases transmission in various habitat types (Vanlalruia et al., 2014).

Oviposition behavior of gravid female mosquitoes was an important step for selecting appropriate oviposition sites because the future offspring mosquitoes based on where eggs were laid (Chou et al., 2016, Kershenbaum et al., 2012). Gravid female mosquito species performed variation and complex oviposition cues in order to explore potential oviposition sites. Some oviposition cues of female mosquitoes had been known such as olfactory, visual, and tactile that associated with abiotic and biotic factors (Grech and Juliano, 2018). These oviposition cues were well recognized to influence on fundamental mosquito female decision. Some studies were demonstrated about mosquito oviposition cues included the present different color and size of containers (Chou et al., 2016, Torrisi and Hoback, 2013); the presence of detritus and types detritus (Norman and Walker, 2018, Yee et al., 2010, Yee and Juliano, 2006); presence of organic chemical from conspecific eggs (Ganesan et al.,2006); presence of the bacteria (Arbaoui and Chua, 2014); and presence of conspecific larvae anuran tadpoles (Mokany and Shine, 2003). Therefore, the oviposition behavior of female mosquitoes for responding a variety of oviposition cues is essential for female mosquitoes to seek suitable site condition for their offspring.

The larval mosquitoes had to cope with environmental factors after they hatched from eggs in their habitat. The survival of larvae mosquitoes based on food resource, which consisted of microorganisms and detritus, competition, predation and resistance with physical factors (Duguma et al., 2017).

There were some observations had been focused on abundance of larval mosquitoes inhabiting in *Nepenthes* spp. pitcher plants and to evaluate some environmental factors inside pitcher impact on survival larval mosquitoes. Especially, characteristics of *Nepenthes* species affected on oviposition behavior of gravid female mosquito decision. The recently an investigation of three different *Nepenthes* species of pitcher plants, *Nepenthes gracilis* (Korth.), *Nepenthes mirabilis* (Lour.), and *Nepenthes ampularia* (Jack) was indicated that *Nepenthes mirabilis* and *Nepenthes gracilis* were lethal for larvae *Aedes aegypti* and *Aedes albopictus* because of high acidity levels and microbial activities. This study was concluded that *Nepenthes* species pitcher plants were not suitable habitats for survival *Aedes albopictus* and *Aedes aegypti* (Chou et al., 2015). Further investigation of Chou et al (2016) was illustrated that both color and size of *Nepenthes ampularia* species were not attractive gravid mosquitoes *Aedes aegypti* and *Aedes albopictus* to deposit their eggs. On the other hand, an investigation of mosquito diversity in peat swamp forest in Narathiwat province of Southern Thailand by cut several *Nepenthes mirabilis* was shown that had three larval mosquito species (Apiwathnasorn et al., 2009).

Although, some observation have been reported already about mosquitoes larvae in *Nepenthes* species but many *Nepenthes* species are still neglected to evaluate diversity larval mosquitoes. Moreover, the relationship of larval mosquitoes and *Nepenthes* species environmental factors such as, pitcher size, high pitcher from ground, abundance microorganisms, detritus, pH, and electrical conductivity have no any investigations.

Research questions

1. How many species and abundance of larval mosquitoes in pitcher plants *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, *Nepenthes mirabilis* var. *mirabilis* in Surat Thani, Trang, and Songkhla province?
2. Do environmental conditions of *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* impact on abundance larval mosquito species?

Hypotheses

1. Environmental conditions of *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* are influence on abundance of larval mosquitoes.
2. Abundance of larval mosquitoes are different between rainy and dry seasons in pitcher plants; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*.
3. Abundance of larval mosquitoes are different between upper pitchers and lower pitchers of *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*.

Objectives

1. To investigate larval mosquito species and abundance in pitcher plants *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* in Surat Thani, Trang, and Songkhla province.
2. To ascertain environmental conditions and characteristics of *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* predict mosquito abundant and impact on abundance larval mosquito species.
3. To observe abundance of larval mosquitoes in upper pitchers and lower pitchers, which pitcher position is the most preference by gravid female mosquito oviposition decision.

Literature review

1. Mosquitoes

Mosquitoes were named in order Diptera and family Culicidae (Harbach and Kitching, 2005). This family had been known as a large abundant group that existed throughout temperate and tropical regions of the world especially in tropical forest environment (Harbach, 2007). Mosquito identification were easily noticed by their parts body such as long legs, slender, long proboscis and the presence of scale on their bodies. Morphology adult of mosquitoes were distinguished into three main parts such as; head, thorax, and abdomen. Mosquito adult's had shape like ovoid and consisted of large compound eyes, two antennae, two maxillary palpi and proboscis. Thorax of adult mosquitoes was divided three segments, prothorax, mesothorax, and metathorax that these segments attached with legs. Addition, each segment of mesothorax and metathorax supported a pair wings location. Abdomen part of adult mosquitoes had 10 segments which three segments were responsible for excretion and reproduction (Rueda, 2008, Rattanarithikul et al., 2005).

Larvae stage of mosquito species were distinguished from larvae other aquatic insects by without legs and the presence of a distinct head bearing mouth brushes and antennae. Moreover, mosquito larvae's thorax comprised a bulbous wider than head and abdomen. Posterior anal papillae and either a pair of respiratory opening or an elongate siphon (subfamily Culicinae) borne near the end of abdomen. Consequently, larval mosquitoes were not same with other larvae insects (Harbach, 2007).

The larvae stage of mosquitoes existed in aquatic habitats. Larval mosquitoes were survival in various habitats such as ground water including permanent or temporary water. However, larval mosquitoes were mostly found in natural containers and artificial containers such as; leaf axils, tree holes, rock holes, bamboo internodes, flower bracts, and pitcher plants (Harbach, 2007). Mosquito larvae species were known to exist in aquatic habitats by consuming microorganisms and particle matter within water. Larval mosquito species consumed microorganisms such as bacteria, fungi, and microalgae as well as protozoa and rotifers (Duguma, 2017, Hoekman, 2011). However, some larval mosquito species were predatory mosquito larvae, *Toxorhynchites*, to feed other larvae species by using their mandibles or maxillae. Larval mosquito species occupied surface water in order to obtain oxygen from the atmosphere. On the other hand, all mosquito larvae species *Mansonia*, *Coquillettidia* and some species *Minomyia* did not receive oxygen on surface water but they used their special siphon to pierce vessels of quantic plants for absorbing air (Harbach, 2007).

Abundance of mosquito species had well been known different abundance each mosquito species because of influencing environmental conditions. Particularly, diversity of vector mosquito species was changed even though in same area through alternating season and included all condition suitable for their breeding and adult survival (Bashar et al., 2016). Furthermore, many studies were demonstrated that mosquito species found high abundance during rainy season, which supported sufficiency food resources and ensured mosquitoes completely development their lifecycle (Mwangangi et al., 2012).

2. Mosquitoes diseases transmission

Mosquito 3,500 species had been classified and some genera were well recognized to play an important role for diseases transmission both animals and human. Female mosquitoes took blood meal from vertebrate in order to support their eggs development. Pathogens were brought inside female mosquito saliva during female mosquito consumed bloods and transferred to mosquito hosts (Tolle, 2009). Some genera of mosquito species were identified vectors human diseases. Mosquito *Aedes* species transferred diseases; Yellow fever, Chikungunya, Dengue, and Zika viruses. Mosquito *Anopheles* species transmitted diseases Malaria and Filariasis, Japanese encephalitis (Subashini et al., 2017, Lemine et al., 2017, Rodriguez et al., 2017).

Recently, *Aedes albopictus* were announced a potential vector to transmit Zika virus (Ajamma, 2016, Wong et al., 2013). Chikungunya virus caused Chikungunya fever in human, this disease was detected in southern Thailand in 2009, which were transmitted by vector *Aedes* mosquito species (Thavara et al., 2009). Thailand country was received impacting malaria disease particularly area near border eastern Myanmar that consisted of seventy percent malaria disease (Tanachai et al.,2012, Rattanarithikul et al., 2006).

Figure 1: Mosquito species *Aedes albopictus* (Charrel et al., 2012)

3. Mosquitoes habitats

Habitats of mosquitoes were divided in two main parts. Firstly, the ground water habitats included streams, swamps, salt marsh, and footprints (Rattanarithikul et al., 2005). Difference of mosquito larvae species preferred different habitats. Mosquito larvae *Anopheles* species; *Anopheles claviger*, *Anopheles hyrcanus*, and *Anopheles superpictus*, were mostly found in habitat, which consisted little plants. On the other hand, mosquito larvae species, *Culiseta longiareolata*, *Culiseta subochrrea*, preferred habitats with vegetation growth (Sofizadeh et al., 2017). In addition, mosquito larvae species *Anopheles* had been known to prefer agricultural habitats including rice field and channel irrigation areas (Sattler et al., 2005). Some habitats of mosquitoes larval were likely unsuitable for larval mosquito development such as cow hoof print and footprint in open areas. These habitats were small size and easily to dry before larval mosquitoes completely their lifecycle development (Munga et al., 2007, Vezzani, 2007). Secondly, the container habitats that included natural container and artificial containers. Natural container habitats of larval mosquitoes such as; death part of palm fronds, coconut shells, tree holes, and split bamboo poles. In addition, ginger flowers as water container that found larval mosquitoes (Rattanarithikul et al., 2005). Phytotelmata is a kind of terrestrial plants that provided special structure for water storage. Many plant types were considered to provide larval mosquito habitats such as pitcher plants, tree holes water, plant axils, bromeliads etc. (Rosa et al., 2016, Chitra et al., 2014, Adebote et al., 2008). Artificial container habitats were highly selected by gravid female mosquitoes to deposit their eggs. Artificial container habitats of larval mosquitoes caused by using people productions such as tires, bottles, earthen jars, cans, plastic containers, concrete pool, water storage tanks, urban trash etc. (Vezzani, 2007, Mcintyre, 2005, Rattanarithikul et al., 2005).

4. *Nepenthes* pitcher plants

Nepenthes pitcher plants were a special natural container habitats that held freshwater by special leaves structure of pitcher plants (Kitching, 2001). The specialized leaves structure of carnivorous *Nepenthes* served as pitfall traps that were essential function to catch and digest preys in order to uptake nutrients (Lam et al.,2017,Yilamujiang et al., 2016). Each species of *Nepenthes* had different both pitcher size and color for attractive preys. The larger size of pitchers were attractive more preys to fall in pitchers, for instance, *Nepenthes ampullaria* was known unattractive *Aedes* species (Chou et al., 2016).

Nepenthes species have unique spot characteristic of morphology for identification. Firstly, pitcher traps were divided in two position lower pitchers and upper pitchers. Lower position of pitchers were identified tendril settle in front of the pitcher trap and pitcher shape was oval form with colorful. However, upper position of pitchers was identified when tendril located behind pitcher and pitchers were cylinder shape with colorless. Secondly, flower characteristics were essential to identify until species levels. The presence of a bract and partial peduncles branching were different between each species *Nepenthes*. Third, existence angular stems and leaf connection on stem provided important characteristics for distinguishing *Nepenthes* species. Finally, pitcher shapes were used to identify for some *Nepenthes* species. Moreover, the variation of peristome characteristics, which were size, shape, and number of glands under lid, was classified species *Nepenthes* pitcher plants (Jebb and Cheek, 1997).

4.1. *Nepenthes thorelii*

Pitcher plants *Nepenthes thorelii* species were a single unique species in Surat Thani province that were recognized some their characteristics such as leaf, peristome, and glandular zone. The leave of *Nepenthes thorelii* were coriaceous in texture linear to lanceolate that measured up to 35 cm length, 4 cm width, and 0.5 mm thick. The peristome was flattened 10 mm wide with teeth and long up to 1 mm. on the other hand, the glandular zone of inner surface was variable ranging from one-third to two third of the pitcher height. Furthermore, male plants had peduncle constitutes 50 cm, rachis 20 cm and flowers were produced around 100 -180. However, female plants produced a short rachis and longer pedicels (Mey, 2010).

4.2. *Nepenthes mirabilis* var. *globosa*

The difference name globose of *Nepenthes mirabilis* species was found in peat swamp meadow in Trang and Phang-Nga provinces. It had been identified depend on their several characteristics. Pitcher plants *Nepenthes mirabilis* var. *globosa* species had pitcher's body round to oval shape and peristome color was usually red or green but inside peristome was dark color. *Nepenthes mirabilis* var. *globosa* species were know large size for lower pitcher and curling for upper pitchers. Addition, color tendril showed conspicuous red color even though pitchers were developing. Lower pitchers were no pitcher hip but pitcher hip appeared in the half of upper pitcher. *Nepenthes mirabilis* var. *globosa* species were no glands under their lids (Catalano, 2010).

4.3. *Nepenthes mirabilis* var. *mirabilis*

Nepenthes mirabilis var. *mirabilis* species were little different from *Nepenthes mirabilis* var. *globosa* species. Pitcher plants *Nepenthes mirabilis* var. *mirabilis* were bright green color of lower and upper pitchers. The upper pitcher position *Nepenthes mirabilis* var. *mirabilis* pitcher plants were tubulose that was different from *Nepenthes mirabilis* var. *globosa*. Moreover, pitcher plants *Nepenthes mirabilis* var. *mirabilis* species contained glands under but this species were without short appendage at the apex of the lid's lower surface (Catalano, 2010).

5. Distribution *Nepenthes* species in Thailand

Nepenthes was a single genus of the family Nepenthaceae, which was carnivorous plants with inconspicuous flow lacking petals, comprised 85 species in the world originating from Southeast Asia. Particularly, this genus was mostly found in Borneo, Sumatra, Java, Malay Peninsula, and Philippine southern islands (Anuniwat et al., 2009). However, the great diversity of *Nepenthes* pitcher plant species was located in Sumatra, which found more than 30 species, and Borneo consisted of 36 species. Philippines was reported 21 *Nepenthes* pitcher plants (Clarles and Moran, 2016, Bauer et al., 2012). *Nepenthes* 14 species had been recognized in Thailand and 11 *Nepenthes* species were found in Southern Thailand. These *Nepenthes* species in Southern Thailand were *Nepenthes suratensis*, *Nepenthes andamana*, *Nepenthes kerrii*, *Nepenthes thai*, *Nepenthes mirabilis* var. globosa, *Nepenthes kongkandana*, *Nepenthes rosea*, *Nepenthes ampullaria*, *Nepenthes mirabilis* var. mirabilis, and *Nepenthes gracilis* (Kanokratana et al., 2016). *Nepenthes krabiensis* species were endemic that grew in limestone rock above 600-700m above sea level on top of mountain in Krabi province (Nuanlaong et al., 2016). *Nepenthes thai* Cleek species were found in Narathiwat province (Cheek and Jebb, 2009). Pitcher plants *Nepenthes mirabilis* species were encountered near the end of trail through primary swamp forest of Tung Kai Botanical garden (Bert et al., 2003).

6. Size pitcher traps

Pitcher size was essential oviposition cue function for attractive preys to come in pitcher traps and correlated with rate of prey capture (Heard, 1998). Pitcher plants were generally to attract more preys with large size of pitchers (Bergland et al., 2005). The more number preys in pitchers caused to increase abundance both protozoa and bacteria that were consequently consumed by larval mosquitoes (Hoekman et al., 2007). The difference of prey capture in pitchers affected strongly in term of different pitcher size. For instance, *Nepenthes mirabilis* pitchers were larger size, pitcher length up to 20 cm, with mouth up to 5.2 cm in diameter that attracted more preys (Hua and Li, 2005). The large diameters of pitchers were necessarily for attractiveness pitchers to capture flying preys especially dipteran. The large diameter pitchers provided reflective polarization that was vital as visual cue to attract gravid female mosquito to lay eggs in pitcher fluids (Gaume et al., 2016). Pitcher plants *Nepenthes* different species had differ diameter size such as *Nepenthes gracilis* was cylinder size 1.6 cm and *Nepenthes rafflesiana* var. *gigantean* was large 7.2cm diameter (Game et al., 2016).

7. Pitcher digesting fluids

Pitcher fluids volume different *Nepenthes* species had generally water storage around 0.2 ml to 1.5L and differ pH levels to sever habitats for species ranging from bacteria to vertebrates (Adlassnig et al., 2011). Interestingly, pitcher plants *Nepenthes gracilis* were known extremely acidity levels pH= 2-3 (Chou et al., 2015). However, some *Nepenthes* species had slightly acidic such as *Nepenthes ampullaria* pH= 5-7, *Nepenthes mirabilis* pH=3.6 (Bauer et al., 2009, Clarke et al., 2007). Furthermore, an observation seven *Nepenthes* species was found that *Nepenthes rafflesiana* and *Nepenthes gracilis* were lowest nearly pH=2. The fluid pH different pitcher plants species had different pH levels and fluid pH were not dependent on *Nepenthes* species (Gaume et al., 2016). Pitcher fluid pH was depended on amount preys capture in pitchers (Kanokratan et al., 2016).

The pH levels in pitcher fluids was realized to impact on mosquito larvae survival in pitcher fluids. Larval mosquitoes had to control their ionic movement in and out haemolymph in order to response environmental conditions (Adlassnig et al., 2011). For instance, larval mosquito *Aedes aegypti* species adapted survival in habitats with pH= 4 -11 (Clark et al., 2004). However, *Aedes albopictus* species were not allowed to exist in *Nepenthes gracillis* and *Nepenthes mirabilis* with acidity lower than pH=3 (Chou, 2015). Mosquito larvae species influenced negatively by lower pH levels through excessive efflux sodium that could not be filled via uptake active of sodium ions from surrounding environment (Paradise and Dunson, 1997). On the other hand, in pitcher fluids consisted numerous ion types that all these ions were obtained from breaking down of death insects, detritus, and as well as all chemical compound reaction. These ion types were illustrated to impact on larval mosquitoes in container as well as in pitchers (Yee et al., 2010).

