



## THESIS APPROVAL

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**TITLE:** The Bees (Hymenoptera: Apoidea) as Insect Pollinators on  
Physic nuts (Euphorbiaceae: *Jatropha curcas* L.) in Thailand

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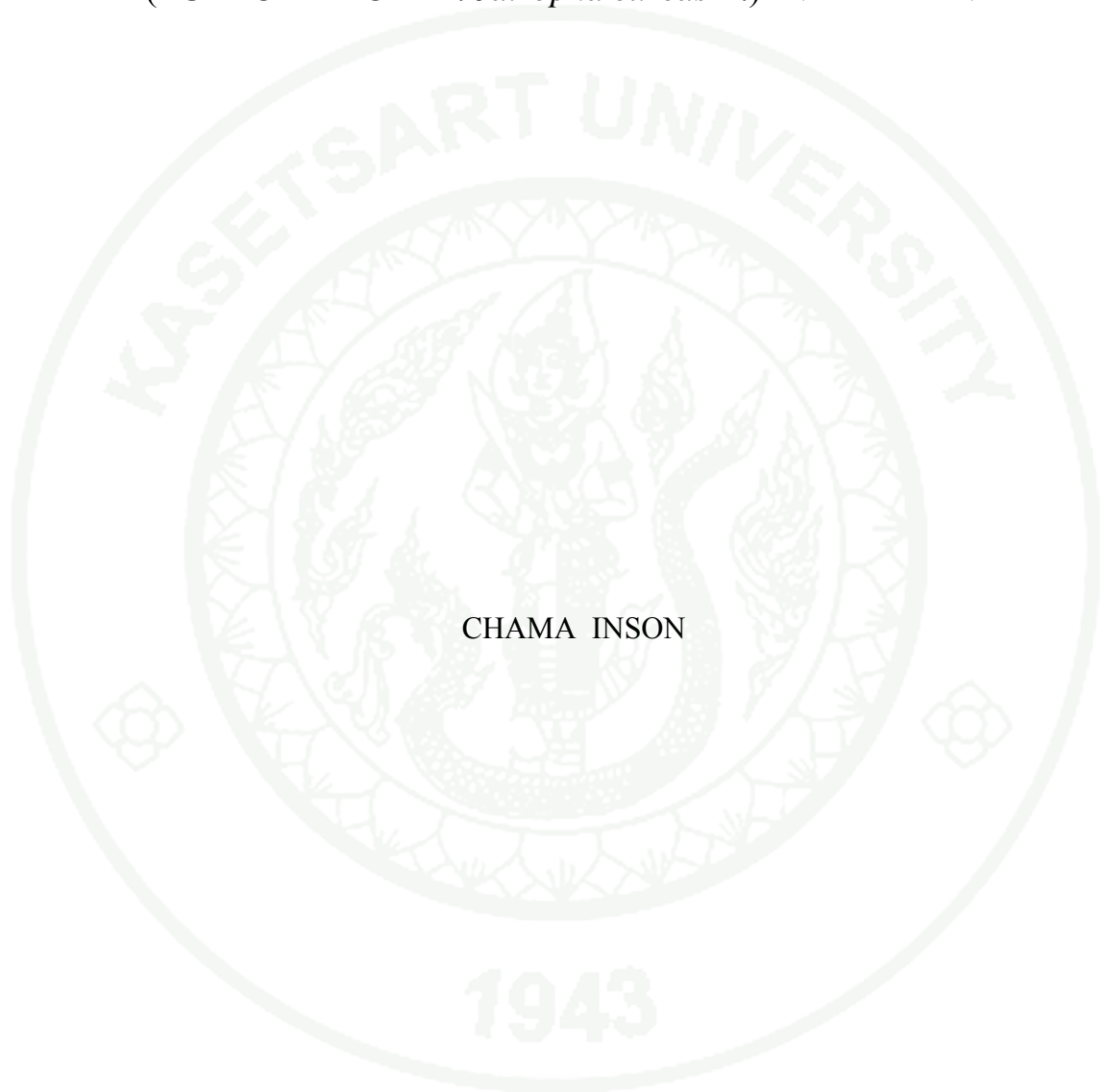
**APPROVED BY THE GRADUATE SCHOOL ON**

#### DEAN

( Associate Professor Gunjana Theeragool, D.Agr. )

THESIS

THE BEES (HYMENOPTERA: APOIDEA) AS INSECT  
POLLINATORS ON PHYSIC NUTS  
(EUPHORBIACEAE: *Jatropha curcas* L.) IN THAILAND



CHAMA INSON

A Thesis Submitted in Partial Fulfillment of  
the Requirements for the Degree of  
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Chama Inson 2012: The Bees (Hymenoptera: Apoidea) as Insect Pollinators on Physic nuts (Euphorbiaceae: *Jatropha curcas* L.) in Thailand. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Savitree Malaipan, Ph.D. 306 pages.

Study on diversity of insect pollinators and pollination on physic nuts (*Jatropha curcas* L.) was conducted in a survey and identification of insect pollinators on physic nut flowers in 20 provinces of Thailand from April to August, 2009. The pollination experiments on toxic and non-toxic varieties of physic nut plantations were conducted during 2007-2011 at four locations (Kamphaeng Saen, Agriculture Research and Development Center, Aviation School, and Bangkhen) in the central part of Thailand. Because of unisexual, female and male flowers separated, insect pollinators are very important for fruit setting of physic nut. The results of this survey revealed 311 species, 138 genera and 64 families in eight orders namely Hymenoptera (with 45.02% of the species) which exhibited the highest diversity followed by Lepidoptera (20.58%), Diptera (15.43%), Coleoptera (9.97%), Hemiptera (7.40%), Mantodea and Orthoptera (0.64%) while the lowest diversity percentage was found in Blattodea (0.32%). All insect species were divided into major and minor groups according to their behavioral activities and importance to physic nuts. The insect pollinators in 20 provinces were compared by species diversity index and distribution. The highest species diversity index was observed in the north while the lowest values were found in the northeast and the south. Sixty species of superfamily Apoidea were identified. The most widely distributed species were *Apis cerana indica* Fabricius, *A. florea* Fabricius and *Trigona pagdeni* Schwarz. These bees were considered to be the most common and effective pollinators. Increasing potential of oil content around 14.16-15.41% was associated with *A. cerana indica*, *A. mellifera ligustica*, and *T. pagdeni*. Physic nut plants absolutely need cross-pollination by honey bees and stingless honey bees. In additional, some factors (variety of physic nuts, location, season, and environment) influenced the botanical characteristics (flower sex ratio, pollen germination, and growth development), species diversity of insect pollinators, bee behavior, weight and oil content of physic nut production.

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Student's signature

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Thesis Advisor's signature

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Chama Inson

April, 2012

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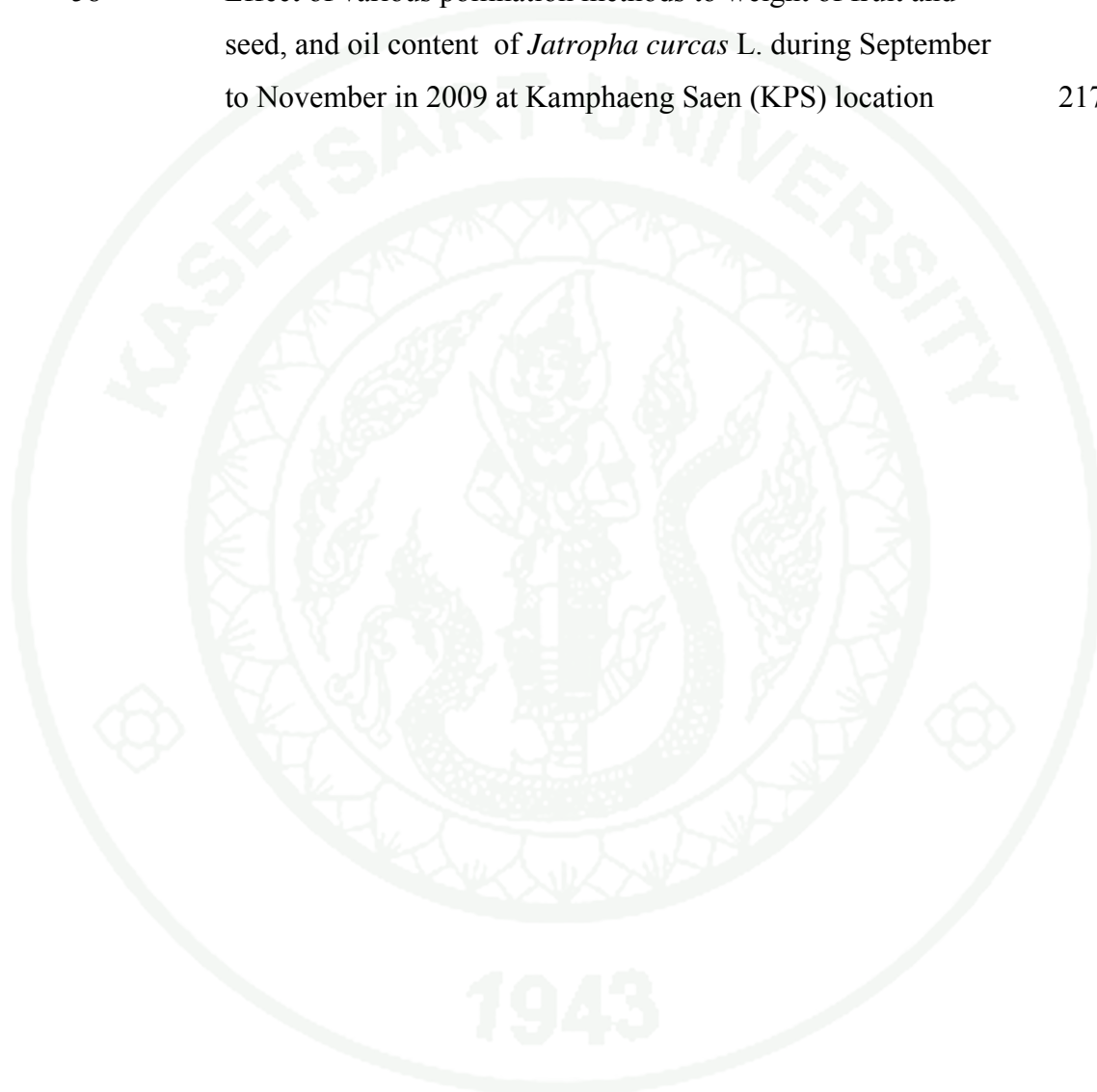
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## LIST OF ABBREVIATIONS

|            |   |  |
|------------|---|--|
| AFLP       | = | Amplified fragment length polymorphism                   |
| ANOVA      | = | Analysis of variance                                     |
| AS         | = | Aviation School  |
| BK         | = | Bangkhen   |
| cm         | = | Centimeter   |
| C.N.P.N.A. | = | Committee on the Status of Pollinators in North America  |
| °C         | = | Degree Celsius   |
| DHPB       | = | Deoxy hydroxyphorbol butadienyl                          |
| DNA        | = | Deoxyribonucleic acid                                    |
| DW         | = | Dry weight   |
| E          | = | Easting  |
| ha         | = | Hectare  |
| KPS        | = | Kamphaeng Saen   |
| KS         | = | Kamphaeng Saen series                                    |
| KU         | = | Kasetsart university                                     |
| kg         | = | Kilogram   |
| km         | = | Kilometer  |
| m          | = | Meter  |
| μl         | = | Microliter   |
| mm         | = | Millimeter   |
| M          | = | Molar  |
| NY         | = | Nong Yai   |
| nt         | = | Non-toxic variety  |
| ASnt       | = | Non-toxic at Aviation School                             |
| ARDCnt     | = | Non-toxic at Agriculture Research and Development Center |
| SSnt       | = | Non-toxic from Mr. Suksan Suttipunhiboon                 |
| N          | = | Nothing  |
| PEs        | = | Phorbol ester  |

**LIST OF ABBREVIATIONS (Continued)**

|                 |   |                                 |
|-----------------|---|---------------------------------|
| pH              | = | Potential of Hydrogen ion       |
| RH              | = | Relative humidity               |
| SSR             | = | Simple sequence repeat          |
| H'              | = | Species diversity index         |
| cm <sup>2</sup> | = | Square centimeter               |
| m <sup>2</sup>  | = | Square meter                    |
| t               | = | Toxic variety                   |
| KPSt            | = | Toxic variety at Kamphaeng Saen |
| NYt             | = | Toxic variety at Nong Yai       |
| USA             | = | United States of America        |

**THE BEES (HYMENOPTERA: APOIDEA) AS INSECT  
POLLINATORS ON PHYSIC NUTS  
(EUPHORBIACEAE: *Jatropha curcas* L.) IN THAILAND**

**INTRODUCTION**

Physic nut (*Jatropha curcas* L.) as a choice crop that has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content and low seed cost (Jones and Miller, 1992; Francis *et al.*, 2005; Kumar and Sharma, 2008). Non-edible oil like physic nut can be used for the production of biodiesel. Physic nut has enormous potential for biodiesel production. Physic nut is a multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low to high rainfall areas and can be used to reclaim land, as a hedge and a commercial crop. Thus, growing it could provide employment, improve the environment and enhance the quality of rural life (Kumar and Sharma, 2005). Added to this, qualitative sustainability assessment, focusing on environmental impacts and strengthened by some socioeconomic issues, is quite favorable as long as physic nut is cultivated on wastelands and degraded lands (Achten *et al.*, 2007).

Until recently, in tradition and successful application of physic nut includes function like soil water conservation, soil reclamation, erosion control, living fences, firewood, green manure, lightning fuel and local use in soap production, insecticide and medicinal application at modest scale (Jongschaap *et al.*, 2007). Physic nut was mainly grown for fencing and local soap production, but awareness of physic nut growing as a cash crop for biofuel is now widely known, as farmers look for alternatives to generate income. However, physic nut has a low and limit yield, that is the important problem for biodiesel production (Jones and Miller, 1992). Because low numbers of female flowers, limited branching and inadequate pollination are the major factors that limit seed production and thus oil yield of physic nut (Abdelgadir *et al.*, 2008).

The mechanism that balances the promotion of out-crossing and minimizes self-pollination is poorly understood (Achten, 2010). Chang-wei *et al.* (2007) categorize physic nut as an out-crosser that is self-compatible and needs pollinators because the adhesiveness of the pollen, the smoothness of the stigma of 1.62 mm in diameter and make wind pollination almost impossible, there are insect-pollinated. Insect pollinators play an essential role in physic nut as carriers of pollen grains from male flowers to the stigma of female flowers. This is a successful pollination and fruits or seeds can be collected for biodiesel production (Bhattacharya *et al.*, 2005).

Insect pollinators and physic nut flowers are protocol-operation, which both of them seem to help each other. Insect pollinators give successful pollination to physic nut that has a complete fruit setting, while these pollinators take some food, such as nectar and pollen from physic nut flowers. Nectar and pollen production are costly to a plant and must be balanced for maximum return (that is, maximum chance of successful reproduction) for the energy spent to produce them (Delaplane and Mayer, 2000).

In Asia, there have been only limited investigations of the diversity of insect pollinators on physic nut. The first observation (more than 50 species of insect pollinators) was obtained in Thailand (Malaipan *et al.*, 2007). Later, 13 species were reported in India by Raju and Ezradnama (2002). Bhattacharya *et al.* (2005) also surveyed physic nut in India but discovered just six species of insect pollinators.

Raju and Ezradanam (2002) reported that bees were the major group there were large population and effective different for flowers. With regard to floral structure and floral visitor adaptive features, it was stated that honey bees (*Apis dorsata*, *A. florea* and *A. mellifera*) were effective pollinators. These bees were the major group belong to the colonization or socialization bees and the most common and effective pollinators. *Eumenes*, *Vespa* and beetles are not considered as effective pollinators as the pollination syndromes of these visitors do not match with physic nut flowers. The bees mostly collected floral rewards on different plants effecting geitonogamous and xenogamous pollinators. Malaipan *et al.* (2007) suggested that

insect pollinators were divided into two groups, major and minor. The major group is social bees, blow fly and syrphid flies, and minor group includes other insects in Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Thysanoptera.

The lack of knowledge will limit pollination programs from using the full potential of physic nut. Establishment and public sharing of pollination knowledge are therefore important for effective domestication of physic nut. Physic nut oil projects are expected to provide income and to increase crop yields, as well as being an ecologically friendly source of alternative energy to rural farmers.

The present study investigates the variation factors that limit physic nut yield, species diversity of insect pollinators has vary applications such as predicting fruit setting or yield, estimating pollination problem, and choose suitable species of insect pollinators for pollination on each province. This study focuses to bees using because bees are the most common, important insect pollinators, belonging in either colonization or socialization and effective pollinators for pollination. The study also seeks to assess whether insect pollinators are adequate for physic nut pollination.

These studies are directly relevant to pollination factors for increasing physic nut yield that are important to rural farmers and energy users. The information acquired forms valuable building blocks for the development of an integrated pollination management program in Thailand. Also, this study attempts to clarify the species of bees and pollination factors in order to give guidelines for both conversation strategies and for pollination techniques for managing bees on physic nut flowers. The results will create baseline data of bees in Thailand that can be used to monitor bee population on physic nut plantation in the future.

## OBJECTIVES

1. To study the species diversity of insect pollinators and flowers of physic nuts (*Jatropha curcas* L.) in 20 provinces of Thailand.
2. To study the general botanical characteristics and pollination factors on toxic and non-toxic varieties of physic nuts at four locations in the central part of Thailand.
3. To study bee behaviors on physic nut flowers.
4. To investigate the effect of various pollination methods and pollinators to fruit set and yield on toxic and non-toxic varieties of physic nuts.