8. Organic compound in pitcher fluids

Pitcher fluids contained organic compounds and some organic compounds was influenced on larval survivorship of foreign species (Chou, 2015). Pitcher fluid of *Nepenthes* had been realized to contain various organic compounds. Neprosin 1 is an enzyme type was isolated from pitcher fluids *Nepenthes* and this enzyme used for a bottom-up proteomic approach, particularly, neprosin 1 was useful for developing sequence coverage for histone mapping and proteome analysis (Athauda et al., 2004). Moreover, protein Pathoge-related (PR) was detected in pitcher fluid *Nepenthes mirabilis* and pathogen-related protein exhibited an antibacterial activity such as *Escherichia coli* in acidity levels pH (3-4) that provided optimal to apply in the medical field (Miguel et al., 2018, Buch et al., 2014). Furthermore, some chitinase of enzyme class in *Nepenthes* pitcher fluids was indicated to protect effectively fungal contamination, especially this enzyme type facilitated on insect exoskeleton degradation (Ishisaki et al., 2012). Chia et al. (2004) indicated function of free radicals in pitcher fluids to assist protein digestion of preys. Plumbagin was polyphenolic compound that played important role for preventing molting of insects and synthesis chitin (Kubo et al., 1983).

9. Mosquito species in pitcher habitats

Pitcher plants *Nepenthes* had been known to provide habitats for female mosquitoes to lay their eggs and supported mosquito offspring development. An observation of mosquito species inhabited in three *Nepenthes ampullaria*, *Nepenthes rafflesiana*, and *Nepenthes gracilis* in Singapore was shown that had 9 mosquito species. Larval mosquitoes *Tripteroides nepenthes* and *Uranotania mouitoni* existed in different *Nepenthes* species. However, other mosquito species preferred in specific pitcher *Nepenthes* species such as *Tripteroides tenax*, *Culex coerulescens*, *Culex cartipalipis*, *Culex eminentia*, *Culex hawitti*, *Culex navalis*, and *Uranotaeia xanthomelaena* (Mogi and Chan, 1997). Mosquitoes *Aedes dyasi* and *Aedes maehleri* same *Stegomyia* subgenus used to *Nepenthes mirabilis* for their larvae habitats development in Yab island of Micronesia (Mogi, 2010). Moreover, mosquito larvae *Aedes albopictus* were found in *Nepenthes ampullaria* at lowland in Peninsular Malaysia (Chou et al., 2016). Mosquito larvae species of *Topomyia nepenthicola* in genus *Topomyia* were discovered in *Nepenthes stenophylla* above 1200m sea level of Northern Sarawak in Malaysia (Miyagi and Toma, 2007). However, Chou et al (2015) suggested that *Nepenthes* species were not suitable habitats for mosquito larvae development. Tsukamoto (1989) reported that mosquito larvae *Culex rajah* and *Toxorhynchites rajah* found in pitcher *Nepenthes rajah* at high elevation 1600 to 1800m that these mosquito larvae species existed with other mosquito larvae such as *Culex jenseni* and *Uranotaenia moultoni* in Malaysia. Southern Thailand, there were currently few reports mosquito larval existing in *Nepenthes* pitcher plants. Mosquito larvae *Tripteroides tenax*, *Toxorhynchites manopi*, and *Culex curtipalpis* were found first time in *Nepenthes mirabilis* at peat swamp forest (Apiwathnasorn et al., 2009).

10. Mosquito larvae identification

Larval mosquitoes were distinguished into three main parts; head, thorax, and abdomen. Head of mosquito larvae was recognized with subparts such as antenna, cranium head capsule, hypostomal suture, lateral palatal brush, median labral plate, mandible, maxillary palpus, mentum, maxilla, and posterior tentorial pit. For thorax part of mosquito larvae was divided into prothorax, mesothorax, and metathorax that these sections consisted setae. On the other hand, abdomen part of each mosquito larvae contained nine apparent segments. Siphon connected to abdomen part of mosquito larvae in some species (Rattanarithikul et al., 2006, 2005).

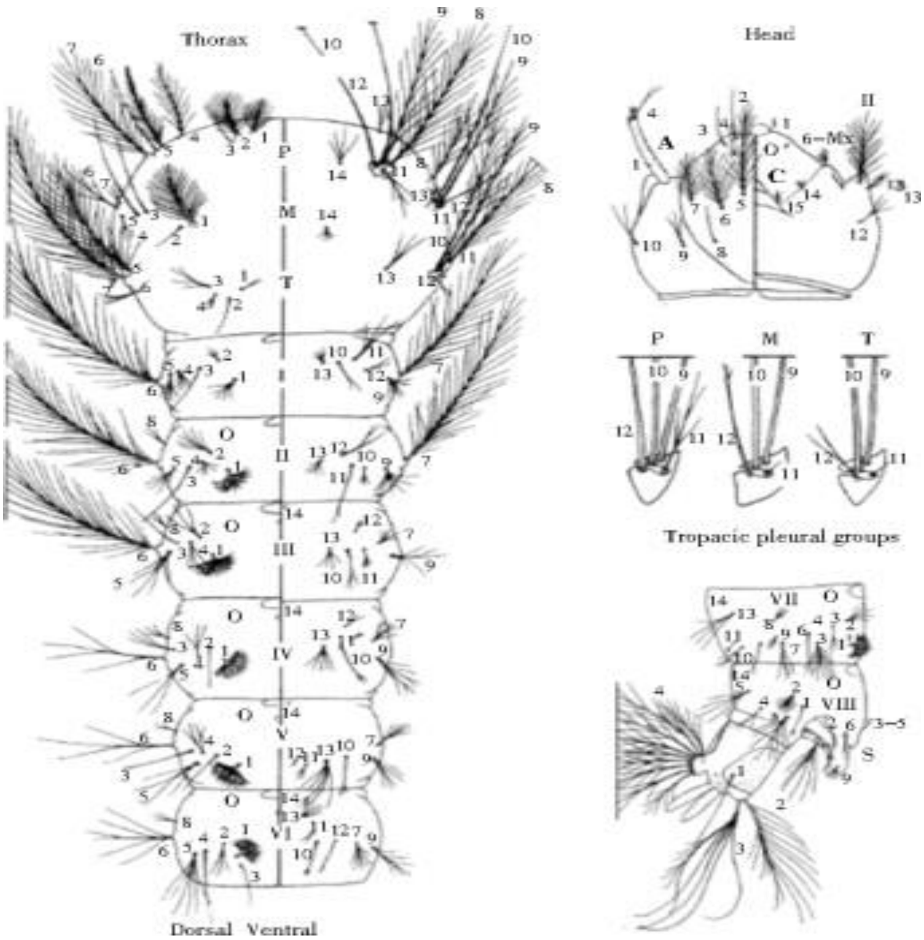

Figure 2: Morphological mosquito larvae and identification (Cunathilak et al., 2014).

11. Mosquitoes oviposition

Almost insects were not deposited randomly their eggs. Insects tried to adjust both abiotic and biotic environmental factors. For instance, gravid female mosquitoes received information abiotic and biotic environmental factors in order to ensure that they did appropriately oviposition quality decision (Edgerly et al., 1998). The gravid female mosquito oviposition had been observed widely that mosquitoes were possible to utilize chemo-tactile, taclitle, and olfactory or visual cues to detect suitable site characteristics (Chou et al., 2015, Heard, 1994). The gravid female mosquitoes decided their decision to deposit their eggs dependent on sit characteristics such as color, reflectance, moisture, texture, surrounding vegetation, salinity, bacterial growth, conspecific other larval insects density, and presence of a different chemical (Bentley and Day 1989). According, reporting Chou et al (2015) indicated that *Aedes aegypti* and *Aedes albopictus* preferred to oviposit in black color and large size of containers. Gravid female mosquito *Culex quinquefasciatus* and *Ochlerotutus australis* utilized biological and chemical cues to detect suitable water for their oviposition. Therefore, these mosquito species were successfully for their eggs laying in water habitats with conspecific other larval (Mokany and Shine, 2003).

The successfulness offspring of female mosquitoes were dependent on female mosquito decision (Crump, 1991). Gravid female mosquitoes used two behaviors preoviposition, which concerned with attraction to deposit eggs on sites, and oviposition behavior referred to actual mosquitoes deposited on substrate (Bentley and Day, 1989). Female mosquitoes of genera *Anopheles*, *Sabethes*, *Toxorhynchites*, and *Wyeomyia* preferred to lay individual eggs on water surface. However, females of genera *Coquillettidia*, *Culex*, and *Culiseta* evaluated water chemistry before they released eggs on raft directly on water surface. On the other hand, some genera of mosquito species oviposit their eggs on above water line (Bentley and Day, 1989).

Some factors had been considered to impact on mosquito oviposition such as color of substrate, type of water, absence of vegetation, presence of microorganisms and volatile compound produced from breeding sites (Huang et al., 2005). Moreover, microorganisms; protozoa, rotifer, bacteria, and fungi microalgae as well as detritus in mosquito habitats were well known influenced both on mosquito decision oviposition and mosquito larvae survival (Duguma et al., 2016). Microorganisms in mosquito habitats produced volatile for serving as semiochemical to providing signal for female gravid mosquito *Anopheles gambiae* (Sumba et al., 2004). On the other hand, predators were investigated to impact on mosquito oviposition of *Culex quinquefasciatus* species. Mosquito larvae *Culex quinquefasciatus* species were declined dramatically when fishes inhabited in mosquito larval habitats (Van Dam and Walton, 2008). The species *Mesoyclops longisetus* in genus *Coppepod* were realized that produced some substances for attractive gravid females *Aedes aegypti*.

Therefore, *Aedes aegypti* species preferred to oviposit in containers with *Mesocyclop longisetus* species (Torres-Estrada, 2001).

12. Life cycle of mosquitoes

Mosquito life cycle had four stages development such as; egg, larvae, pupa, and adult (Figure 2). Female mosquitoes were normally interest water and flowing water places, which were pooling surface, artificial containers and sometime on surface floating vegetation, to deposit their eggs around an egg to several hundred eggs. Eggs of mosquitoes were hatched to larvae that larvae stage consisted first larvae, second larvae, third larvae, and four larvae that these larvae stages distinguished by them size. Mosquito larvae consumed microorganisms and organics for their growing to be pupa during 1 to 3 weeks or depended on temperature in their habitats. Pupa stage was call resting stage during 1-3 day before pupa become adults. Adult females absorbed blood for eggs production but adult males preferred to feed on lower nectar (Rueda, 2008).

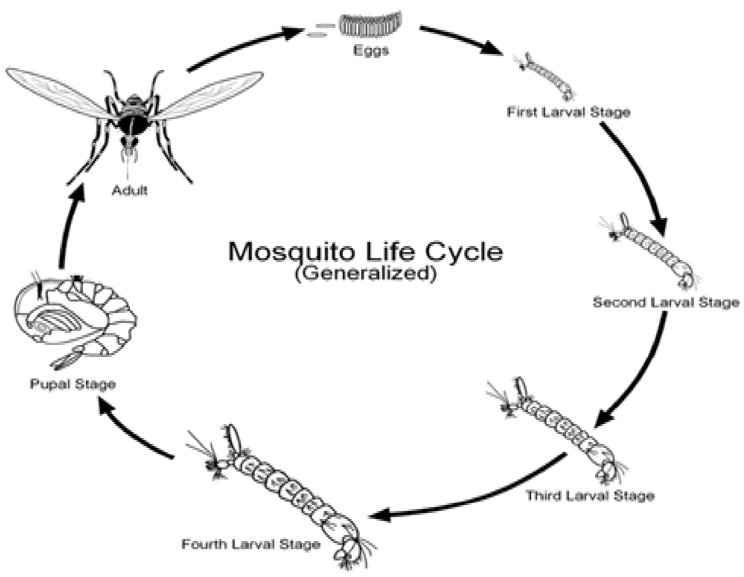

Figure 3: Life cycle of mosquito development (Rueda, 2008)

13. Advantages of mosquito niches

Mosquitoes had been realized essential group of arthropod that served vital functions for ecological food web and transferred pollen for some plant species (Ruedal, 2008). Mosquito larvae in freshwater were main food resource for fishes and larger larvae of dragonflies. Addition, some mosquito larvae served foods for wading birds. Mosquito larvae in water habitats assisted nutrient recycling in term of mosquito larvae consumed organic matter and detritus in water. Furthermore, adult mosquitoes provided part diet of some invertebrates such as birds, mammals, reptiles, and amphibians (Asha and Aneesh, 2014). Male mosquito some species carried out pollens when they visited flower and feed nectar. For example, an observation mosquito *Culex pipien* and plants *Tanacelum* vulgare species were indicated that *Culex pipien* visited *Tanacelum vulgare* flowers and brought pollens between inflorescences same or different plants (Peach and Gries, 2016).

Chapter 2
Materials and methods

1. Study sites

This study were carried out in three sites in Southern Thailand that were covered three *Nepenthes* species pitcher plants, *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*.

1.1. Surat Thani

This study is located at Surat Thani province within Kanchanadit district 9°11'23.8"N 99°32'28.0"E. Surat Thani province has a tropical savanna that temperature is stable throughout the year, which short dry season from January to April and rainy season from May to December (Figure 11) (data supported by Meteorological Station at Surat Thani province). This study site has small population around 300 pitcher plants *Nepenthes thorelii* species.

1.2. Trang

This study site is located in Trang province in Prince of Songkhla University Trang campus that has position 7°30'56.2"N 99°34'46.7"E. Trang province has a tropical monsoon and temperature different slightly per year. The season in Trang province has short dry season from January to March and long rainy season from April to December (Figure 12) (data supported by Meteorological Station at Trang province). Prince of Songkhla University Trang campus has several large *Nepenthes mirabilis* var. *globosa* populations. Each *Nepenthes mirabilis* var. *globosa* population. This study sit is less of shrub trees to cover with these populations.

1.3. Songkhla

The study site is located in Songkhla province within Bangkhlam district 7°01'41.4"N 100°22'27.6"E. Songkhla province has climate high temperature and humidity and dry season from middle of January to April and rainy season from May to January (Figure 13) (data supported by Meteorological Station at Hat Yai, Songkhla province). This study site consist of large *Nepenthes mirabilis* var. *mirabilis* population.

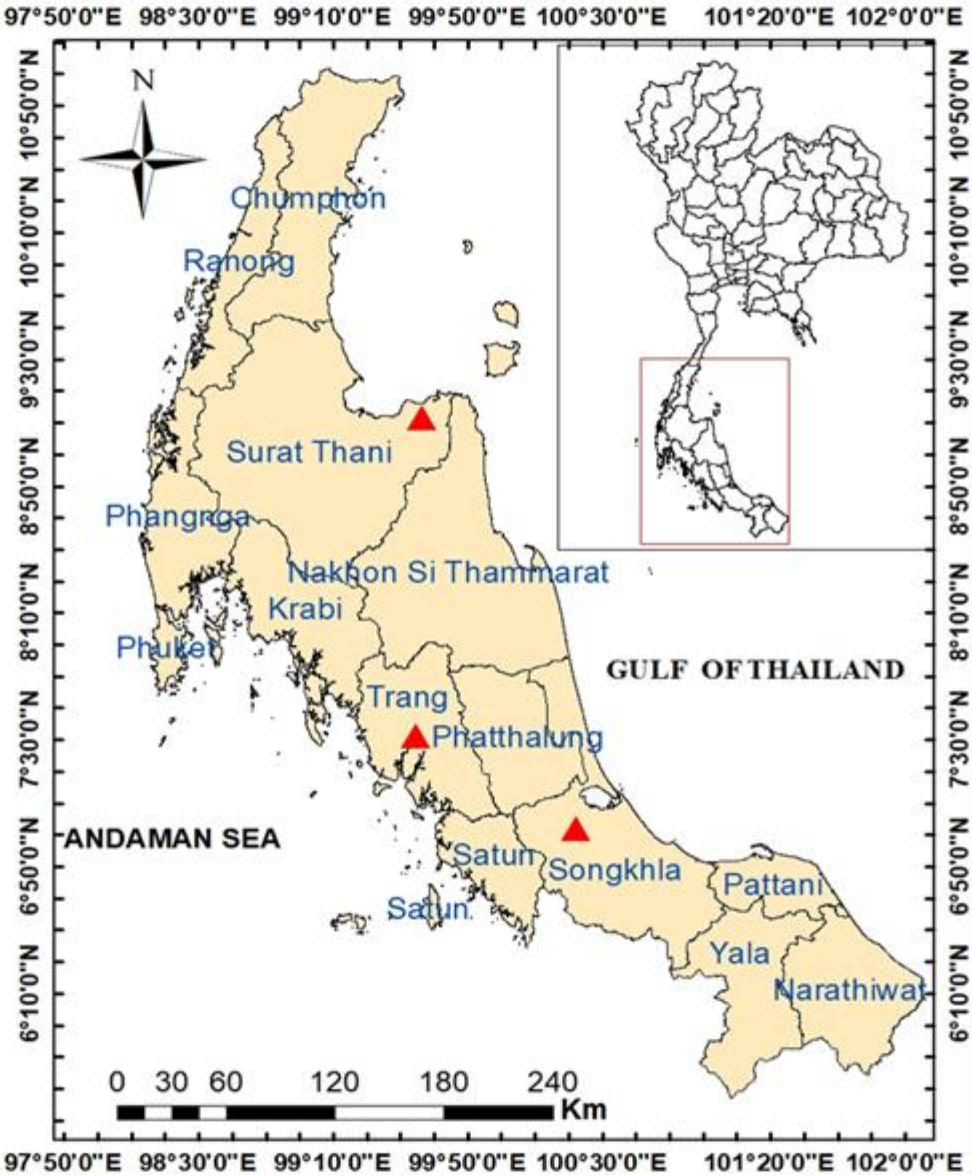

Figure 4: Map study sites, Surath Thani, Trang, and Songkhla province in southern of Thailand.