## LITERATURE REVIEW

### 1. Description of physic nuts (*Jatropha curcas* L.)

#### 1.1 Distribution of physic nuts

*Jatropha curcas* (from Greek language meaning Jatro=physician, and trophe=food; common name physic nut or purging nut) belongs to the family Euphorbiaceae (Becker and Francis, 2010). The delineation of the original area of species distribution of physic nut has been the subject of a long debate (Heller, 1996) but, currently, there is a growing agreement that the physic nut center of origin is Mexico and continental Central America (Rao *et al.*, 2008; Sujatha *et al.*, 2008). The Portuguese learned about physic nut's medicinal properties in the 16<sup>th</sup> century, and later established commercial plantations for soap and lamp oil production on the Cape Verdian Islands and Guinea Bissau. Later, physic nut genotypes adopted in Western Africa were spread across other Portuguese colonies in Africa Mozambique, Angola and into Asia (India, China and Indonesia) (Heller, 1996). Additionally, more than 300 years ago, physic nut was introduced to Thailand by Portuguese sailors (Pasabut and Suttipunphiboon, 2011). Presently, two varieties of physic nuts, toxic and non-toxic or low toxic are widespread in Thailand the toxic varieties are grown all over the country.

#### 1.2 Toxic and non-toxic (low toxic) varieties

An important aspect of domestication of physic nut is the toxicity of the plant (Pamidimarr *et al.*, 2009). Consumption of physic nut seeds may result in various symptoms (Becker and Makkar, 1998; Chimbari and Shiff, 2008) these seeds are toxic to humans and many animals (Gubitz *et al.*, 1999). The most problematic toxic components in physic nut are probably a number of phorbol esters. In general, phorbol esters are found present in high concentrations in the seeds (Makkar *et al.*, 1997; Rakshit *et al.*, 2008). Jongschaap *et al.* (2007) suggested that the detoxification of physic nut organic material was such a complicate process, it had so far only been

successful at laboratory scale. Phorbol esters decompose quickly as they were very sensitive to elevated temperatures, light and atmospheric oxygen (National Institutes of Health, 2007); they decompose completely within 6 days (Rug and Ruppel, 2000). Rakshit *et al.* (2008) demonstrated that phorbol ester was not an easily deployed process and the presence of possibly toxic phorbol ester degradation products after treatment could not be ruled out. The presence of naturally occurring plants with low levels of phorbol esters was very interesting in a domestication context, because that plant material without phorbol esters could easily be developed and applied to by product such as seed cake for animal etc. However, introducing non-toxic material may raise new complications, as toxic and non-toxic physic nuts are morphologically alike (Achten *et al.*, 2010).

Preliminary studies indicated very low variation in microsatellite simple sequence repeat (SSR) markers within populations of physic nut even in its natural distribution (Mexico) (Sun *et al.*, 2008). Pamidimarri *et al.* (2009) applied SSR, amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA markers to discriminate between two Mexican accessions of physic nut (one toxic and one non-toxic). Although they could discriminate between the accessions, they found no variation between individuals within each accession. This could be an indication of a population structure with a high level of homozygosity as well. As toxicity in terms of phorbol esters seems to be expressed, it may be regulated by only one or a few genes. The inheritance of toxicity was not settled, but Sujatha *et al.* (2005) suggested that the phenotype of the mother tree was passed on to the seed (i.e., non-toxic mothers give non-toxic seed and toxic mothers give toxic seeds, independent of the phenotype of the father).

The concentrations of phorbol esters in kernels of toxic Cape Verde, Nicaragua and Ife-Nigeria varieties were 2.70, 2.17 and 2.30 mg/g, whereas kernels of non-toxic Mexican had a very low level (0.11 mg/g) or not detected of phorbol esters (Makkar *et al.*, 1998). Phorbol esters (PEs) (DHPB; 12 – deoxy - 16 - hydroxyphorbol - 4' - [12',14'-butadienyl] - 6' - [16',18',20' - nonatrienyl] bicyclo [3.1.0] hexane - (13-O) - 2'- [carboxylate] - (16-O) - 3' - [8'-butenoic-10']

ate) content were investigated from the leaves, pulps and seeds of non-toxic variety of physic nut from Mexico. These toxic compounds were present in leaves at the greatest level, 1.07 mg/g of dry weight (DW), of all selected parts. The amounts of PEs in pulps and kernel were 0.25 and 0.21 mg/g DW, respectively and the concentration of PEs was low in the seed coat at 0.12 mg/g DW. From the results, they found that physic nut leaves contained high level of PEs (DHPB) but in the seeds, PEs content was lower than those found in toxic variety from Thailand (Junyam *et al.*, 2009).

### 1.3 Cultivation

Physic nut is a drought resistant species, easy to establish, grows relatively quickly, and hardy (Kumar and Sharma, 2005). It is a succulent plant that may shed its leaves during the dry season and attains a height of about 3 m in 3 years (Kumar and Sharma, 2008). Physic nut is adapted to higher temperatures and grows well in well drained soils receiving an average rainfall of 300-1,000 mm. It can however tolerate slight frosts and in the years without rainfall and is well adapted to marginal soils with low nutrient content (Becker and Francis, 2010). Physic nut is a small tree or large shrub, which can reach a height of 3-5 m, but under favorable conditions it can attain a height of 8 or 10 m (Kumar and Sharma, 2008). Physic nut often starts yielding from the first year onwards, but yields are maximum from plants that are about 3-5 years old. The productive age of physic nut is over 30 years (Becker and Francis, 2010). The plant shows articulated growth, with a morphological discontinuity at each increment. The branches contain latex. Normally, five roots are formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetatively propagated plants. Leaves 5-7 lobed, hypostomatic and stomata are of paracytic (Rubiaceous) type (Kumar and Sharma, 2008). In addition, Srinoprakun *et al.* (2011) reported that type of insects and pests were surveyed in the physic nut plantation and ranking of the most found insects and pests were proposed. Mealy bug, aphids, and scale insects are the most found in the physic nut plantations. In the mean time, leaf spots, fungus infections, were the most severe in many areas in Thailand.

Srinoprakun *et al.* (2011) stated that the spacing was important factor along with cutting management program for long time cultivation. Therefore, the three different spacing (2x2, 2x3, and 3x3 m) were tested in Thailand. The result showed that 2x3 and 3x3 m spacing gave high yield of seed weight of 129.2 and 127.1 kg/rai for the first cultivation year. Kumar and Sharma (2008) recorded that the seeds mature about 3-4 months after flowering. The seeds were black and the seed weight/1,000 seeds were about 727 g (0.727 g/seed), there were 1,375 seeds/kg in the average. In like manner, Becker and Francis (2010) reported that the dry seed of physic nut weighed around 0.75 g/seed and contained more than 30% by weight of oil that was suitable for conversion into biodiesel. The kernel forms around 60% of the seed. The deoiled seed cake had a crude protein content of between 53 to over 60 % and a favorable amino acid profile. In additional, Singh (1970) described the microscopical anatomy of fruits. Gupta (1985) investigated the anatomy of other plant parts. Physic nut was a diploid species with  $2n=22$  chromosomes.

#### 1.4 How to use physic nut

Physic nut attracts a lot of interest as triggering large investments, rapid expansion of cultivation areas, a biofuel crop and multipurpose plant. Once, the seeds are used to oil extract for biodiesel production, the oil can be extracted easily with techniques of different sophistication levels (Achten *et al.*, 2008). The crude physic nut oil meets the fuel quality standards of rape seed (Fuels from Agriculture in Communal Technology, 2006) and can be easily converted into biodiesel (Azam *et al.*, 2005). Biodiesel is made from virgin or used vegetable oils (both edible and non-edible) and animal fats through trans-esterification and is a diesel substitute and requires very little or no engine medications up to 20% blend and minor modification for higher percentage blends. Physic nut oil can be used as fuel in diesel engines directly and by blending it with methanol (Gubitz *et al.*, 1999). The seed oil of physic nut was used as a diesel fuel substitute during the World War II. Engine tests with physic nut oil were done in Thailand, showing satisfactory engine performance (Takeda, 1982). For African countries, the feasibility of the production of fatty acid ethyl esters from physic nuts oil was studied (Eisa, 1997). The economic evaluation

has shown that the biodiesel production from physic nut is very profitable provided the by products of the biodiesel production can be sold as valuable products (Foidl and Eder, 1997)

The non-toxic variety of physic nut from Mexico can be a suitable alternative to toxic physic nut varieties, and it is suggested to propagate its cultivation. This non-toxic variety of physic nut could be a potential source of oil for human consumption, and the seed cake can be a good protein source for humans as well as livestock (Makkar *et al.*, 1997). In addition, seeds of physic nut can be produced soap, insecticide and medicinal plants. Another, seed cakes may be applied to fertilizer, fodder (non-toxic variety), and biogas. Other parts of physic nut can be used many purpose such as rearing of Eri silk worm, medicinal production, (Kumar and Sharma, 2005), erosion control and a living fence that is protecting cropland from freely ranging cattle, sheep and goats in tropical countries (Becker and Francis, 2010). To use the full potential of physic nut and to support further expansion and systematic selection, breeding and domestication are a prerequisite (Achten *et al.*, 2010).