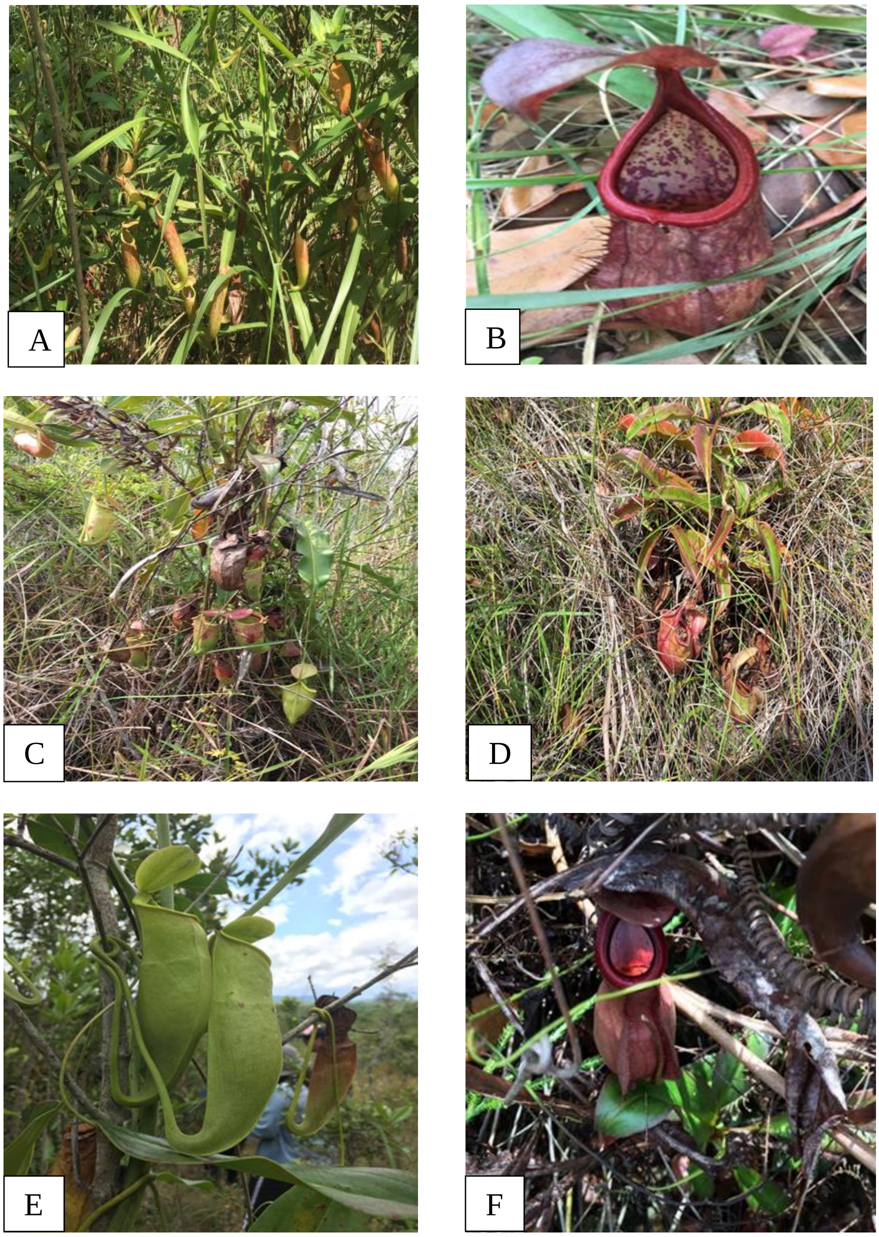

Figure 5: Upper pitcher and lower pitcher of *Nepenthes* pitcher plants. A: upper pitcher *N. thorelli*, B: lower pitcher (A & B in Surat Thani province) *N. thorelli*, C: upper pitcher *N. mirabilis* var. *globosa*, D: lower pitcher *N. mirabilis* var. *globosa* (C & D in Trang province), E: upper pitcher *N. mirabilis* var. *mirabilis*, F: lower pitcher *N. mirabilis* var. *mirabilis* (E & F in Songkhla province).

2. Sample collection

Pitcher plants *Nepenthes* were commonly found in lack nutrient habitats particularly nitrogen and phosphor. Pitcher plants *Nepenthes* species produce jug-shaped that was designed for abilities to attract, catch, retain and digest preys in order to absorbing nutrients pitcher plants growth and reproduction. Pitcher plants *Nepenthes* spp. in our sites were little especially in Surat Thani, *Nepenthes thorelii,* that have only 300 pitcher plants and some pitcher plants were not produce pitchers. Moreover, pitcher plants were lacking water for blossom during summer season and we were not able to control pitcher plants blossom all the time. Furthermore, growth and reproduction of pitcher plants supported by their pitcher traps. Therefore, we needed enough pitcher's number for investigation better than to destroy whole pitcher's populations.

Each study site 50 pitchers including either lower or ground 25 pitcher plants, which have mouth opening towards the tendril and wings situated along the pitcher wall, and 25 upper or aerial pitchers that have no wings and tendril form on backside were collected. A pitcher plant was cut only a pitcher cup and samples were collected two times during rainy (September-November 2017) and dry season (February-April 2018).

25 upper pitchers
50 pitchers $\left\{ \begin{array}{l} \\ \\ \end{array} \right\}$ **a pitcher cut in a pitcher plant**
25 lower pitchers

Figure 6: Pitcher sampling; 50 pitcher cups cut in each study site which have 25 upper and 25 lower, all each pitcher cup cut in only one pitcher plants.

3. Biometric data and environmental parameters measurement

Each study site, temperature and humidity were recorded during samples collection. Longitude and latitude of all pitcher cups were also recorded. All pitcher cups were cut on pitcher's petiole of leaf with pitcher cup from pitcher plants by using knife. Height of each upper pitchers from ground in a pitcher plant was measured by using meter from surface soil to lib tip pitcher cup. Width opening pitchers were measured at broadest point of pitchers using caliper. Moreover, pitcher length was recorded by measuring from pitcher cup's spur to pitcher cup bottom by caliper (Bauer et al., 2012). Pitcher's color was estimated by using colorimeter (HunterLab: L*, A*, B*).

The pitcher fluids were estimated various parts at study sites and continually record in laboratory. The pH levels of pitcher fluids were estimated by Digital pH meter and electrical conductivity measured by electrical conductivity meter. Each pitcher fluid sample was transferred to vial in order to measure the actual fluid by digital balance. Consequently, pitcher fluids were transferred to the 50 mL centrifuge tubes with formalin 7% for working in laboratory. On the other hand, active zone of pitchers, which area internal surface pitcher cup's wall secretion and absorption liquid or nutrients, estimates by filling the empty pitcher with water then transfer water in vail for measuring with digital balance.

In laboratory, larvae mosquitoes were separated from 50 mL centrifuge tubs in order to keep in centrifuge tubes 15 mL with alcohol 75% for identification. All pitcher fluid samples filter by using fold 1mm tulle mesh in order to separate detritus and microorganisms that consisted in pitcher fluids (Hoekman, 2011). The detritus was dried in 50 ^{0}C at least 48 hours and measured by 4 digits electric balance (Yee et al., 2010).

Food source microorganisms, protozoa, rotifera, of larvae mosquitoes in pitcher fluids were estimated all pitcher samples. Each pitcher fluid was centrifuged slightly during 10 minutes then to use auto-pipette to pick up fluid 100µL and drop on hemocytometer slide that protozoa and rotifer abundant were recorded in term of each sample replicated 5 time (Hoekman, 2011).

4. Larval mosquitoes identification

1.4. Slides preparation

Glass slides were cleaned to make sure without dust and debris. Glass slides were immersed in liquid detergent during 4-8 hours and each surface side of glass slides cleaned by rubbing with washing cloth or sponge using between forefinger and thumb. After that, glass slides were washed into water two times in order to remove all traces of liquid detergent then all glass slides were dried. Next step of glass slides cleaning, the glass slides were wrapped in packs of 10 in pieces of cleaning paper then to wrappers turn down. The packs were put in empty boxes and some silica gel put in boxes in order to keep glass slides for using along period. Unfortunately, make pen was used to make label the boxes such as date, box number, number of glass slides per box and person's name.

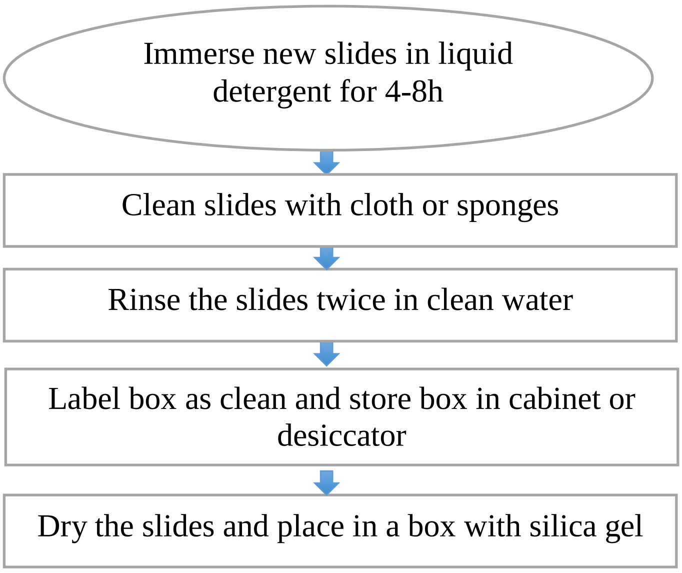

Figure 7: Indicate cleaning slid procession (World Health Organization, 2009).

4.2. Mounting larval mosquitoes procession

Larval mosquitoes were transferred into Threaded Test Tubes (16x125mm) with alcohol 70% for at least two hours. Larval mosquitoes were removed from Treaded Test Tubes alcohol 70% by using pipette and transferred to Threaded Test Tubes (16x125mm) 95% alcohol a least two hours. Finally, larval mosquitoes were kept in Threaded Test Tubes (16x125mm) containing xylene. Some larval mosquitoes were cleaned more than three days with xylene.

Larval mosquito species were placed on center glass slide and arrange larval mosquitoes position with dorsal side up in order to cut between sixth and seventh abdominal segment of larval mosquitoes. Therefore, the view of pecten, teeth, siphonal tufts, anal segment, anal brush, and comb scales of the eighth abdominal segment were clearly visible. The abdomen segment cutting exhibits position of terminal segments below and rest larval mosquito siphon to the right. Moreover, all lateral setae of larval mosquitoes were arranged in a natural position, which larval mosquito show their mid dorsal line in center.

All larval mosquitoes were observed before to start mounting procession in order to recognize whether some larval mosquito’s hairs were broken or appropriate their position. Larval mosquitoes on cleaning glass slide were mounted directly with toluene solution and ensure toluene solution cover all larval mosquitos specimen. Cover slip glass was applied immediately after larval mosquitoes completely covered with toluene solution. Cover slip was held in pair of forceps and place on larval mosquitoes quickly in order to avoid larvae mosquito movement under pressure of cover slip.

All permanent slides were established two labels on both right and left side of the cover glass slid. The label left side comprised specimen scientific name, data collection, locality, and name of collector. Whereas, label at right put chief characteristics that were used to identify species to species level (Burton, 1953). Larval mosquitoes were identified to species level using the keys (Rattanarithikul et al., 2005a, 2005b, 2006, 2007, 2010) base on their external morphological characteristics.

Figure 8: Data collection; A; Activity cut pitcher plants, B & C; Measuring length and width of pitchers, D; measuring dry detritus weights in each pitcher, E; Fixed larval mosquitoes in 70% ,75%, 95% alcohol, F; Counting protozoa and rotifer abundant in each pitcher, G; Larval mosquito identification.

5. Data analysis

Characteristics and environmental conditions of *Nepenthes* pitcher plants were analyzed for correlation and abundance mosquito prediction. Principal components analysis (PCA) was used to reduce the number of variables and create independence variables. Principal component analysis performs on correlation matrix of pitcher's variables. This was equivalent to using the variance-covariance matrix of standardize variables and had advantage of compensating for different units of measurement. The principal components analysis (PCA) was chosen in three factors or principal components with eigenvalues > 1.0. The results of three principal components were later used as independent variables. Multiple linear regression was used to predict abundance mosquito larvae as dependent variable with independent variables of three components PCA.

Spearman Rank correlation coefficient were analyzed to find out influence pitcher variables impact on each abundance of larval mosquito species. For all tests, a *P*-value < 0.05 were considered to represent significant differences (Mangudo et al., 2017).

Analysis of variance (one-way ANOVA) was applied to compare mean abundance of larval mosquitoes in pitchers between rainy and dry season. In addition, it was important to determine whether there were different abundance of larvae mosquitoes between upper pitchers and lower pitchers of *Nepenthes* pitcher plants. Analysis of variance (one-way ANOVA) was also used to compare mean numbers abundance of larval mosquitoes between upper pitchers and lower pitchers *Nepenthes* pitcher plants. All data were transformed following logarithmic transformation in order to assume normal distribution (Bond et al., 2014, Thongsripong et al., 2014). All data analysis were carried out using R program version 3.5.1 (R Core Team, 2018).

Chapter 3

Results

1. Mosquito species and abundance in pitcher *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*

Twice sample collections during rainy and dry seasons of mosquito larvae in *Nepenthes* pitcher plants were collected in Surat Thani, Trang and Songkhla provinces. Pitchers 50, which had 25 upper pitchers and 25 lower pitchers, of three species *Nepenthes* pitcher plants were collected during rainy and dry seasons. The results were showed that 5 mosquito species belong to 3 genera and 1,155 mosquito individuals were identified (Table 1). *Tripteroides tenax* (85%, 991 individuals), *Tripteroides* species.1 (4.59%, 53 individuals), *Toxorhynchites albipes* (1.65%, 19 individuals), *Uranotaenia demeillori* (13.33%, 154 individuals), *Uranotaenia bicolor* (1.3%, 15 individuals). The morphological *Tripteroides tenax* larvae species had large seta 1-P, M, T (P= Prothorax, M= Mesothorax, T= Metathorax) with stellate more than 6 branches and seta 1-IV-VIII had highly branched, and setae 0, 2, 11-IV-VII stellate were presence, that setae 8-VII were smaller. Moreover, seta 4-X were branched and they were short as long as saddle. Furthermore, larval *Tripteroides tenax* comprised comb scales arising from sclerotized plate. Conversely, larval *Tripteroides* species.1 species were identified that seta 1-P, M, T were single or few branches, seta 1-IV-VII were not stellate and seta 4-X had single or double long more than width of saddle two times. Larval mosquito *Toxorhynchites albipes* species identification were recognized by their lateral palatal brush 6-10 thick with simple filaments, comb and pecten were absent. Moreover, larval *Toxorhynchites albipes* species had head darkly pigment and siphon was short, which contained seta 1-S single or double with spine shape. Larval mosquito species identification in genus *Uranotaenia* were ventral brush (seta 4-X) with 3 or more pairs of setae. A single pair of setae were located in siphon and siphon comprise pecten. The head larval had no hypostomal suture or incomplete. Setae 5, 6-C were spin shape and abdominal segment VII contained sclerotized plate. Larval mosquito *Uranotaenia demeilloni* species were identified the presence of comb scales and pecten were simple with spine shape and seta 9-T was single. In contract, larval mosquito *Uranotaenia bicolor* recognized such as; antenna had few small scattered and seta 14-C were stout and frayed apically. A species accumulation curve of mosquito species in pitcher plants *Nepenthes* showed that the species recoded was likely to be considerable underestimate of the real numbers (Figure 10).

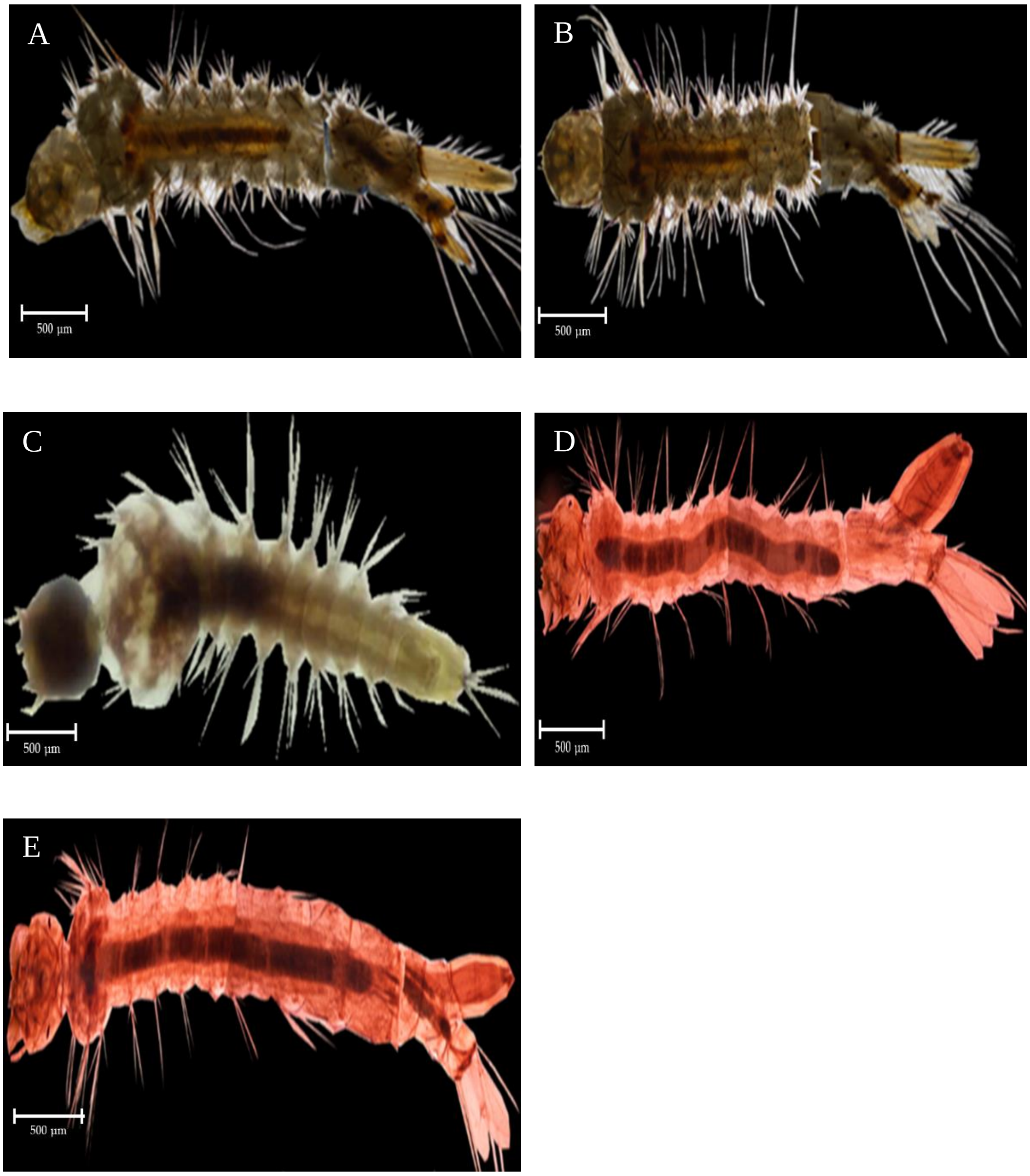

Figure 9: Larval mosquito species from all study sites, A: *Tripterioides* species.1, B: *Tripteroides tenax*, C: *Toxorhynchites albipes*, D: *Uranotaenia demeillori*, E: *Uranotaenia bicolor*.