The primary conservation benefits to be derived from production of physic nut relate to improved soil restoration and management. The findings of Kumar and Sharma (2008), have shown that the heavy metal contaminated soil can be restored by using combination of industrial wastes and suitable bio-inoculants strain. Physic nut in addition to protecting crops from livestock; it reduces wind erosion and pressure on timber resources and increases oil moisture retention. Physic nut oil projects are expected to provide income and organic fertilizer to increase crop yields, as well as being an ecologically friendly source of alternative energy to rural farmers. In additional, Srinoprakun *et al.* (2011) reported that the seed cake of physic nut utilization, different rates of the seed cake (1,600, 800, 400 kg/rai) were applied to the cultivation of Chinese kale, tomato and potato. It was understandable that the mixing of chemical fertilizer and physic nut seed cake (1,600 kg/rai) gave the highest plant performance and no phorbol ester residue left in the cultivation soil and harvested vegetable.

### 1.5 Low yield problem of physic nut

Physic nut has a low and limit yield, that is the important problem for biodiesel production. The economics of the industry depend on production yields. Because of various factors such as variety, cultivation, irrigation, season and pollination are unsuitable to physic nut (Jones and Miller, 1992; Kumar and Sharma, 2008). Malaipan *et al.* (2007) reported that very rare the insect pollinators were found in Lop Buri physic nut plantation, there were lose seed yield because most physic nut flowers were not successfully pollinated.

### 1.6 Botanical characteristics of physic nut flowers

Physic nut is monoecious—with female and male flowers on the same plant and in the same inflorescence (Raju and Ezradanam, 2002; Bhattacharya *et al.*, 2005; Chang-wei *et al.*, 2007a); occasionally hermaphrodite flowers or perfect flowers occur (Dehgan and Webster, 1979).

#### 1.6.1 Morphology of physic nut flowers

The size of the raceme inflorescences, with dichasial cyme pattern, can vary considerably (5-9.5 cm in length and 4.5-12.5 cm in diameter) and with this variable size, the number of flowers also varies (Raju and Ezradanam, 2002).

1) Male flowers are small and plate shaped (Raju and Ezradanam, 2002). The five sepals (0.5 cm long) and five petals (0.7 cm) are free (Chang-wei *et al.*, 2007a). The latter are connivent at the flower base, forming a short tube. Ten diadelphous stamens are arranged in two tiers of five. The flower tier is free, while the upper tier is united. The anthers are yellow, approximately 0.2 cm, ditheous and dorsixed. Five oval-shaped glands are present at the villous flower base (Raju and Ezradanam, 2002).

2) Female flowers show a similar shape as the male flowers. The flowers contain three styles and bifid stigmas. The ovary has three carpels, each with a single locule producing one ovule. The floral base is villous and contains five yellow elliptical glands under the ovary (Raju and Ezradanam, 2002; Chang-wei *et al.*, 2007a).

3) A perfect flower or hermaphrodite flowers of physic nut is formed terminally, individually, with female flowers (tricarpellary, syncarpous with trilocular ovary) usually slightly larger and occurs in the hot seasons (Dehganand and Webster, 1979).

#### 1.6.2 Flowering of physic nut flowers

Dhillon *et al.* (2006) observed flowering at the terminal end of branches after rainy season in India. However, some plants flower even in spring season (March-April). Usual peak period of flowering varies from 3-20 days. Likewise, Chang-wei *et al.* (2007a) reported that normally, the male flowers open the first at the branch joint, followed by female ones. Female flowers at the top of inflorescence bloom earlier than flowers at other places. Some male flowers remain un-opened even when all female flowers have opened. Very few inflorescences had their female flowers open before male ones. Each inflorescence's flower lasts from 13-19 days. Normally, a few male flowers open at the first one or two days, followed by the quick opening of the female flowers. About 60% of the female flowers opened from the 3<sup>rd</sup>-5<sup>th</sup> day. The flowering pattern showed that male flowers open gently with a small peak between the 9<sup>th</sup> and 13<sup>th</sup> day. The results contrast with previously observations in that most female flowers opened from the first (more than 60%), each inflorescence's flower lasts from 10-12 days, and male flowers open gently with a small peak between the 3<sup>rd</sup> and 6<sup>th</sup> day in the summer season, that open was faster than the rainy and the winter season.

### 1.6.3 Flower sex ratio of physic nut

The number of female and male flowers per inflorescence varies a lot among the observations (female:1-19 and male:25-238), the female and male flower ratio of Raju and Ezradanam (2002) research is similar to study of Chang-wei *et al.* (2007a) (1:29 and 2:49), but differ from observation of Bhattacharya *et al.* (2005) (2:17) (Table 1). Although, Prakash *et al.* (2007) reported variation in the F/M ratio during the first year with average F/M ratio of 1:25, and it reduced to 1:13 in the second year indicating a positive trend towards productivity. Raju and Ezradanam (2002) reported that flowering occurs during the wet season and two flowering peaks are often seen, i.e. during summer and autumn. Dhillon *et al.* (2006) reported that the average female to male (F/M) flower ratio was 1:20, which changes drastically (1:108) with the fall in temperature. In additional, Malaipan *et al.* (2007) observed sex ratio of female and male on physic nut flowers (1:9) was higher in March and decreased (1:22) in October.

**Table 1** Flower and pollen characteristics of *Jatropha curcas* L.

|              | Male flower per inflorescence | Female flower per inflorescence | Female: Male | Total P per male | P : O           |
|--------------|-------------------------------|---------------------------------|--------------|------------------|-----------------|
| <sup>1</sup> | 25-93                         | 1-5                             | 1:29         | 655              | 6,332:1         |
| <sup>2</sup> | 17-105                        | 2-19                            | 2:17         | 1,617±100        | 539:1           |
| <sup>3</sup> | 49-238                        | 0-17                            | 2:49         | 1,597-5,763      | 13,015-46,968:1 |

P = Pollen grains; O = Ovule

**Source:** <sup>1</sup> Raju and Ezradanam (2002); <sup>2</sup> Bhattacharya *et al.* (2005);

<sup>3</sup> Chang-wei *et al.* (2007a)

#### 1.6.4 Anthesis and receptive time of physic nut flowers

1) Anthesis time, the anthers dehisce 1 hour after flower opening by longitudinal slits (Raju and Ezradanam, 2002). The pollens from male flower bloom at the first day are adhesived, and do not float even if it is shaken slightly. Chang-wei *et al.* (2007b) said that pollen viability is relatively high up to nine hours after blooming and gets low 33 hours later and pollen hardly has any viability after 48 hours. However, the pollens dispersed in the 2<sup>nd</sup> or 3<sup>rd</sup> day would lose float sometimes by the vibration of foraging insects such as butterflies. The viability of pollens dispersed on the 2<sup>nd</sup> or 3<sup>rd</sup> day flowers considerably (Chang-wei *et al.*, 2007a).

2) Receptive time, the stigma has become receptive 2 hours after anthesis, during 3 days after the opening of the flower (Raju and Ezradanam, 2002; Bhattacharya *et al.*, 2005), and the stigma of the female flower is smooth without secretion to catch and detain them (Chang-wei *et al.*, 2007a). Likewise, Heller (1996) studied the pollen viability, stigma receptivity and reproductive features and found that, the lifespan of the male flower is about two days. The lifespan of the female flower is about 5-12 days. The stigma receptivity is strong during 1<sup>st</sup>-4<sup>th</sup> day and begins to decline 5<sup>th</sup> day onwards and completely loses its receptivity on 9<sup>th</sup> day. There are still many un-open male flowers after all female flowers opened. This provides time for receptive stigma to get pollens from male flowers, and enhance the opportunity of reproductive success. In a few racemes, and the female flowers opened firstly shows a tendency to promote xenogamy (cross-pollination) and minimize geitonogamy (self-pollination). This provides time for receptive stigma to get pollens from male flowers, and enhances the opportunity of reproductive success (Chang-wei *et al.*, 2007b).

#### 1.6.5 Pollen of physic nut flowers

The pollen produced globular and their size ranges from 52-89  $\mu\text{m}$  and its exine is verrucate. The floating of pollen grains from stamens is relative to

the stage of flowering. The plant is unlikely to be pollinated by wind. Pollen can be dispersed by wind to a place 4-5 m away or even farther, but wind pollination is almost impossible for physic nut (Chang-wei *et al.*, 2007a). In addition, Bhattacharya *et al.* (2005) examined the fore noon pattern of anthesis with subsequent pollen release with each male flower producing  $1,617 \pm 100$  pollens with pollen: ovule ratio of 539:1.

Malaipan *et al.* (2007) reported that there were variation of pollen germination on the clones, ages stem, levels, and direction of plants canopy. Pollens were collected from florets at the apex of inflorescence (68%) which had better germination than at the middle (64%) or at the base (58%) of inflorescence. Percent germination of the late blooming inflorescence stage (74%) was higher than that of the early blooming inflorescence stage (54%). Pollens from the florets at the first group blooming on the stem (55%) had lower germination than the second (77%) or the third (75%) group. The first day blooming floret pollen (30%) had percentage of germination close to the dropping pollen grains on the leaves (36%) and also greater than the second day blooming floret (15%). Pollen germination test was conducted on the 16<sup>th</sup> November, 2006 (the sun compass moved slightly 23 degree to the south). The peak of germination occurred on inflorescences that faced the sun for example pollen at the south side germinated better than at the north side of the plant canopy. In the morning, pollen from florets at the east side germinated was the best, similarly to getting the best again at the west side in the afternoon. Correspondingly, Srinoprakun *et al.* (2011) reported that percentage of pollen germination differ among dissection of canopy and time of the day. In the morning the higher percentage of pollen germination was found in direction exposing to the sun. Likewise, in the late afternoon the higher percentage of pollen germination was found at the southwest where the sun was.