Table 1: Abundance larval mosquito species in *Nepenthes* species; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* during rainy and dry seasons.

Province	Surat Thani		Trang		Songkhla	
Nepenthes species	*Nepenthes thorelii*		*Nepenthes mirabilis* var. *globosa*		*Nepenthes mirabilis* var. *mirabilis*	
Season	Rainy	Dry	Rainy	Dry	Rainy	Dry
Tripteroides tenax	0	0	262	203	484	42
Tripteroides species.1	0	0	18	24	11	0
Toxorhynchites albipes	0	0	7	3	7	2
Uranotaenia demeillori	32	45	0	0	0	0
Uranotaenia bicolor	5	10	0	0	0	0
Total	37	55	287	230	502	44

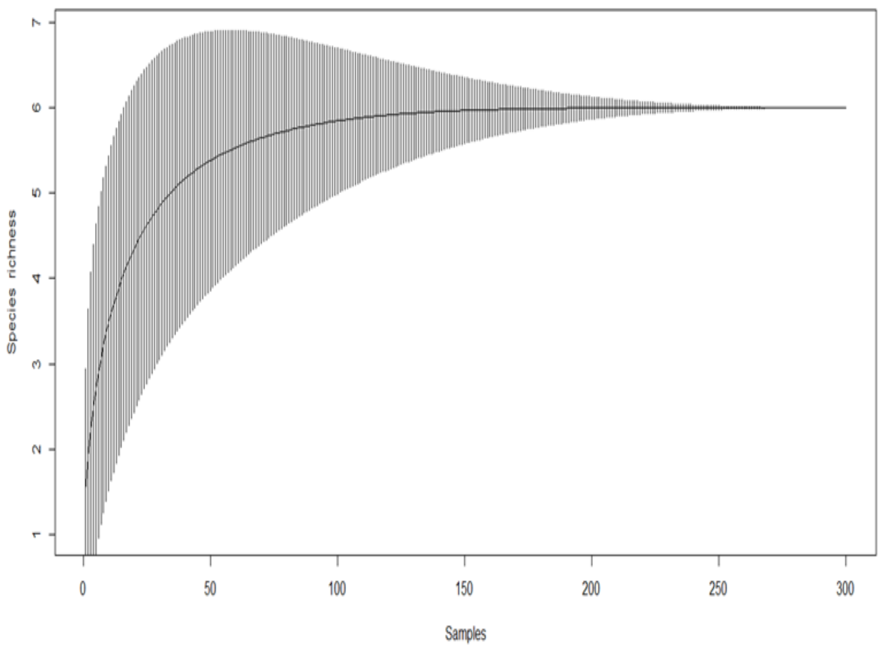

Figure 10: Species accumulation curve of larval mosquito species in each sample.

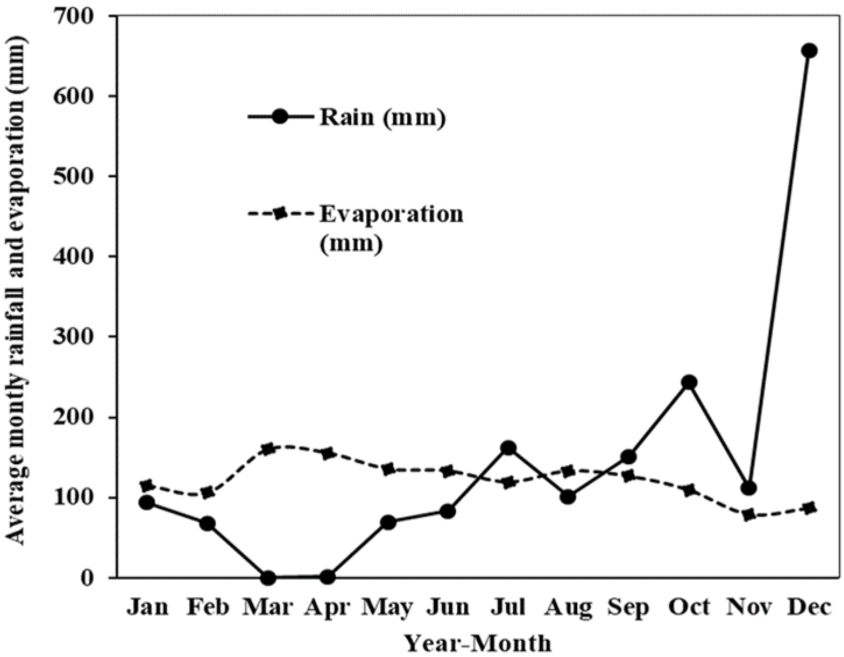

Figure 11: Average monthly rainfall and evaporation in Surat Thani province 2017 (data supported by Meteorological Station at Surat Thani province)**.**

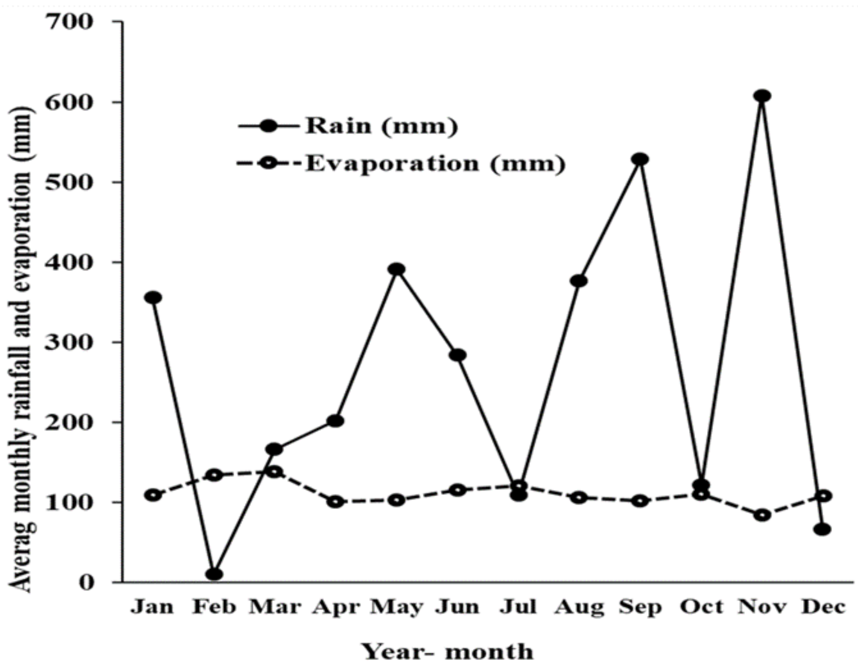

Figure 12 Average rainfall and evaporation data in Trang province during 2017 (data supported by Meteorological Station at Trang province).

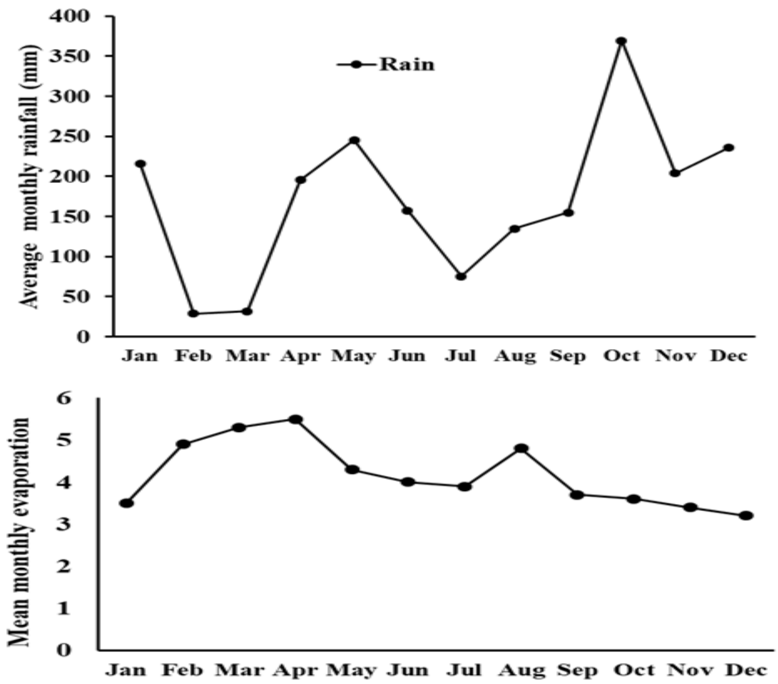

Figure 13: Average monthly rainfall and mean evaporation in Songkhla province 2018 (data supported by Meteorological Station at Hat Yai, Songkhla province).

Table 2: Pitcher's variables measurement (Mean ± SE) of *Nepenthes thorelii* in Surat Thani province (Abbreviation; SE: Standard Error).

Nepenthes thorelii					
	Rainy season		**Dry season**		**Number pitcher**
	Upper	**Lower**	**Upper**	**Lower**	
Length (cm)	16.78±0.33	10.83±0.25	15.17±0.35	10.23±0.23	50
Width opening (cm)	3.33±0.07	3.50±0.08	4.84±1.09	2.91±0.06	50
Dry detritus (g)	0.03±0.01	0.14±0.05	0.03±0.01	0.09±0.02	50
pH	3.12±0.21	6.20±0.19	3.25±0.18	5.15±0.29	50
Actual fluid (ml)	5.42±0.63	11.30±0.71	7.04±0.51	8.45±0.88	50
Active zone (ml)	39.30±2.11	40.67±2.76	23.27±0.93	20.24±1.27	50
Electrical conductivity (µs/cm)	81.40±4.45	1025.58±142.67	127.79±14.18	128.52±16.82	50
Protozoa abundance	2277.80±634.01	3075.64±1107.95	1958.68±612.14	1147.92±274.43	50
Rotifera abundance	567.64±178.25	1039.72±514.54	975.44±354.90	340.72±978.20	50

Table 3: Pitcher's variables measurement (Mean ± SE) of *Nepenthes mirabilis var. globosa* in Trang province (Abbreviation; SE: Standard Error).

Nepenthes mirabilis var. globosa					
	Rainy season		**Dry season**		**Number pitcher**
	Upper	**Lower**	**Upper**	**Lower**	
Length (cm)	12.86±0.25	14.53±2.28	12.63±0.27	10.26±0.20	50
Width opening (cm)	4.34±0.10	4.27±0.10	5.26±1.02	3.72±0.07	50
Dry detritus (g)	0.07±0.07	0.08±0.03	0.04±0.02	0.01±0.004	50
pH	2.90±0.27	4.24±0.23	2.85±0.11	4.52±0.16	50
Actual fluid (ml)	13.61±1.12	27.61±1.84	14.70±2.17	20.60±1.55	50
Active zone (ml)	25.89±1.51	40.95±2.94	23.39±2.22	29.46±1.78	50
Electrical conductivity (μs/cm)	3056.24±346.09	827.96±174.99	387.20±22.21	210.28±13.7	50
Protozoa abundance	4630.56±1036.12	14898.16±3420.12	2251.96±597.47	2437.88±732.55	50
Rotifera abundance	1944.64±625.67	7076.66±2114.77	538.00±384.29	1890.92±709.67	50

Table 4: Pitcher's variables measurement (Mean ± SE) of *Nepenthes mirabilis var. mirabilis* in Songkhla province (Abbreviation; SE: Standard Error).

	Nepenthes mirabilis var. mirabilis				
	Rainy season		**Dry season**		**Number pitcher**
	Upper	**Lower**	**Upper**	**Lower**	
Length (cm)	12.62±0.27	10.26±0.20	11.91±0.40	10.31±0.28	50
Width opening (cm)	5.26±1.02	3.72±0.07	2.90±009	2.43±0.09	50
Dry detritus (g)	0.04±0.02	0.01±0.004	0.32±0.01	0.27±0.05	50
pH	2.85±0.11	4.52±0.16	6.05±0.09	5.96±0.12	50
Actual fluid (ml)	14.70±2.17	20.60±1.55	5.58±0.48	5.72±0.41	50
Active zone (ml)	23.39±2.22	29.46±1.78	13.91±0.85	17.71±1.53	50
Electrical conductivity (µs/cm)	387.20±22.21	210.18±13.7	99.76±6.7	85.32±6.43	50
Protozoa abundance	2251.96±597.46	2437.88±732.55	2524.12±815.41	3077.88±766.61	50
Rotifera abundance	538.00±384.29	1890.92±709.67	420.96±182.39	596.24±211.37	50

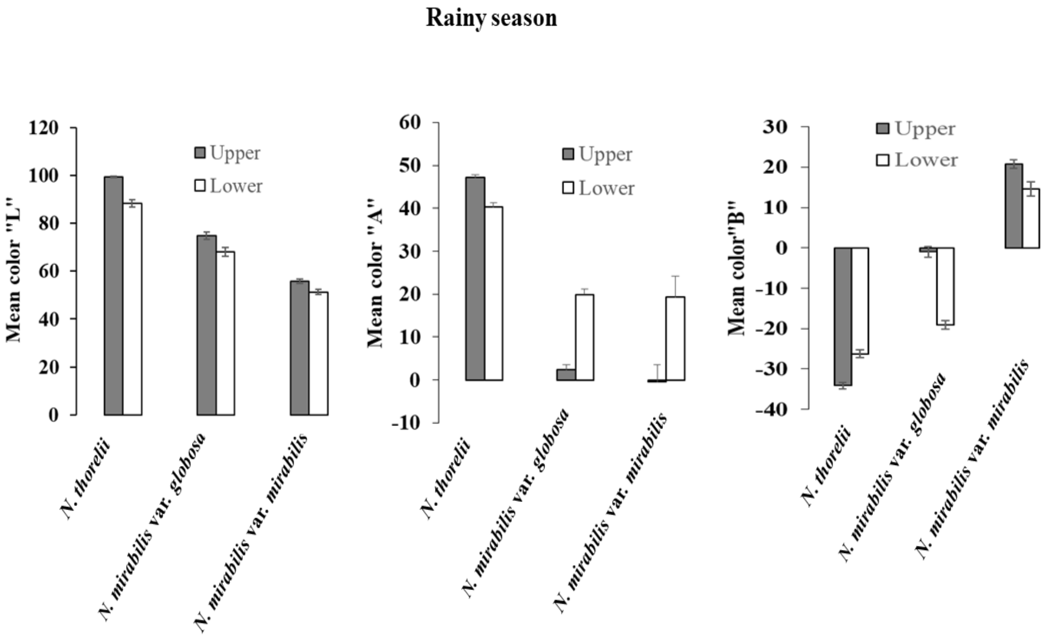

Figure 14: Mean color “L”, ”A”, “B” of pitcher plants; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* in rainy season.

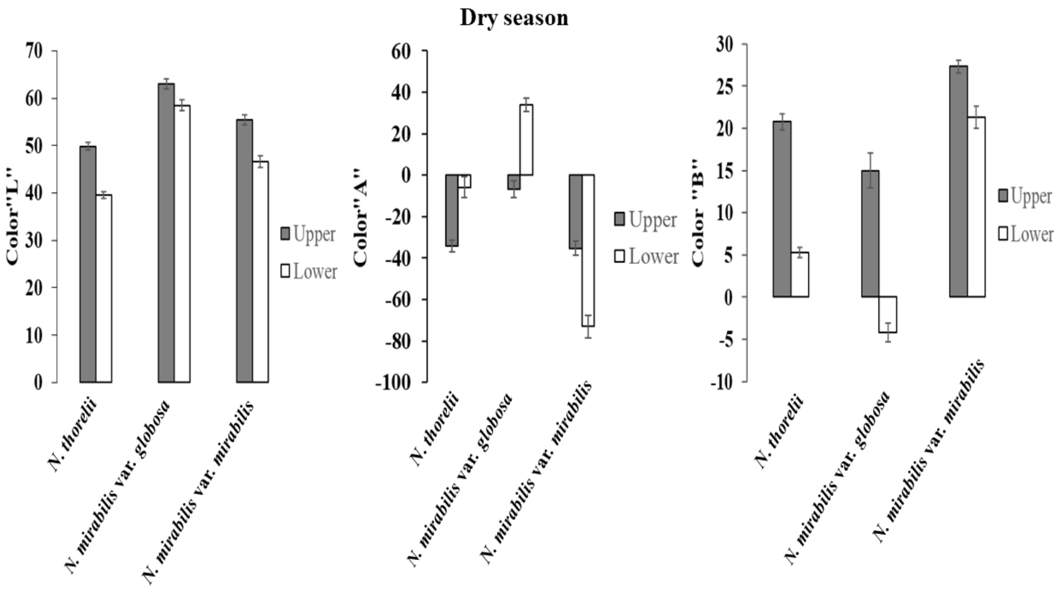

Figure 15: Mean color “L”,”A”, “B” of pitcher plants; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* in dry season.

2. Environmental conditions and characteristics of *Nepenthes* pitcher plants predict mosquito abundant and impact on abundance larval mosquito species

2.1. Environmental conditions and characteristics of *Nepenthes* pitcher plants predict mosquito abundant

The analysis of relationship of environmental factors and larval mosquito species were carried out by principal component analysis. PCA results were indicated that the first three principal components explained 57% of the total variance for the data set in three sites. There was positive strong loading of variables, length pitchers, width opening of pitchers, active zone of pitchers, actual fluid in pitchers, and electrical conductivity on PC1 which accounted for 27% of the total explained variance and negatively influenced by pH. PC2 accounted for 17% of the total variance and consisted of a strong loading of variables: dry detritus, actual fluid, protozoa abundance, and rotifera abundance. On the other hand, PC3 represented 13% of the total variance and had loading positively with dry detritus and influenced negative by protozoa abundance and rotifer abundance (Table 5).

Table 5: Loading coefficients for pitcher's variables and three principal components (Abbreviation; PC: Principal component).

Variables	PC1	PC2	PC3
Length pitchers	**0.36**	-0.02	0.26
Width opening	**0.46**	-0.10	0.04
pH	**-0.38**	0.35	0.27
Dry detritus	0.01	**0.48**	**0.41**
Active zone	**0.46**	0.17	0.22
Actual fluid	**0.37**	**0.38**	0.13
Electrical conductivity	**0.39**	-0.26	-0.24
Protozoa abundance	0.08	**0.51**	**-0.42**
Rotifera abundance	0.06	**0.36**	**-0.61**
Eigenvalue	1.55	1.23	1.09
Percent variance	0.27	0.17	0.13
Cumulative % variance	0.27	0.44	0.57

Multiple regression analysis was indicated correlation coefficient of 0.13 between larval mosquito abundance and first result principal component. The coefficient 0.07 was illustrated larval mosquito abundance and second principal component. Moreover, multiple regression analysis showed correlation coefficient 0.01 between larval mosquito abundance and third principal component (Table 6).

Table 6: Multiple regression analysis resulted in a partial correlation between mosquito abundance and principal component results (Abbreviation; PC: Principal component).

Principal components	Correlation coefficient R^2	Adjust R^2	*F*- statistic	*P*-value
PC1	0.13	0.1102	7.17	$P < 0.05$
PC2	0.07	0.06	4.71	$P < 0.05$
PC3	0.01	-0.001	0.90	$P > 0.05$

2.2. Environmental conditions and characteristics of *Nepenthes* pitcher plants impact on larval mosquito abundant

The effective pitcher variables on abundance larval mosquito species was considered using spearman rank correlation. The results of spearman rank revealed that variables of pitchers seemed to have less impacting on larval mosquito species in *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* pitcher plants. Spearman rank correlation indicated that larval mosquito species *Tripteroides tenax* corrected positively with actual fluid ($r = 0.33$, $P = 0.003$) and larval mosquito *Tripteroides* species.1 corrected slightly with electro conductivity ($r = 0.12$, $P = 0.03$). On the other hand, larval mosquito *Uranotaenia demeillori* was associated negatively with electrical conductivity ($r = -0.12$, $P = 0.02$).

Table 7: Spearman's correlation coefficient among the pitcher characteristics and abundance of larval mosquitoes.

Variables	Mosquito larvae	
	r_s	*p*
Length (cm)	0.22*	0.03
Width opening (cm)	0.12*	0.32
pH	-0.24*	0.01
Dry detritus weight (g)	0.26*	0.23
Active zone (ml)	0.21*	0.02
Active fluid (ml)	0.34***	0.00
Electrical conductivity (µs/cm)	-0.02*	0.78
Protozoa	0.34**	0.001
Rotifera	0.25*	0.03

*=$P < 0.05$;**=$P < 0.01$; ***=$P < 0.001$

2.1.1. Seasons are influence on abundance of larval mosquitoes

The mean abundance of larval mosquitoes in each *Nepenthes* species or in each study sites were compared between rainy and dry season by using (one-way ANOVA). The results of analysis of variance (one-way ANOVA) was indicated that there were significant difference between seasons and abundance of larval mosquitoes (one-way ANOVA, F = 16.08, P < 0.05) (Figure 12).

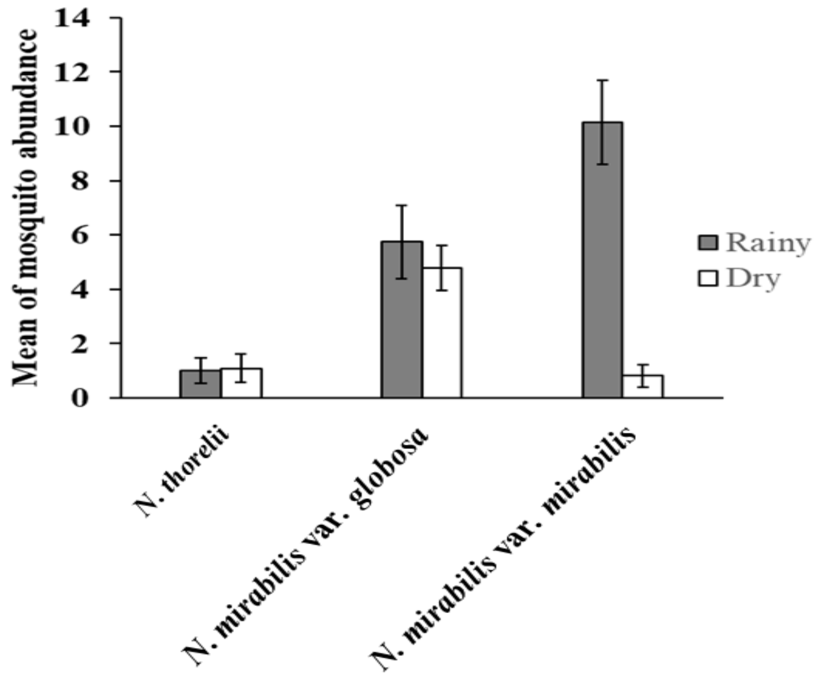

Figure 16: Comparison larval mosquito abundance between rainy and dry seasons in each study sites.

3. Abundance of larval mosquitoes in upper pitchers and lower pitchers

The analysis variance (one-way ANOVA) was indicated that the abundance of larval mosquitoes in upper pitchers and lower pitchers were not significant difference (F = 2.54, P > 0.05) (Figure 13).

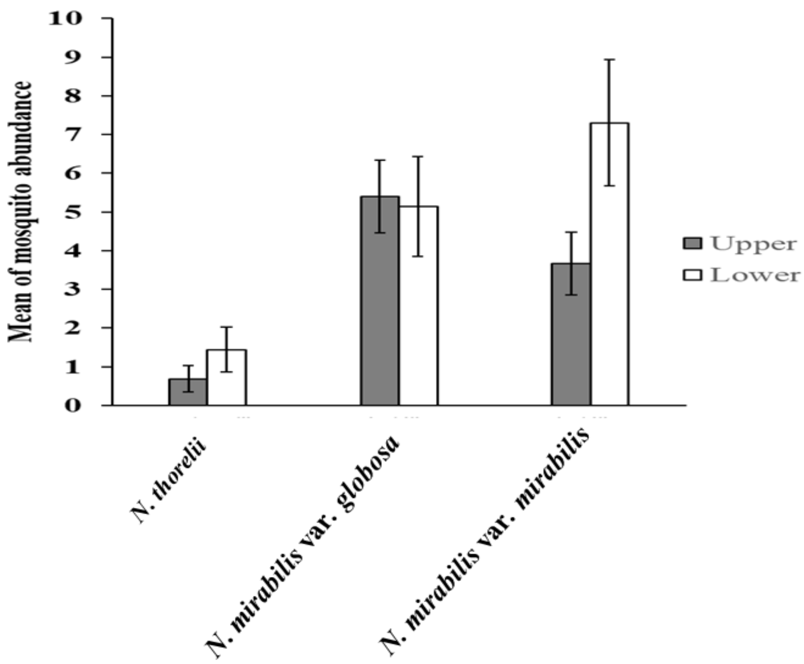

Figure 17: Comparison of larval mosquito abundance between upper pitcher and lower pitcher in each study site.

Chapter 4

Discussion

Mosquito species and abundance in *Nepenthes* pitcher plants

In this study, 3 genera following 5 species and 1,155 individuals of mosquito species were collected from three *Nepenthes* species pitcher plants; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*, in Surat Thani, Trang, and Songkhla province. Mosquito species *Tripteroides tenax* (526 individuals) was the most abundant in *Nepenthes mirabilis* var. *mirabilis* in Songkhla and *Nepenthes mirabilis* var. *globosa* (465 individuals) Trang province. Mosquito species *Tripteroides* species.1 was also found both *Nepenthes mirabilis* var. *mirabilis* (11 individuals) in Songkhla and *Nepenthes mirabilis* var. *globosa* (42 individual) in Trang province. Moreover, larval mosquito species *Toxorhynchites albipes* were consistent discovered both *Nepenthes mirabilis* var. *mirabilis* (9 individuals) in Songkhla and *Nepenthes mirabilis* var. *globosa* (10 individuals) in Trang province. Conversely, larval mosquito species *Uranotaenia demeillori* (57 individuals) and *Uranotaenia bicolor* (10 individuals) were found in *Nepenthes thorelii* in Surat Thani province that these mosquito species were not encountered in *N. mirabilis* var. *globosa* and *N. mirabilis* var. *mirabilis* in Trang and Songkhla province.

It was not surprising that mosquito species *Tripteroides tenax* and *Tripteroides* species.1 had higher number individuals in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis*. Owing to, mosquito larvae *Tripteroides tenax* species were dominant filter feeders in many communities and they were absent in their habitats because of predatory insects or predatory mosquito larvae coexistence (Mogi and Yong, 1992, Clarke and Kitching, 1993, Apiwathnasorn et al., 2009). The reason of mosquito larvae *Tripteroides tenax* species dominant in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis* could be interpreted following the processing of competitive exclusion. This present observation, we found larval mosquito *Tripteroides* species.1 species was lowest abundance than larval mosquito *Tripteroides tenax* species abundant. In this case, larval mosquito *Tripteroides* species.1 species seemed suppression less than mosquito larvae *Tripteroides tenax* with predatory larval mosquito *Toxorhynchites albipes* species that was also discovered in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis*. The competitive exclusion was supported by Mogi and Chan (1997) that showed that occurrence mosquito larvae *Tripteroides tenax* species prevents larval mosquito *Tripteroides nepenthis* from predatory larval mosquito *Toxorhynchites acaudatus* in *Nepenthes* pitcher plants. Mogi and Chan (1996) indicated that mosquito larvae *Tripteroides nepenthis* were higher resistance attacking by predatory larval mosquito *Toxorhynchites* more than mosquito larvae *Tripteroides tenax* species. Consequently, Mosquito larvae *Tripteroides tenax* species might be withstand with predatory larval mosquito *Toxorhynchites albipes* species higher than mosquito larvae *Tripteroides* species.1 thereby predators larval mosquito *Toxorhynchites albipes* consumed larval mosquito *Tripteroides* species.1 instead of mosquito larvae *Tripteroides tenax* species. Therefore, mosquito larvae *Tripteroides tenax* species was detected dominantly in pitcher plants *Nepenthes mirabilis* var. *glosbosa* and *Nepenthes mirabilis* var. *mirabilis* because of competitive exclusion. A mosquito larvae species with large size body was well known *Toxorhynchites* genus which all members in this genus played important role for predatory larval mosquitoes to feed on other larval mosquito species and all members of this genus had their potential of biological agent for controlling vector mosquito species diseases transmission (Danlel et al., 2001, Tyagi et al., 2015). The present study detected mosquito larvae *Toxorhynchites albipes* species first time in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis*. Mosquito larvae species in genus *Toxorhynchites* were realized to inhabit in plant cavities particularly bamboo stumps, tree holes, and *Nepenthes* pitcher plants (Tsukamoto, 1989, Kitching, 1993, Mogi and Chan, 1997, Focks, 2007, Munirathinam et al., 2014). Gravid female mosquitoes *Toxorhynchites* species were well known to lay an egg in one time (Mogi and Chan, 1997). Hence, we were no doubts that this results illustrated lowest abundance *Toxorhynchites albipes* in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis* pitcher plants. On the other hand, pitcher plants *Nepenthes thorelii* species were found two mosquito species; *Uranotaenia bicolor* and *Uranotaenia demeillori*. The mosquito larvae species in genus *Uranotaenia*, which were larval mosquito filter-feeding, played an important role for facilitating food webs and some larval mosquito species in this genus were often found in *Nepenthes* pitcher plants (Beaver, 1985, Mogi and

Yong, 1992, Sota and Mogi, 1998, Thongsripong et al., 2013). Interestingly, our results discovered two larval mosquito species, *Uranotaenia demillori* and *Uranotaenia bicolor*, in *Nepenthes thorelii* in Surat Thani province with lower larval mosquito abundant in pitcher fluids while any predatory insects and predatory larval mosquito species had been yet detected coexistence in *Nepenthes thorelii* pitcher plants. The lower abundance and species richness of immature mosquito species in *Nepenthes thorelii* in Surat Thani site could be interpreted following a reason of specialization attractive prey capture of *Nepenthes* species pitcher plants. Pitcher plants *Nepenthes* species produced their traps specialization with prey-capture. Chin et al. (2014) stated that different pitcher traps of *Nepenthes* species pitcher plants performed attractiveness to different arthropod preys, although, when differentiation of *Nepenthes* species pitcher plants were appeared in the same area. Gaume et al. (2016) pointed out that the specialization in each *Nepenthes* species of pitcher characteristics, which were pitcher shapes, attraction of pitchers, capture traits of pitchers, fluid volumes in pitchers, rewards of pitchers, and different degree of ontogenetic pitcher dimorphism, were the main component to attractive specific preys. For instance, the results of this observation were shown precisely *Nepenthes* species pitcher plants displayed attractive specifically mosquito species that *Nepenthes thorelii* species was found immature *Uranotaenia bicolor* and *Uranotaenia demeillori* mosquitoes. Whereas, pitcher plants *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis* were detected the same three immature mosquito species; *Tripteroides tenax*, *Tripteroides* species.1, and *Toxorhynchites albipes*. Furthermore, pitcher plants *Sarracenia purpurea* species exhibited specifically with *Wyeomyia smithii* mosquito species that this larval mosquito were always found inhabiting in pitcher fluids *Sarracenia purpurea* species pitcher plants (Hoekman, 2007, Trzcinski et al., 2005, Nastase et al,1995, Miller et al.,1994).

Interestingly, this study were impossible to detect immature *Aedes* species in *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* but this results and previous observations brought to highlight that *Aedes albopictus* had been tried to adapt in aquatic *Nepenthes* pitcher plants. Similarly, immature *Aedes albopictus* history were originated from forest habitats such as tree holes, bamboo stumps and consequently they had adapted well to suburban and urban habitats environment including discard vehicle tires, cemetery urns, and water storage containers (Bonizzoni et al., 2013). Milne et al. (2000) showed that *Aedes albopictus* mosquitoes were adaptive to complete their lifecycle in *Sarracenia purpurea* pitcher plants. Indeed, mosquito *Aedes* species (*Aedes dybasi* Bohart and *Aedes maehleri* Bohart) were discovered to adapt in *Nepenthes mirabilis* pitcher plants on the islands of Palau and Yap (Mogi, 2010, Sota and Mogi, 2006). Moreover, Mogi and Yong (1992) showed that *Aedes albopictus* larvae species existed in *Nepenthes ampullaria* in Melaka Zoo in Peninsular Malaysia. Immature mosquito *Aedes albopictus* were reported possible colonization in *Nepenthes ampullaria* pitcher plants on very rare occasions (Chou et al., 2015). Furthermore, recently Chou et al. (2016) pointed that *Nepenthes ampullaria* pitcher plants were unattractive by gravid mosquito *Aedes albopictus* owing to smaller size and green color of *Nepenthes ampullaria* pitcher plants. Therefore, immature *Aedes albopictus* mosquitoes will might be successfully adapted in *Nepenthes* pitcher plants in future coming soon and this results were essential basic knowledge for designing and planning immature *Aedes albopictus* controlling in *Nepenthes* pitcher plants.

Why were few mosquito species in *Nepenthes* pitcher plants?

In this study 5 mosquito larvae species were identified from *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* pitcher plants. An observation of mosquito diversity in peat swamp forest in term of different habitats mosquito larval in Narathiwat province Southern Thailand was found 3 mosquito larvae species; *Tripteroides tenax*, *Toxorhynchites manopi*, *Uranotaenia edwardsi* in *Nepenthes mirabilis* (Apiwathnasorn et al., 2009). Moreover, Cresswell (1998) found 4 mosquito larvae species; *Culex coerulescens*, *Uranotaenia moultoni*, *Toxorhynchites* spp., *Tripteroides nepenthis* in *Nepenthes ampullaria* in heath forest in the Sughei Ingei conservation area of Brunei. Clarke and Kitching (1993) showed genera mosquito larvae; *Culex*, *Tripteroides*, *Uranotaenia*, *Toxorhynchites* in 6 *Nepenthes species* in lowland forests of Borneo. Moreover, Sota and Mogi (1998) detected mosquito larvae 6 species; *Culex navalis*, *Culex curtipalpis*, *Uranotaenia moultoni*, *Uranotaenia gigantean*, *Tripteroides* sp. in *Nepenthes alata* in

West Sumattra. Conversely, Mogi and Chan, (1997) illustrated 10 mosquito larvae species; *Toxorhynchites acaudatus*, *Tripteroides nepenthis*, *Uranotaenia moultoni*, *Uranotaenia xanthomelaena*, *Tripteroides tenax*, *Culex eminentia*, *Culex coerulescens*, *Culex curtipalpus*, *Culex hewitti*, *Culex navalis*, in 3 *Nepenthes* species pitcher plants in Singapore. Mogi and Yong (1992) found 11 mosquito larvae species; *Aedes albopictus*, *Aedes brevitibia*, *Culex coerulescens*, *Culex hewitti*, *Culex navalis*, *Tripteroides nepenthis*, *Tripteroides tenax*, *Tripteroides* sp.2, *Tripteroides* sp.3, *Uranotaenia moultoni*, *Uranotaenia* sp.1, *Toxorhynchites indicus* in *Nepenthes ampullaria* in Malacca zoo Peninsular Malaysia. However, difference data mosquito species collection from *Nepenthes* species pitcher plants may be cause from some problems such as; different *Nepenthes* species pitcher plants, different size sample collections, different *Nepenthes* population regions, different time collection, and including asking various research questions. Interestingly, all studies mosquito species collection from *Nepenthes* pitcher plants seemed to indicate lower both mosquito species richness and density including mosquito species vector diseases transmission when compare with other larval mosquito habitats collections. Larval mosquito species richness and density collection were likely higher in habitats such as; Yee et al. (2010) found 13 mosquito species from discarded vehicle tires from four countries in central Illinois, Bond et al. (2014) discovered 14 mosquito species from along the pacific coast of Mexico, Selvan et al. (2016) detected 30 mosquito species from trees holes in India, Muturi et al. (2006) showed 25 mosquito species from rice land agroecosystem in Mwea, Kenya.