#### 1.6.6 Nectar of physic nut flowers

The male floral base contains 0.3  $\mu$ l nectar (Achten *et al.*, 2010). The female flower base secretes nectar that the female flowers secrete the same

quantities of nectar as the male flowers (Raju and Ezradanam, 2002). Although, Bhattacharya *et al.* (2005) observed a higher nectar production at 12.00 h on female flowers ( $4.54 \pm 0.82 \mu\text{l}$ ) than male flowers ( $1.92 \pm 0.44 \mu\text{l}$ ). Perhaps this might have led to bees prefer female flowers than male flowers in the afternoon (15.00-17.00 h). Chang-wei *et al.* (2007a) stated that both types of flowers had nectar glands, which can pressurize nectar. The nectar dew in a bagged female flower could cover the ovary. Nectar could absorb ultraviolet rays, which could be sensitively observed by bees during its pollination. In addition, Julia (1964) recorded that nectar of physic nut abundant flow during April to May that was sought by bees but difficult to collect. Honey is dark-amber, strong, but agreeable in flavor.

## 1.7 Pollination of physic nut

### 1.7.1 Breeding system

Self-pollinated fruits are lighter in general (Abdelgadir *et al.*, 2008) and aborted before maturation in 25% of cases (Raju and Ezradanam, 2002). This could be due to early acting inbreeding depression thus may reflect a high natural out-crossing rate (Chang-wei *et al.*, 2007a). In addition, the rare hermaphrodite flowers can be self-pollinating. The flowers are pollinated by insects especially honey bees (Kumar and Sharma, 2008). Abdelgadir *et al.* (2008) indicated that to understand the breeding system of physic nut, bagged and open, and self- and cross-pollination were studied. Fruits from open-pollinated flowers were significantly more numerous, larger and heavier than those produced from autogamous self-pollinated flowers. Cross-pollinated flowers had significantly higher fruit set than self-pollinated flowers. However, supplemental self- and cross-pollination did not significantly increase fruit set. However the fruit sets of artificial self-pollination, artificial cross-pollination and natural cross-pollination were 87.93%, 86.66% and 76.42%, respectively, which indicated that physic nut was self-compatible and tended to cross-pollinate (Qing *et al.*, 2007). Also, the ability to self-pollinate through geitonogamy is considered to be adaptive for physic nut for colonization (Raju and Ezradanam, 2002).

### 1.7.2 Pollinators of physic nut

1) Wind pollination, the plant is unlikely to be pollinated by wind. Pollen can be dispersed by wind to a place 4-5 m away or even farther, as tested by Gravity slide method. The distance and intensity of pollen flow changed in different wind velocities, air humidity, and barriers. The maximum of the pollen flow provided by a blooming plant reaches only 2.8 grains/cm<sup>2</sup>. It is not effective to a stigma with a diameter of only 1.62 mm. Thus, wind pollination is almost impossible for physic nut (Chang-wei *et al.*, 2007a).

2) Insects pollination, Chang-wei *et al.* (2007a) suggested that insect pollinators were the major pollination of physic nut. There are many foraging insects on physic nut, whose species belong to the order Diptera, Hymenoptera, Hemiptera, Coleoptera, and Lepidoptera. By the pollination affectivity comparison and the number of these foraging insects, that the main pollination insects of this population included *Chrysomyia megacephala*, *Catopsilia pomona*, and *Apis cerana*. Likewise, Bhattacharya *et al.* (2005) reported that the flowers attracted the insect visitors of Hymenoptera (*Apis dorsata*, *A. florea*, *A. mellifera*, *Eumenes conica* and *Vespa* sp.) and Coleoptera. Among these insect visitors, *Apis* spp. were the most frequent. Correspondingly, Malaipan *et al.* (2007) reported more than 50 species of pollinators were found on physic nut flowers. These were divided into two groups, major and minor. The major group was social bees such as *Apis* spp. and *Trigona* spp. and a large population of Diptera such as Calliphoridae (*Chrysomyia megacephala*), and Syrphidae (*Eristalis arvorum*, *E. obscuritarsis*, *Helophilus bengaliensis* and *Rhingia* spp.), whereas the minor group was other Hymenoptera, other Diptera, Coleoptera, Lepidoptera, Hemiptera, Mantodea and Thysanoptera. Similarly, Qing *et al.* (2007) reported 17 species of floral visitors among which 11 species were pollinators with two floral visiting peaks at 10.00-12.00 h and 16.00-17.00 h every day. Among the different insect visitors *Apis* spp. were the most frequent. Likewise, Raju and Ezradanam (2002) observed that of the total foraging visits made by insects on female flowers, bees contributed 28%, ants 70% and flies 2%. On male flowers, bees made up 34%, ants 61% and flies 5% of the total, while Bhattacharya *et al.*

(2005) observed the major abundance in the genus *Apis* (honey bee; 71%). Similarly, Malaipan *et al.* (2007) reported that insect pollinators preferred visiting on female flowers (21%) less than male flowers (31%) in March, but in October, they visited female flowers (2.2-3%) more than male flowers (1.7-2%). Population of insect pollinators in October was high on the east side of plant canopy in the morning and on the west side in the afternoon.

In conclusion, the pollination factors are important occurrence for the physic nut flowers, these are unisexual (monoecious), female and male separate each flower on the same inflorescence (Jones and Miller, 1992; Gurcharan, 2004). The mechanism that balances the promotion of out-crossing and minimizes self-pollination is poorly understood (Achten *et al.*, 2010). Chang-wei *et al.* (2007a) categorize physic nut as an out-crosser that is self-compatible and needs pollinators. Indeed, the insect pollinators play an essential role in physic nut as carriers of the pollen grains from male flowers to the stigma on female flowers. This is a successful pollination and seed or fruit can be collected for biodiesel production.

## 1.8 Yield

### 1.8.1 Cultivation factors

Propagation of physic nut is direct seeding, pre-cultivation of seedlings (nursery raising), transplanting of spontaneous wild plants, and direct planting of cuttings. Wider spacing (3x3m) was reported to give larger yields of fruit, at least in early years (Heller, 1996). Correspondingly, Kobilke (1989) in Cape Verde, conducted comparative research on the influence of different propagation methods on survival and vegetative development. In better rainfall or good irrigation the plantation could also be established by direct seeding. The survival rate depended not only on sowing time and depth of sowing, but also on the trial year. When establishing a physic nut crop, the survival rate can be influenced by the choice of cultivation method. Likewise, Hartmann and Kester (1983) stated that two factors were generally responsible for sprouting: the age of the plant from which cuttings

were taken and the position of the cutting within the plant, it might had been due to declines of rooting ability of many woody plants with age, when the source was a seedling derived mother plant. Distal portion of the stock plants were the first to exhibit this reduced rooting potential, while cuttings from the lower or juvenile regions of the plants generally maintain a higher rooting capacity than those from the upper regions. Similarly, Kamphaengkaew and Chinawong (2007) indicated that study on patterns of pruning and nutrition management for increase yield in physic nut in Thailand. Four different patterns of pruning have no different with plant canopy diameter which pattern un-pruned (control), and nutrition management with five different patterns had no effect with plant height, plant canopy diameter, stem diameter and yield.

Abdelgadir *et al.* (2008) indicated that low number of female flowers, limited branching and inadequate pollination were the major factors that limit seed production and thus oil yield of physic nut. Likewise, Jongschaap (2007) suggested that flowering was one of the most important crop stages for physic nut oil production, as the number of female flowers and their fertilization determine how many fruits and seeds eventually will develop. Flowering normally starts after a dry and dormant period and was induced and continued by prolonged periods of soil water availability, either by precipitation or irrigation. Nutrient limitation seems to provoke the end of flowering (Aker, 1997)

### 1.8.2 Fruit setting

Dhillon *et al.* (2006) indicated that 32.9% fruit setting under selfing and 89.7% under natural pollination in physic nut. The high fruit setting under open pollination revealed that the plant is capable of producing fruits through self and cross-pollination. Such a breeding system represents facultative cross-pollination. Bhattacharya *et al.* (2005) reported that 50% of female flowers set fruit with 53% fecundity rate, 32% apomixis rate and 2:3 (seed:ovule). Chang-wei *et al.* (2007a); Abdelgadir *et al.* (2008) suggested that fruit production could be boosted by manipulating biological processes of pollination and growth.

### 1.8.3 Quality of seed

Dhillon *et al.* (2006) stated that period of fruit development and maturity ranged from 55-61 days from date of first fruit initiation. Seeds of physic nut were harvested at physiological maturity, which color of fruits turn yellow was proof to be the best seed germination and seedling vigor comparing to the seeds of immature green fruits and dark brown fruits, which passed through timing of physiological maturity (Tongpumnug *et al.*, 2007).

KU Biodiesel (2010) in Thailand reported various yield of physic nut in 1-3 years that showed seeds product 200-700 kg/rai/year. The Committee on Development of Biofuel (2003) in India stated that physic nut gave 400-12,000 kg/ha/year (64-1,920 kg/rai/year). Kumar and Sharma (2008) reported that the different countries and regions the seed yields of physic nut might range from 100-15,000 kg/ha/year (16-2,400 kg/rai/year). Similarly, Makkar *et al.* (1997) reported that large variations in contents of crude protein, crude fat, neutral detergent fiber and ash on 18 different provenances of physic nut from countries in the West and East Africa, America and Asia. Wani *et al.* (2006) recorded variation in India accessions for oil content (27.8-38.4%) and seed weight (0.44-0.77 g). Kaushik *et al.* (2007) explored the variability in Haryana-India accessions to find wide variation in seed weight (0.49-0.69 g) and oil content (28-39%). Rao *et al.* (2008) found wide variation in seed weight (0.57-0.79 g) and oil content (30-37%) for Andhra Pradesh, India, accessions. Likewise, Pramanik, (2003) indicated that the oil content of physic nut seed ranges from 30-50% by weight and the kernel itself ranges from 45-60%. Srinophakun *et al.* (2011) reported that oil content was about 35% in the seed or about 50% in the kernel of physic nut from Thailand plantation.

## 2. Insect visitors

### 2.1 Diversity of insect pollinators

Although more than 750,000 insect species have been described (Grimaldi and Engel, 2005), possibly as many as 30 millions were await discovery and formal description. Insects comprise the most diverse assemblage of terrestrial animals, including within their ranks some of the most economically important pollinators and the dominant pollinators in a variety of natural systems. In some communities, insects pollinate as many as 93% of the flowering plants (Committee on the Status of Pollinators in North America, 2007).

#### 2.1.1 Order Coleoptera (beetles)

With about 350,000 described species, Coleoptera (beetles) constitute the largest insect order (Grimaldi and Engel, 2005). Beetles often are ignored as pollinators because both the larvae and adults of so many species destroy the reproductive organs of wild and domesticated seed plants. However, beetle pollination is believed to have contributed to the pollination of different lineages of flowering plants for over 120 million years (Bernhardt, 2000). In the United States and Canada, beetle pollination is economically important in the context of significant numbers of annual and bulbous ornamental flowers-garden favorites on hybrids such as poppy anemone, orange buttercup, red tulip, and corn poppy (C.S.P.N.A., 2007).

Some beetles are important pollinator for oil palm. The most effective are *Elaeidobius kamerunicus* and *E. plagiatus*. Declining populations of pollinating insects in the palm oil plantation is often the cause of the low yields observed in the production of seeds (Syed, 1979; Yalamoussa *et al.*, 2011). In additional, five families were observed on physic nut flowers, including Coccinellidae (*Illeis indica* and *Coccinella* spp.), Curculionidae (*Hypomeses squamosus*, and *Hypomeses* spp.), Elateridae (*Fornax* spp.), Lycidae (*Calochromus* sp.), and Scarabaeidae (*Glycyphana* spp.) (Malaipan *et al.*, 2007).

### 2.1.2 Order Diptera (flies)

The true flies (order Diptera) are among the most diverse of the insects, with more than 150,000 species described (Thompson, 2006). Flies are ancient the oldest fossils are known to have come from the Permian (250 million years ago), and flies might have been the first pollinators. The group, however, underwent its greatest diversification along with flowering plants from the late Cretaceous onward. Higher flies (suborder Cyclorrhapha) are the result of this radiation and now account for about two-thirds of the extant Diptera (Grimaldi and Engel, 2005). Extant flies are classified into 153 families and 148,416 species, with 124 families and 24,219 species found in Canada, Mexico, and the continental United States (Thompson, 2006). The total number of identified Diptera species and the estimated number of Diptera species, that are high diversity pollinators, such as 2,005 species in the family Tachinidae, 1,459 species in the family Dolichopodidae, 1,286 species in the family Asilidae, 1,095 species in the family Bombyliidae 1,001 species in the family Syrphidae, and 124 species in the family Calliphoridae (C.S.P.N.A., 2007). The pollinators on physic nut flowers in Diptera were Calliphoridae (*Chrysomya megacephala*), Sarcophagidae (*Sarcophaga* spp.), and Syrphidae (*Eristalis arvorum*, *Helophilus bengaliensis*, *Rhingia* spp.) (Malaipan *et al.*, 2007)

### 2.1.3 Order Hemiptera (true bugs)

Only a few Hemiptera of value are Anthocoridae (minute pirate bugs), Phymatidae (ambush bugs) and Reduviidae (kissing bugs). The Anthocoridae prey on thrips in flowers; a few Reduviidae prey on bees in flowers and most Phymatidae prey on bees and flies in flowers. Anthocoridae are found in almost any flowers that are visited by thrips. Phymartids and reduvids are found primarily on Compositae and flowers that are grouped into tight heads (Anonymous, 2012). Malaipan *et al.* (2007) recorded that visitors in Coreidae and Scutelleridae (*Callidea* sp.) were found on physic nut flowers.

#### 2.1.4 Order Lepidoptera (butterflies and moths)

With approximately 150,000 species described worldwide Lepidoptera (Grimaldi and Engel, 2005), Lepidoptera are among the most species-rich orders of insects, as measured by formally described and documented species. The Lepidoptera include many of the most easily recognized insects, and they are relevant to understanding pollination systems and their origins. Many flowering plants rely primarily on butterflies or moths, most Lepidoptera visit a wide variety of nectar sources. Although lepidopteran pollinators largely are generalists and often transfer only small amounts of pollen, they tend to move longer distances to visit flowers of the same species than do other pollinating insects, such as bees, and thus are important in maintaining gene flow within and among populations (Herrera, 1987, 2000). The monarch butterflies, *Danaus plexippus*, pollinates many milkweeds and other plant species. It has been the subject of intense interest, in part because of its dramatic ecology (sequestering cardenilides from asclepiaceous host plants and serving as the central model in an extensive mimicry system) and migratory behavior (Halpern, 2001). A variety of moths are known to be pollinators, the families Geometridae, Noctuidae, and Sphingidae are among the best studied. The Sphingidae and the highly diverse Noctuidae are among the most efficient of the lepidopteran pollinators. Migratory noctuids feed on the floral nectar of many species of plants (C.S.P.N.A., 2007). Geometrids have been recorded as pollinators of orchids among other species (Thien and Utech, 1970). In addition, yuccas have a very specialized, mutualistic pollination system, being pollinated by yucca moths (*Tegeticula maculata*: Prodoxidae). Insect purposefully transfers the pollen from the stamens of one plant to the stigma of another, and at the same time lays an egg in the flower; the moth larva then feeds on some of the developing seeds, always leaving enough seed to perpetuate the species (Adrienne *et al.*, 1985). Visitors on physic nut flowers were found in six families, such as Arctiidae (*Syntomis divisia*), Danaidae (*D. chrysippus chrysippus*, and *D. genutia genutia*), Hesperidae (*Pithauria marsena*), Papilionidae (*Papilio memnon agenor*, and *Papilio* spp.), Pieridae (*Derias descombesi descombesi*), and Yponomeutidae (*Ateva* sp.) (Malaipan *et al.*, 2007).

### 2.1.5 Order Thysanoptera (thrips)

The thrips (Thysanoptera) are slender, small insects arranged into nine families of living species distributed worldwide, largely in the tropics and temperate regions, with a few species in Arctic regions (Mound, 1997). As minor pollinators, thrips also pollinate such agricultural plants as beets, beans, onions, and cacao (Kirk, 1997). Although thrips can pollinate plants in the absence of other pollinators, their importance in open-pollinated crops depends on whether other insects pollinate the flowers first (Kirk, 1997). Thrips can enter unopened buds, but the peak number of thrips can occur after peak visits by other insects. The grooming behavior of thrips contributes to both self- and cross-pollination in plants. As thrips arrange the fringe hairs before and after flight, pollen grains are shed from their bodies. The stigma is prominent in many flowers and because it is used by thrips for take-off and landing, the pollinator thus places pollen directly on the stigma (C.S.P.N.A., 2007).

Thrips pollination would be important in many plants, especially those of the Ericaceae family. The most abundant species were *Ceratothrips ericae*, previously cited as a potential pollinator of many Ericaceae in northern Europe, and *Haplothrips setiger*, a widely distributed Mediterranean Phlaeothripidae commonly cited in flowers of Asteraceae (Patricio and Goldarazena, 2008). Likewise, Scott (1984) reported that thrips, in particular *Thrips obscuratus*, were collected from the flowers of 13 species of trees common in the lowland forest of New Zealand. Many New Zealand trees have flowers which are small, shallow, clustered, and lack bright colors. This unspecialized floral form is suitable for pollination by small insects, such as flies and thrips. *T. obscuratus* is among the most frequent visitors to the flowers of some trees. The pollen loads of the thrips and the breeding system of the plants indicate thrips may function as effective pollinators. Thrips on physic nut flowers were found in previous surveys by Raju and Ezradanam (2002) and Malaipan *et al.* (2007). Raju and Ezradanam (2002) reported two species in Thripidae such as *Scirothrips dorsalis* and *T. hawaiiensis*.