The lower mosquito species survival in *Nepenthes* pitcher plants could be interpreted in several cases by following environmental conditions and characteristics of *Nepenthes* pitcher plants. Firstly, color of pitcher plant; *Nepenthes thorelii*, *Nepenthes mirabilils* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* was not highly attractive gravid female mosquito to deposit eggs. The results of color measurement by colorimeter were showed that *Nepenthes mirabilis* var. *mirabilis* and *Nepenthes mirabilis* var. *globosa* were slightly green or green color, whereas *Nepenthes thorelii* was slightly green mixing red color (Figure 8, Figure 9). This pitcher's color; green, green mixing red color, were not influence to attractive gravid female mosquitoes to lay their eggs. Chou et al. (2016) indicated that green color of pitchers *Nepenthes ampullaria* was not attractive to gravid female mosquito species particularly *Aedes albopictus*. Gravid female mosquitoes had recognized to prefer egg laying in container's black or dark color. Second, pitcher plants *Nepenthes* species had fluids higher acidity levels. Pitcher plants *Nepenthes* species had to maintain their fluids acidity for digesting preys and material organics in order to absorb nutrients for sustaining their growth and development. Pitcher plants *Nepenthes* species were generally realized highly acidity levels such as; *Nepenthes gracilis* Korth pH= 2-3, *Nepenthes mirabilis* (Lour.) pH = 3-6, and *Nepenthes ampullaria* pH = 5-7 that were typically slightly acidic (Adlassnig et al., 2010, Chou et al., 2015). Even though, the result of the present study was indicated highly acidity of pitcher fluids; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis* (Table 2, Table 3, Table 4). Accordingly, Selvan et al. (2015) suggested that female mosquitoes preferred to deposit their eggs in habitats with pH range from 7.1 to 7.3 and pH range 6.94 -7.41 were suitable for survival of mosquito larvae *Culex quinquefasciatus* including larval mosquito *Aedes* species preferred pH=7.4. As a consequence, the present study cannot detect any larval mosquito *Aedes* species in pitcher plants, *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*. Larval mosquitoes existing in aquatic habitats had to maintain ionic compositions in their haemolymph in order to react correctly with a variety of environmental conditions. However, the low pH levels in water mosquito larvae habitats impacted on osmoregulation and oxygen transportation of larval mosquitoes, particularly, larval mosquitoes were affected negatively excessive sodium ions in larval mosquito's body (Chou et al., 2015, Clark et al., 2007, Boudko et al., 2001). Third, pitcher plants *Nepenthes* species provide habitats for predators, which exist inside freshwater of pitchers and some predators survive around pitchers, were conspicuous cause to decrease both larval mosquito species richness and abundance in pitchers. Inside freshwater of pitchers *Nepenthes* species was generally found genus larval mosquitoes *Toxorhynchites* that all members in this genus had potential consumed other mosquito larvae including larval mosquito *Aedes* species coexistence in the same pitchers (Mogi and Chan, 1996, Focks, 2007, Albeny et al., 2011, Nyamah et al., 2011, Chou et al., 2015). The results of present study were consistent with previous observation, larval mosquito species in genus *Toxorhynchites* found in *Nepenthes* pitcher plants, that larval mosquitoes *Toxorhynchites albipes* species were detected in *Nepenthes mirabilis* var. *globosa* and *Nepenthes mirabilis* var. *mirabilis*, exception *Nepenthes thorelii*. On the other hand, some spider species in family Thomasina had been recognized coexistence outside freshwater *Nepenthes* pitchers and they consumed all mosquito larvae species in pitcher fluids *Nepenthes* species (Clarke and Kitching, 1993, Chou et al., 2015). Moreover, our results indicated that during samples collections were found red crab spiders *Misumenops nepenthicola* existed and constructed their nest on surface wall inside *Nepenthes* pitcher plants. Furthermore, the experiment of Chua and Lim (2012) supported that red crab spiders *Misumenops nepenthicola* were predatory spiders to feed *Tripteroides* spp. in pitchers *Nepenthes* and larval mosquito densities were decreased dramatically in *Nepenthes* pitcher plants. Finally, the present digestive enzymes and microbes in pitcher fluids of *Nepenthes* species had been known as barrier obstacle for larval mosquito species survival in *Nepenthes* pitcher fluids. Numerous enzymes were isolated from *Nepenthes* pitcher fluids species such as; ribonuclease, phosphatases, acid proteinases, and esterase that these enzymes caused higher acidity levels in *Nepenthes* pitcher fluids (Hatano and Hamada, 2012). The microbes survival in *Nepenthes* pitcher fluids produces some enzymes and microbes metabolize activities had controlled the organisms that existed in *Nepenthes* pitcher plants (Takeuchi et al., 2015). For instance, Chou et al. (2015) provided evidence the higher activity microbes in pitcher fluids *Nepenthes gracilis* and *Nepenthes mirabilis* affected on larval mosquito *Aedes* species to be higher mortality. Furthermore, Sota and Mogi (2006) indicated larval mosquito *Aedes* species used to adapt in aquatic of *Nepenthes mirabilis* species pitcher plants and particularly, in pitcher fluids low acidity levels of *Nepenthes* species pitcher plants. The discovery of larval mosquito *Aedes* species in some *Nepenthes* species pitcher plants was feasible that *Nepenthes* pitcher plants provided breeding habitats for mosquito *Aedes* specie in the future while habitats artificial of mosquito *Aedes* had been actively eliminated. Especially, urban expansion to rural areas and degradation of the forest habitats *Nepenthes* pitcher plants seemed to increase opportunity encounters between *Nepenthes* and gravid female *Aedes* mosquitoes (Chou et al., 2016).

Environmental conditions and characteristics of *Nepenthes* pitcher plants predict and impact on abundance of mosquito larvae species

The association between environmental conditions and characteristics (length of pitchers, width opening of pitchers, pH, dry detritus in pitchers, active zone in pitchers, actual fluid in pitchers, electrical conductivity, protozoa abundant, and rotifer abundant) of *Nepenthes* species pitcher plants were detected using principal component analysis, multiple linear regression, and spearman’s correlation coefficient (Table 5) (Table 7). The results indicated all variables recording from environmental factors and characteristics of *Nepenthes* pitcher plants appeared to have affecting 21% on abundance of larval mosquito species. The results of principle component analysis for variables pitcher measurement indicated two category, positive and negative, that correlated on abundance of larval mosquito species. Length of pitchers, width opening of pitchers, dry detritus, active zone in pitchers, active fluid in pitchers, and electrical conductivity were positively associated with larval mosquito abundant. Whereas pH, abundance of protozoa and rotifera were indicated negatively with abundance of mosquito larvae.

The amount detritus in pitchers were essentially to sever as food resources to sustain microorganisms such as, bacteria, protozoa, rotifera, and including larval mosquito density (Duguma et al., 2017). Hoekman (2007) found that the density of larval mosquitoes in pitchers based on amount detritus that were digested by microorganisms in order to obtain nutrients for supporting their population. Alternatively, an experiment was showed that abundance of microorganisms such as protozoa and rotifer in pitchers was declined dramatically when larval mosquitoes present in pitchers (Hoekman, 2011, Trzcinski et al., 2005). Additionally, the different detritus condition in mosquito’s habitats affected on larval mosquito density different period detritus condition in pitchers. The early detritus condition in mosquito’s habitats induced microbial activities and consequence larval mosquito grew faster and larger body size. Conversely, the low degree microbial activities were a main part to lead density of larval mosquito decrease during amount detritus existence many days in habitats of mosquito larvae (Norman and Walker, 2018, Pelz-stelinski et al., 2010, Yee et al., 2007). Thus, amount detritus in pitchers were essential food resource and correlated positively with abundance of larval mosquitoes. Whereas, microorganisms correlate negatively with abundance of larval mosquito species with consistent results in this study.

The characteristics of *Nepenthes* species pitcher plants were known to impact on gravid female mosquitoes in selecting their oviposition sites. Gravid female mosquitoes preferred to deposit their eggs in larger size pitchers that ensured larval mosquitoes to have lowest survival mortality (Heard, 1994). Our results indicated that pitcher characteristics measurement in term of pitcher size such as; length of pitchers, width opening pitchers, actual fluids in pitchers, and active zone in pitchers, were not highly attractive gravid female mosquito species when compare to other habitats including ponds, streams, discarded vehicle tires and tree holes etc. Consistently, an observation behavior mosquitoes in pitcher plants *Nepenthes ampullaria* was suggested that the smaller pitcher size was not highly attractive gravid female mosquitoes to deposit their eggs (Chou et al., 2016). Furthermore, pitcher plants species *Sarracenia purpurea*, which differ from *Nepenthes* genus pitcher plants, were found higher larval mosquito abundant in large pitcher size consisting plenty water in pitchers (Bhattarai and Horner, 2009, Miner and Taylor, 2002, Heard, 1998, Nastase et al., 1995). Moreover, the higher abundance of immature mosquito were noted in other large containers size owing to gravid female mosquitoes preferred generally to lay their eggs in larger containers including discarded vehicle tires and even though tree holes in forests (Mangudo et al., 2017, Yee et al., 2010). Interestingly, the present study showed negatively correlation between pitcher fluids pH levels and abundance of larval mosquitoes (Table 1). In this case, it might be shown that immature mosquitoes were trying tolerance in pitcher fluid with higher acidity levels. The ability of immature mosquito struggling in extreme acidity levels permitted larval mosquito to exploit various habitats in nature (Clark et al., 2004, 2007). For instance, immature mosquito *Aedes* species, particularly *Aedes albopictus* species, had struggled survival in extremely pH=3 environment and some *Nepenthes* species pitcher fluids with pH=4 (Chou et al., 2015, Clark, et al., 2007). However, the strongly acidity levels in mosquito's habitats affects immature mosquito to disable for maintaining ions in their body. Unfortunately, immature mosquitoes were death during strong acidity or when pitcher fluid pH decreased abruptly in certain condition.

However, there had been still found mosquito larvae survival in *Nepenthes* pitchers that an evidence, only pitcher low fluids and smaller pitcher size, high acidity levels and microbial activity, cannot deter gravid mosquitoes to oviposit eggs in pitchers. Giusto et al. (2008) and Chin et al. (2014) suggested that *Nepenthes* pitchers had ability to emit sweet scent for insect attraction particularly flying insects that found mostly in upper pitchers. Furthermore, odor decaying insects in *Sarracenia alata* pitcher plants were found to effect on attractive insect capture (Bhattarai and Horner, 2009). Additionally, the evolutionary ability of immature mosquito adapted in extremely pitcher's environmental conditions. For instance, some immature mosquito species, *Uranotaenia moultoni* and *Culex* spp., performed virtually their behavior to escape from predators larval mosquito *Toxorhynchites acaudatus* in pitchers pitcher fluids. Immature mosquito *Uranotaenia moultoni* and *Culex* spp. moved slowly for long period of time and relaxing frequency against pitcher's wall in order to move for breathing the surface pitcher fluids. In contrast, immature mosquito *Aedes albopictus* moved actively with their whole body without resting and extended period movement that produced vibration in pitcher fluid column and predator larval mosquito *Toxorhynchites acaudatus* species detected easily immature *Aedes albopictus* (Chou et al., 2015, Steffan and Evenhuis, 1981). Similarly, pitchers *Sarracenia purpurea* were always colonized by mosquito larvae *Wyeomyia smithii* and larval midge *Metriocnemus knabi* which they were coexistence without preying upon one another (Hoekman, 2007, Trzcinski et al., 2005, Nastase et al, 1995). Petersen et al. (2000) indicated that larval *Aedes albopictus* were consumed by *Metriocnemus knabi* larvae when *Aedes albopictus* added in pitcher *Sarracenia purpurea* that consisted of both larvae *Wyeomyia smithii* and *Metrocnemus knabi*. Larval mosquito *Wyeomyia smithii* escaped from predatory *Metriocnemus knabi* owing to this mosquito larvae had long setae that prevented predators to detect the larvae *Wyeomyia smithii* body. Conversely, *Aedes albopictus* were without long setae and predators detected easily *Aedes albopictus* larvae (Petersen et al., 2000).

Effect of seasons on abundance larval mosquito in pitchers *Nepenthes* species pitcher plants

This study were shown that seasons affected on abundance of immature mosquito species (one-way ANOVA, $F = 16.08$, $P < 0.05$). Immature mosquito species were illustrated higher abundance in pitchers *Nepenthes* species pitcher plants during rainy season period more than dry season. The water existence in pitchers played important role to serve for eggs oviposition sites of gravid female mosquitoes and water in pitchers supported development for immature mosquitoes until adult mosquitoes stage (Bauer et al., 2009). The fluid in pitchers were either created by pitcher plant itself or rain water flowed into pitchers and water in pitcher traps contained mostly 30 mL of rainwater (Adassnig et al., 2011, Kingsolver, 1979). Pitcher fluids were influenced by pitcher size, which the smaller pitcher size was lose water quickly in short time than larger pitcher size, and microhabitat where pitcher plants had been occupied (Nastase et al., 1995, Kingsolver, 1981). Pitcher fluids was ran drying or evaporation after 5-30 days without precipitation and desiccation risk of pitcher fluids was always occurred quickly when pitcher plants were growing in full sun places particularly during dry season more than under shade trees (Adassnig et al., 2011, Bergland et al., 2005). However, in this study, pitcher plant populations were virtually found under shape trees even though pitcher fluids evaporated easily during dry season, but a little water had remained for sustaining some pitcher plants growth and a few larval mosquitoes existence. The desiccation of pitchers were likely to receive less eggs of mosquitoes and larval mosquitoes could not survival for completing their lifecycle. Thus, immature mosquitoes were found less abundance in pitchers during dry season than rainy season.

Effect of upper pitchers and lower pitchers on abundance of larval mosquito of *Nepenthes* species pitcher plants

The results of this study indicated abundance of larval mosquitoes was not different between dimorphic upper pitchers and lower pitchers (one-way ANOVA, $F = 2.54$, $P = 0.11$). It could be interpreted that both upper pitchers and lower pitchers had same potential to provide oviposition sites for gravid female mosquitoes and immature mosquito survival. All pitchers in upper pitchers position were generally funnel shaped and these pitchers climbed stems at least 1 meters above the ground (Gaume et al., 2016). Moran (1991) pointed out that the height above ground of upper pitchers *Nepenthes* species exhibited pitchers as visual cue for wing preys and upper pitchers utilized neither fragrances nor UV pattern that accounted for catching efficiency flying preys. Moreover, the attraction system by using olfactory cues of *Nepenthes* species particularly *Nepenthes rafflesiana* pitcher plants was suggested to have strongly effective for attraction of flying insects (Moran, 1996). Indeed, Chou et al. (2016) and Giusto et al. (2008) supported olfactory cue of *Nepenthes* pitchers were essential for attractive flying insects and especially, the sweet fragrance was secreted from peristome region of upper pitchers which was odour source attractive for flying insect target. Furthermore, several meter height above ground of upper pitchers position was likely indicated less or without present of predatory spider existence. Therefore, upper pitchers were potential oviposition sites and suitable habitats for immature mosquito development by gravid female mosquito decision. On the other hand, lower pitchers were generally found on the ground and virtually lower pitchers were larger pitcher size with water plentiful in pitchers more than upper pitchers. Addition, almost where lower pitchers resident were shade trees particularly under shade pitcher plants itself and inversely upper pitchers were directly exposed with sunlight. Furthermore, pitcher plants *Nepenthes* might be able to utilize light as light traps to either capture or attract flying insect particularly gravid female mosquitoes while lower pitchers were mostly resident shade. Pitcher plants *Nepenthes* species exhibited unusual pitcher morphology; tubular or ovoid in shape, pitcher mouth at the top was open and surround by the peristome, and lid situate above pitcher mouth opening. Especially, *Nepenthes aristolochioides* showed expanding their morphology into a pronounced dome, which was translucent, with the mouth sitting at the front. Moran et al. (2012) supported that *Nepenthes aristolochioides* pitcher plants created light by light transmitting via the translucent dome for lower pitchers attractive flying insects. Consequently, lower pitchers *Nepenthes* species may process light creation of attractive gravid female mosquitoes. Thereby, lower pitchers would be secure oviposition sites and immature mosquito growth successful as well as upper pitchers.

Although, pitcher plants *Nepenthes* species showed dimorphic pitcher types such as lower pitchers and upper pitchers that upper pitchers prey capturing were mostly found wing preys and wingless preys were discovered in lower pitchers (Chin et al, 2014, Rembold et al., 2010, Giusto et al, 2008, Adam, 1997, Moran, 1996). According to this results, the higher larval mosquito abundance would be found in upper pitchers more than lower pitchers. However, the higher number of prey flying insects in upper pitches was discovered because flying insects exploited foods in pitchers as well as higher abundance of wingless insect preys in lower pitchers. However, gravid female mosquitoes utilized both visual cue and olfactory cue to seek suitable and secure habitats for their immature full lifecycle development (Farajollahi et al., 2009). Thereby, both lower pitchers and upper pitchers had equal opportunity to serve oviposition sites and habitants for gravid female mosquito and immature mosquito species development.