### 2.1.6 Order Hymenoptera (wasps, ants, and bees)

The order Hymenoptera is diverse and economically important group of approximately 125,000 described species comprising plant-feeding sawflies, parasitic and non-parasitic wasps, ants, and bees (C.S.P.N.A., 2007). The order includes within its ranks the principal managed pollinators of the world.

1) Most of the approximately 100,000 described wasp species worldwide are carnivorous, preying on other insects. Many others are parasites, laying their eggs on or inside immature insects or other living hosts. Many adults of sphecids visit flowers to search for nectar might serve as pollinators. Similarly, some species within the large spider wasp family Pompilidae seek out flowers for nectar. Their spiny legs pick up and transport the specialized pollinia of milkweed plants (*Asclepias* spp.) and they can be effective pollinators (Kephart and Theiss, 2003). One group of large wasps that has adopted a flowers-visiting, pollen-collecting, larval-provisioning lifestyle includes the subfamily Masarinae within the family Vespidae (C.S.P.N.A., 2007). Fig wasps in family Agaonidae are the sole pollinators of fig trees and in turn, fig wasps can breed nowhere else but inside figs, a relationship that is a classic example of an 'obligate mutualism' where neither party can survive without the other, and which has evolved over ninety million years. Each species of fig tree is usually pollinated by one fig wasp species that is only associated with that fig species, a host-specific relationship that plays a major role in the prevention of hybridization between different species of fig trees. However, only 300 of a potential 750 fig wasp pollinator species are currently known (there are 750 species of *Ficus* in the world) (Simon, 2004). Species list of fig wasps in Kasetsart University, Bangkhen Campus, Bangkok, showed that there were 17 genera 29 species in *Ficus benjamina* *F. microcarpa*, and *F. concinna*, as follows: *Acophila* sp.1, Agaonid, *Aprostocetus* sp.1, *Camarothorax* sp.1, *Eupristina koningsbergeri*, *Eupristina verticillata*, *Eurytoma* sp.1, *Micranisa* sp.1, *Odontofroggatia corneri*, *Odontofroggatia galili*, *Ormyrus* spp. (2 spp.), *Otitesella* sp.1, *Philotrypesis* spp. (3 spp.), *Platyscapa* sp.1, *Sycobia* sp.1, *Sycophila* spp. (5 spp.), *Sycoscapter* spp. (3 spp.), Torymid, *Walkerella temeraria*, *W. kurandensis*, *Walkerella* sp.1. The fig

wasps in family Eulophidae, genus *Aprostocetus*, were first found in *Ficus* fruits in this study which had no report elsewhere (Sangtongpraow, 2007).

2) All 11,844 named ant species are eusocial, ecologically important insects. Common in most plant communities, ants visit plants and collect sugars. Interactions with flowering plants involve mutualisms that include dispersing seeds, visiting extra-floral nectarines, serving as guards against seed predators or herbivores, and in some cases pollinating flowers. Although ants are collectively involved in thousands pollinate few species (about 20) of angiosperms. Nevertheless, many ant-plant mutualisms other than pollination greatly increase the reproductive success of plants and ants (C.S.P.N.A., 2007). Jones (2012) stated that *Iridomyrmex* sp., *Meranoplus* sp., and *Rhytidoponera tasmaniensis* were observed feeding on the abundant nectar secreted by the labellum of *Microtis parviflora* (Orchidaceae). All three ant species effectively pollinate the *Microtis* blossoms, but *Iridomyrmex* sp. is the most frequent pollinia-bearing visitor.

3) Nearly 17,000 species of bees in superfamily Apoidea have been formally described, and as many as 30,000 are estimated worldwide (Michener, 2000). Although other species are often more efficient pollinators than are honey bees on a flower-by-flower basis, honey bees are for many reasons, the pollinator of choice for most North American crops. *Apis mellifera* is highly suitable as a commercial pollinator because of its biology (Hoopingarner and Waller, 1992; Winston, 1987). Honey bees' behavior increases the efficiency of pollination by ensuring that compatible pollen is transferred among conspecific flowers when needed.

## 2.2 Insect pollinators in superfamily Apoidea

### 2.2.1 Classification of the Bee-Sphecoid Clade

The placement of sphecoid wasps and bees in the same superfamily has led authors such as Gauld and Bolton (1988); Melo and Gonçalves (2005) to

recognize only two major families in the superfamily, Sphecidae and Apidae, thus correcting the inconsistent classificatory treatment of the sphecoid wasps and the bees. Both the Sphecidae and the Apidae in this sense seem diverse; subjectively, they seem to contain groups at least as different from one another as the families of Chalcidoidea. Moreover, the Sphecidae—in the sense of sphecoid wasps or Spheciformes—is paraphyletic. A better idea, therefore, is to divide both sphecoid wasps and bees into several families. Lomholdt (1982) divided the sphecoid wasps into Sphecidae and Larridae (=Crabronidae), as noted above. This eliminates the paraphyly of the Sphecidae. Several families of bees are long established and already have subordinate taxa considered as subfamilies, tribes, etc., however, have in general reduced bees, or Apiformes bee families to subfamilies of Apidae, many subfamilies to tribes, and various tribes to subtribes.

Because a family-group name based on the generic name *Apis* antedates a name based on *Sphex* (Michener, 1986), the name of the bee-sphécoid superfamily should be Apoidea, not Sphecoidea as most previous works have had it. This is not unreasonable, since the bees are a much larger group than the sphécoid wasps. The Apoidea in this sense is divided into families, some of which (Sphecidae, Crabronidae, etc.) are wasps and constitute the informal paraphyletic group or Series Spheciformes of Brothers (1975), while the others (Colletidae, Apidae, etc.) are bees, the Series Apiformes of Brothers and the Anthophila of some older authors. This classification is summarized in Table 2.

1943

**Table 2** Classification of the superfamily Apoidea

| Families                                    | Series                             |
|---|------------------------------------|
| Family Ampulicidae                          | sphecoid wasps,<br>or Spheciformes |
| Family Sphecidae                            |                                    |
| Family Crabronidae<br>(could be subdivided) |                                    |
| Family Stenotritidae                        | bees,<br>or Apiformes              |
| Family Colletidae                           |                                    |
| Family Andrenidae                           |                                    |
| Family Halictidae                           |                                    |
| Family Melittidae                           |                                    |
| Family Megachilidae                         |                                    |
| Family Apidae                               |                                    |

**Source:** Michener (2007)

### 2.2.2 Bees taxa

A classification for the bees of the world is summarized in Table 3. Some principal features of this classification were the placement of *Melitturgain* the Panurginae, of the Nomiinae and Dufoureae in the Halictidae, of *Chilicola* in the Colletidae, and of *Oxaea* in the Andrenidae. Note also the enlarged Melittidae and the recognition of the Lithurginae as a distinct subfamily of the Megachilidae. The broad Apidae including all long-tongued bees except the Megachilidae, was also novel. The enormous number of tribes in the Anthophorinae had not been anticipated by previous general bee classifications, although most of the tribes had been named before 1944. The tribe Caupolicanini was transferred to the Diphaglossinae. The subfamily Euerbstiinae was added to the Andrenidae. The estimate, about 20,000 species of Apoidea worldwide are arranged into 7 families, 21 subfamilies, 57 Tribes and 425 genera. The most important genera are *Apis* and *Trigona*. *Apis* consist of about 11 species (Michener, 2007) and are confined to the Neotropical region. *Trigona* is the largest genus and is the most widely distributed with about 130 species (Heard, 1999).