Chapter 5
Conclusion

Pitcher plants *Nepenthes* species provide habitats for a variety of arthropods species and among these arthropod species, mosquito larvae are also found. There are 1,155 individuals, 3 genera, and 5 mosquito species have been discovered in pitcher plants; *Nepenthes thorelii*, *Nepenthes mirabilis* var. *globosa*, and *Nepenthes mirabilis* var. *mirabilis*. Mosquito species are found such as; *Tripteroides tenax*, *Tripteroides* species.1, *Toxorhynchites albipes*, *Uranotaenia demeillori*, and *Uranotaenia bicolor* in pitcher plants *Nepenthes* species. The most dominant in number of mosquito individual is *Tripteroides tenax* species.

Effect of pitcher's environmental conditions on immature mosquitoes is calculated by multiple linear regression obtained from three components of PCA and multiple linear regression indicate that pitcher's environmental conditions impact 21% on abundance of immature mosquitoes. Furthermore, pitcher's aquatic environment is found predators such spiders *Misumenops nepenthicola* and larval mosquito *Toxorhynchites albipes* that these predators consume larval mosquitoes. Moreover, principal component analysis (PCA) illustrates that pitcher variables have significant influences both positive and negative on abundance immature mosquitoes. Pitcher size, dry detritus, active zone in pitchers, actual fluids in pitchers, and electrical conductivity are positive correlated with abundance of immature mosquitoes. Whereas, pH, protozoa abundant and rotifer abundant are found negatively correlation with abundance of immature mosquitoes. Spearman rank correlation indicate that pitcher's variables seem to have less influence on each larval mosquito species. Actual fluid in pitchers is positive correlated slightly with larval mosquito *Tripteroides tenax* ($r = 0.33$, $P = 0.003$) and electrical conductivity correlate slightly positive with *Tripteroides* species.1 ($r = 0.12$, $P = 0.03$) but electrical conductivity correlate negative with *Uranotaenia demeillori* ($r = -0.12$, $P = 0.02$).

Different seasons affect exactly on differentiation of immature mosquito abundant. Abundance of immature mosquito species are highest during rainy season more than dry season (one-way ANOVA, $P < 0.05$). Water rain in pitchers plays an important role for oviposition sites of gravid female mosquitoes, facilitation foods immature mosquitoes, and immature mosquito full life cycle development.

Pitcher plants *Nepenthes* species exhibit dimorphic pitcher types, upper pitchers and lower pitchers, that these pitcher types are not significant difference consisting of immature mosquito abundant (one-way ANOVA, $P > 0.05$). The reasons may be that (1) upper pitchers are higher several meter from ground and upper pitchers produces fragrance that are easily detected by gravid female mosquito using their visual cue and olfactory cue, (2) upper pitchers may less or without predators, (3) lower pitchers have virtually larger size and resident in shade, (4) lower pitchers may process light creation as light traps by pitcher's morphology for attractive gravid female mosquitoes.

Consequently, it could indicate that the pitcher plants *Nepenthes* species habitants have low species richness of larval mosquitoes resident and *Nepenthes* species may become the secure and suitable habitats in the future for larval mosquitoes colonization, particularly, *Aedes* species existence. These results of larval mosquitoes in *Nepenthes* pitcher plants are crucial knowledges and understanding how these factors effect on larval mosquitoes might be important for designing and planning strategies for vector mosquito management and apply in other larval mosquito habitats.

References

Adlassnig, W.,Peroutka, M.and Lendl,T.2011. Trap of carnivorous pitchers plants as a habitat: composition of the fluid, biodiversity and mutualistic activities. *Annals of Botany* 107: 181-194.

Adebote, D. A., Abolude, D. S., Oniye, S. J. and Wayas, O. S. 2008. Studies on some physicochemical factors affecting the breeding and abundance of mosquitoes (diptera: Culicidae) in phytotelmata on *Delonix regia* (Leuminosae: Caesalpinoidea). *Jouranl of Biological Sciences* 8 (8): 1304-1309.

Albeny, D.S.,Martins, G.F.Andrade,M.R.,Kruger,R.Fand Vilela,F.2011.*Aedes aegypti* survival in the presence of *Toxorhynchites violaceus* (Diptera: Culicidae) fourth instar larvae. *Zoologia* 28 (4):530-540.

Allan, S.A. and Kline, D .L .1998. Larval rearing water and preexisting egg influence oviposition by *Aedes aegypit* and *Aedes Albopictus* (Diptera: Culicidae). *Journal Medical Entomology* 35 (6):943-947.

Ajamma, Y.U., Villinger,J., Omondi,D., Salifu, D.,Onchuru, T.o.,Njoroge,L.,Muigai, A.W.and Masiga, D.K.2016. Composition and genetic diversity of mosquitoes (Diptera: Culicidae) on islands and mainland shores of Kenya's lakes Victoria and Baringo. *Journal of Medical Entomology* 53 (6):1348-1363.

Anuniwat, A., Chaveerach, A., Tannee, T. and Sudmoon, R. 2009. Development of scar marker for species identification of the genus *Nepenthes* (Nepenthaceae). *Pakistan Journal of Biological Science* 12 (22):1455-1461.

Apiwathnasorn, C., Samug , Y., Prummongkol, S., Panasoponkul, C. and Loymek, S.2009. Mosquito fauna of “fauna of Daeng” swamp forest, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 40 (4):720-726.

Asha, A. V. and Aneesh , E . M . 2014 . Diversity of mosquito species (Diptera: Culicidae) at Irinjalakuda, Thrissur with special reference to their breeding habitats. *International Journal of Current Microbiology and Applied Sciences* 3 (2):536-541.

Athauda, S.B.P.,Matsumoto.K.,Rajapakshe,S.,Kuribayashi,M.,Kojima,M.,Kubomura-Yoshida,N.,Iwamastsu, A., Shibata,C.,Inoue,H.,Takahashi,K.2004. Enzymic and structural characterization of nepenthesin, a unique member of a novel subfamily of aspartic proteinases. *Biochemical Journal* 381 (1):295-306.

Bauer, U., Clemente , C . J., Renner , T . and Federle , W. 2012 . Form follows function: morphological diversification and alternative trapping strategies in carnivorous *Nepenthes* pitcher plants. *Journal of Evolutionary Biology* 25: 90-102.

Bashar, K.,Rahman,M.S.,Nodi,I.J., and Howlader, A.J.2016.Species composition and habitat characterization of mosquito (Diptera: Culicidae) larvae in semi-urban areas of Dhaka, Bangladesh. *Pathogens and global health* 110 (2):48-61.

Bauer, U. Willems., C, Federle , W.2009. Effect of pitcher age on trapping efficiency and natural prey capture in carnivorous *Nepenthes rafflesiana* plants. *Annals of Botany* 103:1219-1226.

Beaver, R.A.1985. Geographical variation in food web structure in *Nepenthes* pitcher plants. *Ecological Entomology* 10: 241-248.

Bentley, M . D . 1989 . Chemimcal ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology* 34: 401-421.

Bergland, A. O., Agotsch, M.,Mathias,D., Bradshaw, W.E. and Holzapfel, C.M.2005. Factors influencing the seasonal life history of the pitcher-plant mosquito, *Wyeomyia smithii*. *Ecological Entomology* 30:129-137.

Bert, W., De Ley, I. T., Van Driessche, R., Segers, H. and De Ley, P.2003. *Baujardia mirabilis* gen.n.,sp.n. from pitcher plants and its phylogenetic position within panagrolaimidae (Nematoda: Rhabditida). *Nematology* 5 (3):405-420.

Bhattarai, G.P. and Horner, J.D. 2009. The importance of pitcher size in prey capture in the carnivorous plant, *Sarracenia alata* Wood (Sarraceniaceae). *The American Midland Naturalist* 161 (2):264-272.

Bond, J. G ., Casas - Martinez , M ., Quiroz – Martinez , H ., Novelo - Gutierrez , R., Marina,C.F.,Ulloa,A., Orozoco-Bonilla,A.,Munoz,M.and Williams, T.2014. Diversity of mosquitoes and the aquatic insects associated with their oviposition sites along the Pacific coast of Mexico. *Parasites & Vectors*,7(1),41.

Bonizzoni, M., Gasperi, G., Chen, X. and James , A. A. 2013 . The invasive mosquito species *Aedes albopictus*: current knowledge and future perspective. *Trends in parasitology* 29 (9):460-468.

Boudko, D. Y ., Moroz , L. L ., Linser , P. J ., Trimarchi , J . R ., Smith, P. J . S . and Harvey,W.R.2001. In situ analysis of pH gradients in mosquito larvae using non-invasive, self-referencing, pH-sensitive microelectrodes. *The Journal of Experimental Biology* 204:691-699.

Buch, F., Pauchet , Y., Rott , M ., and Mithofer, A. 2014. Characterization and heterologous expression of a PR-1 protein from traps of the carnivorous plant *Nepenthes mirabilis*. *Phytochemistry* 100:43-50.

Bunawan, H., Yen, C.C., Yaakop, S. and Noor, N. M.2017. Phylogenetic inference of *Nepenthes* species in penisular Malasia revealed by chloroplast (trn L intron) and nuclear (ITS) DNA sequences. *BMC research notes*, 10 (1),67.

Burnham, K.P.,Overton,W.S.1979.Robust estimation of population size when capture probabilities vary among animals. *Ecology* 60:927-936.

Burton, G. J. 1953. Some techniques for mounting mosquito eggs, larvae, pupae, and adults on slides. *Mosquito News* 13 (1):8-15.

Catalano, M. 2010. *Nepenthes mirabilis* var. *globosa* M . Catal.Var . nov : *Nepenthes della Thailand*: *Diario diriaggio*.Prague.P.40.

Charrel, R, N., De Lamballerie , X., and Raoult , D.2007. Chikungunya outbreaks-the globalization of vector borne diseases. *New England Journal of Medicine*,356 (8),769.

Cheek, M. and Jebb , M . 2009. *Nepenthes* group *Montanae* (Nepenthaceae) in Indo-China, with *Nepenthes thai* and *Nepenthes bokor* describe as new. *Kew Bulletin* 64:319-315.

Chia, T. F., Aung, H. H., Osipov, A.N., Goh,N . K. and Chia, L.S. 2004. Carnivorous pitcher plant uses free radicals in the digestion of prey. *Redox Report* 9:255-261.

Chin, L., Chung , A . Y. and Clarke , C. 2014 . Interspecific variation in prey capture behavior by co-occurring *Nepenthes* pitcher plants: evidence for resource portioning or sampling-scheme artifacts?. *plant signaling& Behavior*,9(1),e27930.

Chitra, S.,Ravindram, K. Rajkuberan, C., Janagaraj,K. and Sivaramakrishnan,S.2014. A survey report on baseline data of mosquito distribution in tree holes of discrete ecosystem during different seasonal patterns. *America Journal of Entomology* 7 (2):70-75.

Chou, L.Y., Dykes, G. A., Wilson, R. F. and Clark, C.M.2016. *Nepenthes ampullaria* (Nepenthaceae) pitcher are unattractive to gravid *Ades aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Environmental Entomology* 45 (1): 201-206.

Chou, L. Y., Wilson , R .F., Dykes , G. A ., and Clarke , C. M . 2015. Why are *Aedes* mosquitoes rare colonisers of *Nepenthes* pitcher plants?. *Ecological entomology* 40 (5):603-611.

Clarke, C. M . and Kitching , R. L .1993. The metazoan food webs from six Bornean *Nepenthes* species. *Ecological Entomology* 18:7-16.

Clark, T. M., Flis, B.J. and Remold, S.K. 2004. pH tolerances and regulatory abilities of freshwater and euryhaline *Aedes* mosquito larvae. *Journal of Experimental Biology*: 2297-2304.

Clark, T. M., Marcus, A.L., Vieira, K.L., Huegel, D.F. and Carper,M.2007. Strategies for regulation of hemolymph pH in acidic and alkaline water by the larval mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of Experimental Biology*: 4359-4367.

Clarke, C. and Moran , J.A . 2016. Climate, soils and vicariance-their roles in shaping the diversity and distribution of *Nepenthes* in Southeast Asia. *Plant and soil* 403: 37-51.

Clemons, A., Haugen , M ., Flannery, E ., Kast , K., Jacowski , C., Severson , D . and Duman- Scheel, M.2010. Fixation and preparation of developing tissues from *Aedes aegypti*. *Cold Spring Harbor Protocols* (10), pdb-prot5508.

Cresswell, J.E.1998.Morphological correlates of necromass accumulation in the traps of an eastern tropical pitcher plant, *Nepenthes ampullaria* Jack, and observations on the pitcher infauna and its reconstitution following experimental removal. *Oecologia* 113: 383-390.

Crump, M. 1991 . Choice of oviposition site and egg load assessment by a treefrog. *Herpetologica* 47:308-315.

Edgerly, J.S., Mcfarland, M., Moran, P. and Livdahl, T.1998. A seasonal shift in egg-laying behavior in repose to cues of future competition in a treehole mosquito. *Journal of Animal Ecology* 67: 805-818.

Farajollahi, A., Kesavaraju, B, Price,D.C.,Williams, G.M., Healy,S.P., Gaugler,R.and Nelder, M.P.2009. Field efficacy of BG-sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. *Journal of medical entomology* 46 (4):919-925.

Focks, D. A . 2007 . *Toxorhynchites* as biocontrol agents . *Journal of the American Mosquito Control Association* 23 (2):118-127.

Ganesan, K., Mendki , M . J., Suryanaryana , M . V.S ., Prekash, S. and Malhotra , R. C.2006. Studies of *Aedes aegypti* (Diptera: Culicidae) ovipositional response to newly identified semiochemicals from conspecific eggs. *Australian Journal of Entomology* 45: 75-80.

Gaume, L., Bazile, V., Huguin, M. and Bonhomme ,V. 2016. Different pitcher shapes and trapping syndromes explain resource partitioning in *Nepenthes* species. *Ecology and Evolution* 6 (5): 1378-1392.

Gunathilaka, N ., Fernando , T ., Hapugoda , M ., Abeyewickreme , W . and Wickremasinghe, R.2014. Revised morphological identification key to the larval anopheline (Diptera: Culicidae) of Sri Lanka. *Asian Pacific Journal of Tropical Biomedicine* 4: S222-S227.

Hamilton IV, R., and Duffield, R.M.2002.Novel observations of midge and mosquito larval population dynamics in leaves of the northern pitcher plants, *Sarracenia purpurea* L. *Hydrobiologia* 482 (1): 191-196.

Hatano, N. Hamada , T. 2012. Proteomic analysis of secreted protein induced by a component of prey in pitcher fluid of the carnivorous plant *Nepenthes alata*. *Journal of Proteomics* 75:4844-4852.

Harbach, R . E . and Kitching , I . J . 2005. Reconsideration of anopheline mosquito phylogeny (Diptera: Culicidae: Anophelinae) based on morphological data. *Systematics and Biodiversity* 3 (4):345-374.

Harbach, R.E.2007. The Culicidae (Diptera):A review of taxonomy,classification and phylogeny. *Zootaxa* 1668 (1):591-538.

Harrison, B. A.1980 . Medical entomology studies . XIII. The Myzomyzia series of *Anopheles* (*Cellia*) in Thailand, with emphasis on intra-interspecific variations (Diptera: Culicidae). *Contribution America Entomology Institute* 17:1-195.

Heard, S. B. 1998. Capture rates of invertebrate prey by the pitcher plant , *Sarracenia purpurea* L. *The America Midland Naturalist* 139:79-89.

Heard, S . B. 1994a . Imperfect oviposition decisions by the pitcher plants mosquito (*Wyeomyia smithii*). *Evolutionary Ecology* 8:493-502.

Hoekman, D. 2011. Relative important of top-down and bottom-up forces in food webs of Sarracenia pitcher communities at a northern and southern sit. *Oecologia* 165: 1073-1082.

Hoekman, D. 2007. Top-down and bottom-up regulation in a detritus-based aquatic food web: a repeated field experiment using the pitcher plant (*Sarracenia purpurea*) inquiline community.*The American midland naturalist*157 (1):52-62.

Hoekman, D., Terhorst , C., Bauer, A., Braum , S., Gignac , P., Hopkins, R. Joshi, S., Laskis, K., Sanscrainte,N., Travis,J. and Miller,T.E.2007.Oviposition decreased in respons to enriched water: a field study of the pitcher-plant mosquito *Wyeomyia smithii*. *Ecological Entomology* 32:92-96.

Hua, Y. and Li , H . 2005 . Food web and fluid in pitchers of *Nepenthes mirabilis* in Zhuhai, China. *Acta Botanica Gallica* 152 (2):165-175.

Huang, J., Walker, E.D ., Giroux , P. Y.,Vulule,J. and Miller. J.R.2005. Ovipositional site selection by *Anopheles gambiae*: influence of substrate moisture and texture. *Medical and Veterinary Entomology* 19: 442-450.

Ishisaki, K., Arai, S., Hamada, T., Honda, Y.2012. Biochemical characterization of a recombinant plant class III chitinase from the pitcher of the carnivorous plant *Nepenthes alata*. *Carbohydrate research* 361:170-174.

Jebb, M . and Cheek , M . 1997. A skeletal revision of *Nepenthes* (Nepenthaceae). *Blumea-Biodiversity, Evolution and Biogeography of Plants* 42 (1):1-106.

Kanokratana, P., Mhuanthon , W., Laothanachareon , T ., Tangphatsornruang , S ., Eurwilaichitr, L., Kreuetreepradit ,T., Mayes,S. and Champerda . 2016. Comparative study of bacterial communities in *Nepenthes*. *Microbial ecology* 72 (2) :381-393.

Kengluecha, A., Singhasivanon , P., Tiensuwan , M ., Jones, J. W . and Sithiprasasna, R. 2005. Water quality and breeding habitats of *Anopheline* mosquito in northwestern Thailand. *Southeast Asian Journal Tropical Medical Public Health* 36: 46-53.

Kingsolver, J. G.1979. Thermal and hydric aspects of environmental heterogeneity in the pitcher plant mosquito. *Ecological Monographs* 49 (4):357-376.

Kingsolver, J.1981. The effect of environmental uncertainty on morphological design and fluid balance in *Sarracenia purpurea* L. *Oecologia* 48:364-370.

Kitching, R.L.2001. Food web in phytotelmata: bottom-up and top-down explanation for community structure. *Annual Review Entomology* 46:729-760.

Kneitel, J. M . and Miller , T . E . 2002 . Resource and top - predator regulation in the pitcher plant (*Sarracenia purpurea*) inquiline community. *Ecology* 83 (3):680-688.

Kubo, I ., Uchida , M . and Klocke , J. A . 1983 . An insect ecdysis inhibitor from the African medicinal plant, *Plumbago capensis* (Plumbaginaceae); a naturally occurring chitin synthetase inhibitor. *Agricultural and Biological Chemistry* 47: 911-913.

Lam, W.N., Chong, K.Y., Anand , G.S. and Tan , H . T. W. 2017.Dipteran larvae and microbes facilitate nutrient sequestration in the *Nepenthes gracilis* pitcher plants host. *Biology Letter* 13 (3):2016928.

Lee, L., Zhang , Y., Ozar , B ., Sensen , C .W. and Schriemer, D.C.2016. Carnivorous nutrition in pitcher plants (*Nepenthes spp*.) via an unusual complement of endogenous enzymes. *Journal of proteome research* 15 (9):3108-3117.

Lemine, A.M.M., Lemrabott, M.A.O., Ebou, M.H.,Lekweiry, K.M.,Salem, M.S.O.A., Brahim, K. O., Moukah, M. O., Bouraya, I. N. O., Berngues,C.,Trape,J.,Basco,L.,Bogreau, H., Simard, F.,Faye,O.and Boukhary,A.O.M.2017. Mosquitoes (Diptera: Culicidae) in Mauritania: a review of their biodiversity, distribution and medical importance. *Parasites & vectors* 10 (1), 35.

Mangudo, C., Aparicio, J.P.,Rossi, G. C.and Gleiser, R.M.2018. Three hole mosquito species composition and relative abundance differ between urban and adjacent forest habitats in northwestern Argentina. *Bulletin of Entomological Research* 108 (2):203-212.

Mcintyre, N . E . 2000 . Ecology of urban arthropods : a review and a call to action. *Annals of the Entomological Society of America* 93 (4):825-835.

Mey, F. S . 2010 . The elusive *Nepenthes thorelii*. *Carnivorous Plants their Habitats*: 1-340.

Milne, M.A.,Grefe III, N.A., and Waller, D.A.2008.Colonization and development of the Asian tiger mosquito (*Aedes albopictus*) in the purple pitcher plant (*Sarracenia purpurea*). *The American Midland Naturalist* 160 (1):110-116.

Miyagi, I. and Toma, T . 2007. A new mosquito of the genus *Topomyia* (Diptera: Culicidae) from a *Nepenthes* pitchers plant in Baro highland of Sarawak, Malaysia. *Medical Entomology Zool* 58(3):167-174.

Miller, T., Cassill , D ., Johnson , C ., Kigdell . C ., Leips , J ., Mcinnes , D., Bevis,T., Mehlman,D.and Richard,B.1994. Intraspecific and Interspecific composition of *Wyeomyia smithii* (Coq.) (Culicidae) in pitcher plant communities. *American Midland Naturalist* : 136-145.

Milne, M. A. and Schwarz, M . 2014 . Size matters: purple pitcher plant (*Sarracenia purpurea*) pitcher size influences the presence of *Aedes albopictus* larvae. *America Midland Naturalists* 172:219-226.

Miner, J. A. and Taylor , P . D . 2002 . Effects of peatland size and exposure on two species of Diptera inhabiting the pitcher plant *Sarracenia purpurea* L. *Ecoscience* 9 (3): 347-354.

Mogi, M. 2010. Unusual life history traits of *Aedes* (*Stegomyia*) mosquitoes (Diptera: Culicidae) inhabiting *Nepenthes* pitchers. *Annals of the Entomological Society of America* 103 (4):618-624.

Mogi, M. and Chan, K . L. 1997 . Variation communities of Dipterans in *Nepenthes* pitcher in Singapores: predators increase preys community diversity. *Annals Entomology Society of American* 90 (2):177-183.

Mogi, M. and Chan , K . L . 1996 . Predatory habitats of dipteran larvae inhabiting *Nepenthes* pitchers. *The raffles bulleting of zoology* 44 (1):233-245.

Mogi, M. and Yong, H.S.1992. Aquatic arthropod communities in *Nepenthes* pitcher: the role of niche differentiation, aggregation, predation and competition in community organization. *Oecologia* 90:172-184.

Mokany, A. and Shine, R. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larva and anuran tadpoles. *Australia Ecology* 28:33-37.

Moran, J.A.1996. Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. *Journal of Ecology* 84:515-525.

Moran, J. A., Booth, W. E. and Charles,J.K.1999. Aspects of pitcher morphology and spectral characteristics of six Bornean *Nepenthes* pitcher plant species: implications for pry capture. *Annals of Botany* 83:521-528.

Moran, J.A., Clarke, C. and Gowen,B.E. 2012. The use of light in prey capture by the tropical pitcher plant *Nepenthes aristolochioides*. *Plant signaling & behavior* 7(8):957-960.

Munga, S., Minakawa, N., Zhou, G., Githeko, A.K. and Yan,G.2007. Survivorship of immature stages of *Anopheles gambiae* sl (Diptera: Culicidae) in natural habitats in western Kenya highlands. *Journal of medical entomology* 44(5):758-764.

Munirathinam, A.,Krishnamoorthi,R.,Baskaran, G.,Govindarajan,R.,Venkatest,A.and Tyagi,B. K.2014. Mosquito species biodiversity in phytotelmata from western Ghats,south India. *Halters* 5:56-63.

Muturi, E.J.,Shililu,J.,Jacob,B.,Gu,W.,Githure,J.and Novak,R.2006.Mosquito species diversity and abundance in relation to land use in a Riceland agroecosystem in Mwea,Kenya. *Journal of Vector Ecology* 31(1):129-137.

Mwangangi, J.M., Midega, J.,Kahindi, S., Njoroge, L., Nzovu, J., Githure, J., Mbogo, C. M. and Beier, J.C.2012. Mosquito species abundance and diversity in Malindi, Kenya and their potential implication in pathogen transmission. *Parasitology research* 110 (1): 61-71.

Nastase, A. J., De La Rosa, C. and Newell, S. J .1995. Abundance of pitcher - plant mosquitoes, *Wyeomyia smithii* (Coq.)(Diptera: Culicidae) and midges, *Metrocnemus knabi* Coq. (Diptera: Chironomidae),in relation to pitcher characteristics of *Sarracenia purpurea* L.*American Midland Naturalist*: 44-51.

Ngente, L., Nachimuthu, S. K. and Guruswami, G. 2012 . Insecticidal and repellent activity of hiptage benghalensis L. Kruz (Malpighiaceae) against mosquito vectors. *Parasitology research* 111(3):1007-1017.

Norman, B. C. and Walker,E.D.2018.Conditioning of leaf detritus modulates density-dependent growth of *Aedes triseriatus* larvae (Diptera:Culicidae). *Journal of Medical Entomology* 55(2):342-350.

Nsa, D., Nanthasane, V., Razak, S., Audrey, L., Thor, A. S., Theeraphap, C., Hans, J.O. 2013. Relationship between *Aedes aegypti* production and occurrence of *Escherichia coli* in domestic water storage containers in rural and suburban villages in Thailand and Lao. *Acta Tropical* 126:177-185.

Nuanlaong, S., Onsanit, S., Chusangrach , C. and Suraninpong, P. 2016 . A new species of *Nepenthes* (Nepenthaceae) from Thailand. *Thai Forest Bull Bot* 44 (2): 128-133.

Nyamah, M. A., Sulaiman, S.and Omar, B. 2011. Field observation on the efficacy of *Toxorhynchites splendens* (Wiedemann) as a biocontrol agent against *Aedes albopictus* (Skuse) larvae in a cemetery. *Tropical Biomedicine* 28(2):312-319.

Paradise, C. J. and Dunson, W. A. 1997. Effects of pH and sulphate on insects and protozoans inhabiting treeholes. *Archives of Environmental Contamination and Toxicology* 33:182-187.

Peach, D. A.H. and Gries, G.2016. Nectar thieves or invited pollinators? A case study of tansy flowers and common house mosquitoes. *Arthropod-plant Interaction* 10:497-506.

Pelz-stelinski, K. S., Walker, E. D. and Kaufman, M. G. 2010. Senescent leaf exudate increases mosquito survival and microbial activity. *Ecological Entomology* 35:329-340.

Piyaratne, M. K., Amerasinghe, F. P., Amerasinghe, P. H. and Konradsen, F. 2005. Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. *Journal of vector borne diseases*, 42(2), 61.

Preechaporn, W.,Jaroensutasinee, M.and Jaroensutasinee, K.2006. The larval ecology of *Aedes aegypti* and *Aedes albopictus* in three topographical areas of Southern Thailand. *Dengue Bulletin* 30:204-213.

Rattanarithikul, R., Harrison, B.E., Panthusiri, P., and Coleman, R.E.2005a. Illustrate keys to the mosquitoes of Thailand I. background; geographic distribution; lists of genera, subgenera, and species;and a key to the genera. *Southeast Asian Journal of Tropical Medicine and Public Health* 36:1-80.

Rattanarithikul, R., Harbach, R. E. Harrison, B.A., Panthusiri, P., Jones, J. W. 2005b. Illustrated keys to the mosquitoes of Thailand II.genera *Culex*. *Southeast Asian Journal Tropical Medical Public Health* 36:1-97.

Rattanarithikul, R., Harrison, B.A., Panthusiri, P., Peyton, E.L., Coleman, R. E. 2006. Illustrated keys to the mosquitoes of Thailand III.genera *Aedeomyia*, *Ficalbia*, *Mimomyia*, *Hodgesia, Coquillettidia*, *Mansonia*, and *Uranotaenia*. *Southeast Asian Journal Tropitcal Medicine Public Health* 37:1-85.

Rattanarithikul, R.,Harrison, B. E., Panthusiri, P. and Coleman, R.E.2007. Illustrated key to the mosquitoes of Thailand V.genera *Orthopodomyia*, *Kimia*, *Malaya*, *Topomyia*, *Tripteroides*, and *Toxorhynchites*. *The Southeast Asian Journal of Tropical Medicine and Public Health* 38:1-58.

Rattanarithikul, R. Harbach, R. E, Harrison, B. A., Panthusiri, P., Coleman, R. E. and Richardon, J.H.2010.Illustrated keys to the mosquitoes of Thailand VI. tribe *Aedini*. *The Southeast Asian Journal of Tropical Medicine and Public Health* 41:1-152.

Rattanarithikul, R. Harrison , BA, Harbach RE, Panthusiri P, Coleman, RE. 2006. Illustrated keys to the mosquitoes of Thailand.IV. *Anopheles*. *Southeast Asian Journal of Tropical Medicine Public Health* 37:1-128.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rodriguez, S. D., Chung, H. N., Gonzales, K. K., Vulcan, J., Li, Y., Ahumada , J.A.,Romero, H.M.,De La Torre, M., Shu,F. and Hansen, I.A.2017. Efficacy of some wearable devices compared with spray-on insect repellents for the yellow fever mosquito, *Aedes aegypti* (L.)(Diptera: Culicidae). *Journal of Insect Science* 17(1):1-6.

Rosa, E., Dahelmi., Salmah, S. and Syamsuardi. 2016. Density of different dipteran larvae inhabiting phytotelmata from some locations of west Sumatera, Indonesia. *American Journal of Zoological Research* 4(1):13-16.

Rosenweig, M. L. 1995 . Species diversity in space and time . Cambridge UniversityPress, New York,NY.

Rueda, L. M. 2008. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Hydrobiologyia*: 477- 487.

Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F. and Lengeler, C. 2005.Habitat characterization and spatial distribution of *Anopheles* sp. mosquito larvae in Dares Salaam (Tanzania) during an extended dry period. *Malaria journal*, 4(1), 4.

Sarakham, M., Et, R., Chaiyaphum, Y., Charoen, A., Ratchathani, U., Sa, S., Buri, S. 2015. The climate of Thailand. *Climatological Group Meteorological Development Bureau Meteorological Department*: 1-7.

Schulze, W., Schulze, E. D., Pate, J. S. and Gillison, A. N. 1997. The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californican*. *Oecologia* 112(4): 464-471.

Sofizadeh, A., Moosa - Kazemi , S. H. and Dehgham, H . 2017. Larval habitats characteristics of mosquitoes (Diptera: Culicidae) in North-East of Iran. *Journal of arthropod-borne diseases* 11(2):211-225.

Sota, T., Mogi, M. and Kato, K. 1998.Local and regional-scale food web structure in *Nepenthes alata* pitcher. *Biotropica* 30 (1):82-91.

Sota, T. and Mogi, M.2006. Origin of pitcher plant mosquitoes in *Aedes* (*Stegomyia*): a molecular phylogenetic analysis using mitochondrial and nuclear gene sequences. *Journal Medical Entomology* 45 (5):795-800.

Subashini, K., Sivakami, R. and Jeyasankar, A. 2017. Phytochemical screening and ovicidal activity of *Scutellaria violacea* (Lamiaceae) leaf extract against vector mosquitoes (Diptera: Culicidae). *International Journal Advanced Research in Biological Science* 4(3):152-158.

Sumba, L.A.,Guda, T.O.,Deng, A.L., Hassanali, A.,Beier, J.C.and Knols, B.G.J.2004. Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science* 3(24):260-265.

Takeuchi, Y., Chaffron, S., Salcher, M. M., Instsugi, R.S., Kobayashi,M.J.,Diway,B., Mering, C.V., Pernthaler, J. and Shimizu, K, K. 2015. Bacterial diversity and composition in the fluid of pitcher plants of the genus *Nepenthes. Systematic and Applied Microbiology* 38:330-339.

Tananchai, C., Tisgratog , R ., Juntarajumnong , W., Grieco , J. P ., Manguin, S., Prabaripai, A. and Chareonviriyaphap, T.2012. Species diversity and biting activity of *Anopheles dirus* and *Anopheles baimaii* (Diptera: Culicidae) in a malaria prone area of western Thailand. *Parasites&vectors*, 5(1),211.

Thavara, U., Tawatsin , A., Pengsakul, T., Bhakdeenuan, P ., Chanama , S ., Anantapreecha, S., Molito, C., Chomopoosri, J., Thammapalo, S., Sawanpanyalert, P.and Siriyasatien, P.2009. Outbreak of chikungunya fever in Thailand and virus detection in field population of vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health*, 40(5), 951.

Thongsripong, P., Green,A., Kittayapong,P.,Kapan,D.,Wilcox,B.and Bennett,S.2013. Mosquito vector diversity across habitats in central Thailand endemic for dengue and other arthropod-borne diseases. *PLos neglected tropical diseases*, 7(10), e2507.

Torres-Estrada, J. L., Rodriguez, M. H ., Cruz - Lopez, L. and Arredondo - Jimenez, J. I.2001. Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to *Mesocyclops longisetus* (Copepoda: Cyclopoidea) under laboratory and field conditions. *Journal Medical Entomology* 38(2):188-192.

Tolle, M. A. 2009. Mosquito - borne diseases. *Current problems in pediatric and adolescent health care* 39(4):97-140.

Trzcinski, M. K.,Walde, S.J. and Taylor, P.D.2005. Local interaction in pitcher plant communities scale-up to regional patterns in distribution and abundance. *Environmental Entomology* 34(6):1464-1470.

Tsukamoto, M.1989.Two new mosquito species from a pitcher plant of M t.Kinabalu, Sabah,Malaysia: *Culex rajah* and *Toxorhynchites rajah* (Diptera: Culicidae). *Japanese Journal of Tropical Medicine and Hygiene* 17 (3):215-228.

Tyagi, B.K.,Munirathinam,A.,Krishnamoorthy,R.,Baskaran,G.,Govindarajan,R.,Krishnamoorthi, R.,Mariappan,T., Dhananjeyan, K.J and Venkatest, A. 2015. A revision of genus *Toxorhynchites* Theobald,1901,in the South-East Asian countries, with description of a new species *Toxorhynchites* (*Toxorhynchites*) *darjeelingensis* from West Bengal, India (Diptera,Culicidae). *Halteres* 6:13-32.

Van Dam, A. R. and Walton, W. E. 2008. The effect of predatory fish exudate on the ovipositional behavior of three mosquito species: *Culex quinquefasciatus*, *Aedes aegypti* and *Culex tarsalis*. *Medical and Veterinary Entomology* 22:399-404.

Vanlalruia, K., Senthilkumar , N . and Gurusubramanian, G. 2014. Diversity and abundance of mosquito species in relation to their larval habitats in Mizoram, North Eastern Himalayan region. *Acta tropica* 137:1-18.

Vezzani, D. 2007. Artificial container-breeding mosquitoes and cemeteries : a perfect match. *Tropical Medicine & International Health* 12(2):299-313.

Wong, P.S.J., Li, M, Z.I.,Chong, C.S.,Ng, L.C. and Tan,C.H.2013.*Aedes* (*Stegomyia*) *albopictus* (Skuse): a potential vector of Zika virus in Singapor. *PLoS neglected tropical diseases* 7 (8), e2348.

Yee, D.A., Kesavaraju, B. and Juliano,S.A.2007. Direct and indirect effects of animal detritus on growth, survival, and mas of invasive container mosquito *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* 44(4):580-588.

Yee, D. A.,Kneitel, J.M.Juliano,A.A.2010. Environmental correlates of abundance of mosquito species and stages in discarded vehicle tires. *Journal Medical Entomology* 47:53-62.

Yilamujiang, A., Reichelt, M. and Mithofer, A. 2016. Slow food : insect prey and chitin induce phytohormone accumulation and gene expression in carnivorous *Nepenthes* plants. *Annals of botany* 118(2):369-375.

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