#### Scientific classification

Class: Insecta

Order: Hymenoptera

Superfamily: Apoidea

**Table 3** The bee taxa

| Families                              | Subfamilies                      | Tribes                                       |  |
|---------------------------------------|----------------------------------|--|--|
| Stenotritidae ( <i>Stenotritus</i> )  |                                  |  |  |
| Colletidae                            | Colletinae                       | Colletini ( <i>Colletes</i> )                |  |
|                                       |                                  | Paracolletini ( <i>Paracolletes</i> )        |  |
|                                       | Diphaglossinae                   | Caupolicanini ( <i>Caupolicana</i> )         |  |
|                                       |                                  | Diphaglossini ( <i>Diphaglossa</i> )         |  |
|                                       |                                  | Dissoglottini ( <i>Mydrosoma</i> )           |  |
|                                       |                                  | Xeromelissinae                               |  |
|                                       | Xeromelissinae                   | Chilicolini ( <i>Chilicola</i> )             |  |
|                                       |                                  | Xeromelissini ( <i>Xeromelissa</i> )         |  |
|                                       | Andrenidae                       | Hylaeinae ( <i>Hylaeus</i> )                 |  |
|                                       |                                  | Euryglossinae ( <i>Euryglossa</i> )          |  |
| Alocandreninae ( <i>Alocandrena</i> ) |                                  |  |  |
| Andreninae ( <i>Andrena</i> )         |                                  | Protandrenini ( <i>Protandrena</i> )         |  |
|                                       |                                  | Panurgini ( <i>Panurgus, Protandrena</i> )   |  |
| Panurginae                            |                                  | Melitturgini ( <i>Melitturga</i> )           |  |
|                                       |                                  | Protomelitturgini ( <i>Protomelitturga</i> ) |  |
|                                       |                                  | Perditini ( <i>Perdita</i> )                 |  |
|                                       |                                  | Calliopsini ( <i>Calliopsis</i> )            |  |
| Halictidae                            |                                  | Oxaeinae ( <i>Oxaea</i> )                    |  |
|                                       | Rophitinae ( <i>Rophites</i> )   |  |  |
|                                       | Nomioidinae ( <i>Nomioides</i> ) |  |  |
|                                       | Nomiinae ( <i>Nomia</i> )        |  |  |
|                                       | Halictinae                       | Halictini ( <i>Halictus</i> )                |  |
| Augochlorini ( <i>Augochlora</i> )    |                                  |  |  |
| Melittidae                            | Dasypodainae                     | Dasypodaini ( <i>Dasypoda</i> )              |  |
|                                       |                                  | Promelittini ( <i>Promelitta</i> )           |  |
|                                       |                                  | Sambini ( <i>Samba</i> )                     |  |

**Table 3** (Continued)

| Families                            | Subfamilies                                | Tribes  |             |  |
|-------------------------------------|--|---|-------------|--|
| Melittidae                          | Meganomiinae ( <i>Meganomia</i> )          |   |             |  |
|                                     | Melittinae ( <i>Melitta</i> )              |   |             |  |
| Megachilidae                        | Fideliinae                                 | Pararhophitini ( <i>Pararhophites</i> )                               |             |  |
|                                     |  | Fideliini ( <i>Fidelia</i> )  |             |  |
|                                     | Megachilinae                               | Lithurgini ( <i>Lithurgus</i> )                                       |             |  |
|                                     |  | Osmiini ( <i>Osmia</i> )  |             |  |
|                                     |  | Anthidiini ( <i>Anthidium</i> , <i>Stelis</i> )                       |             |  |
|                                     |  | Dioxyini ( <i>Dioxys</i> )  |             |  |
|                                     |  | Megachilini ( <i>Coelioxys</i> , <i>Heriades</i> , <i>Megachile</i> ) |             |  |
|                                     |  | Apidae  | Xylocopinae | Manuelini ( <i>Manuelia</i> )                    |
|                                     |  |   |             | Xylocopini ( <i>Xylocopa</i> )                   |
|                                     |  |   |             | Ceratinini ( <i>Ceratina</i> , <i>Exoneura</i> ) |
| Nomadinae                           | Allodapini ( <i>Allodape</i> )             |   |             |  |
|                                     | Hexepeolini ( <i>Hexepeolus</i> )          |   |             |  |
|                                     | Brachynomadini ( <i>Brachynomada</i> )     |   |             |  |
|                                     | Nomadini ( <i>Nomada</i> )                 |   |             |  |
|                                     | Epeolini ( <i>Epeolus</i> )                |   |             |  |
|                                     | Ammobatoidini ( <i>Ammobatooides</i> )     |   |             |  |
|                                     | Biastini ( <i>Biastes</i> )                |   |             |  |
| Apinae                              | Townsendiellini ( <i>Townsendiella</i> )   |   |             |  |
|                                     | Neolarrini ( <i>Neolarra</i> )             |   |             |  |
|                                     | Ammobatini ( <i>Ammobates</i> )            |   |             |  |
|                                     | Caenoprosopidini ( <i>Caenoprosopina</i> ) |   |             |  |
|                                     | Isepeolini ( <i>Isepeolus</i> )            |   |             |  |
|                                     | Osirini ( <i>Osiris</i> )                  |   |             |  |
|                                     | Protepeolini ( <i>Leiopodus</i> )          |   |             |  |
| Exomalopsini ( <i>Exomalopsis</i> ) |  |   |             |  |

**Table 3** (Continued)

| Families | Subfamilies | Tribe   |
|----------|-------------|---|
| Apidae   | Apinae      | Ancylini ( <i>Ancyla</i> )<br>Tapinotaspidini ( <i>Tapinotaspoides</i> )<br>Tetrapediini ( <i>Tetrapedia</i> )<br>Ctenoplectrini ( <i>Ctenoplectra</i> )<br>Emphorini ( <i>Melitoma</i> , <i>Ptilothrix</i> )<br>Eucerini ( <i>Eucera</i> )<br>Anthophorini ( <i>Anthophora</i> )<br>Centridini ( <i>Centris</i> )<br>Rhathymini ( <i>Rhathymus</i> )<br>Ericrocini ( <i>Ctenioschelus</i> , <i>Ericrocis</i> ,<br><i>Mesoplia</i> )<br>Melectini ( <i>Melecta</i> )<br>Euglossini ( <i>Aglae</i> , <i>Euglossa</i> ,<br><i>Eulaema</i> , <i>Exaerete</i> )<br>Bombini ( <i>Bombus</i> , <i>Psithyrus</i> )<br>Meliponini ( <i>Austroplebeia</i> ,<br><i>Cephalotrigona</i> , <i>Dactylurina</i> ,<br><i>Hypotrigona</i> , <i>Lestrimelitta</i> ,<br><i>Lisotrigona</i> , <i>Melipona</i> , <i>Meliponula</i> ,<br><i>Meliwillea</i> , <i>Nannotrigona</i> ,<br><i>Nogueirapis</i> , <i>Oxytrigona</i> ,<br><i>Paratrigona</i> , <i>Pariotrigona</i> ,<br><i>Partamona</i> , <i>Plebeia</i> , <i>Plebeina</i> ,<br><i>Scaptotrigona</i> , <i>Trichotrigona</i> ,<br><i>Trigona</i> , and <i>Trigonisca</i> )<br>Apini ( <i>Apis</i> ) |

**Source:** Michener (2007)

### 2.2.3 Morphology of Apoidea

Bees are usually robust and pilose, although most parasitic forms and a few nonparasitic genera are relatively bare and slender like many sphecoid or vespoid wasps. The presence of pollen-transporting scopae or corbiculae on the posterior tibiae, or scopae on other parts of the body, is characteristic of females of most non-parasitic forms. *Hylaeus* (Colletidae) and *Lestrimelitta* (Apidae) are exceptions in that they carry pollen in the honey stomach. Bees can often be distinguished from otherwise similar appearing sphecoids by most absence of metallic body and fascial hairs; and *Eulonchopria* fold their wings in vespid fashion.

The most diagnostic feature of bees is the presence of at least slightly plumose hairs on various parts of the body. The most consistent areas to examine are near the thoracic spiracle and sides of the propodeum. Sometimes high magnification (70x or higher) is required to see plumosity. Broadened posterior tibiae and metatarsi are obvious characteristics of the genera that transport pollen. Even the genera that carry it elsewhere usually have broader tibiae and metatarsi than are found on wasps. (Stephen *et al.*, 1969)

Bees of many genera can be identified to genus at a glance, or at least very promptly, by a person familiar with the bees of the relevant region. This is possible in part because of the diverse body shapes of bees. Michener (2000) have indicated general body shape in the text by a series of terms such that with a single word a person who knows a few common genera of bees can get an idea of what bees of an otherwise unknown genus look like. These terms, are listed below, arranged in a general way from slender and relatively hairless to robust and hairy. References to the colored plates, as well as to the habitus drawings and photographs, are offered so that the reader might gain a better idea of the meanings of these terms. The terms are subjective, however, and can be used only to give a general idea of body form and often hairiness. (Terms marked by asterisks apply primarily to megachilids and therefore indicate the large-headed megachilid body form.). The bees included thirteen body forms (Figure 1).

1) Hylaeiform. Body form of *Hylaeus*, also suggestive of a pemphredonine wasp. Slender, the hairs inconspicuous without magnification; scopa inconspicuous or absent.

2) Nomadiform. Body form of *Nomada*. Slender, wasplike, not noticeably hairy, often with yellow or red markings; scopa absent.

3) Epeoliform. Body form of *Epeolus* or *Doeringiella*. Somewhat more robust than *Nomada* but nonetheless wasplike parasitic bees; scopa absent. Body often with areas of short, pale pubescence forming a conspicuous.

4) Andreniform of *Andrena*, *Halictus*, or *Colletes*. Male often slender, its metasoma more parallel-sided than that of female.

5) Heriadiform. See hoplitiform.

6) Hoplitiform. Body form of *Hoplitis* (*Alcidamea*), *Heriades*, or more slender species of *Megachile* (*Callomegachile*). Similar to megachiliform but more slender, metasoma parallel-sided. The term heriadiform has been used for this body form, but implies greater slenderness.

7) Chalicodomiform. Between hoplitiform and megachiliform.

8) Megachiliform. Body form of *Megachile* or *Anthidium*. Body heavy, head thick, metasoma rather wide, not parallel-sided.

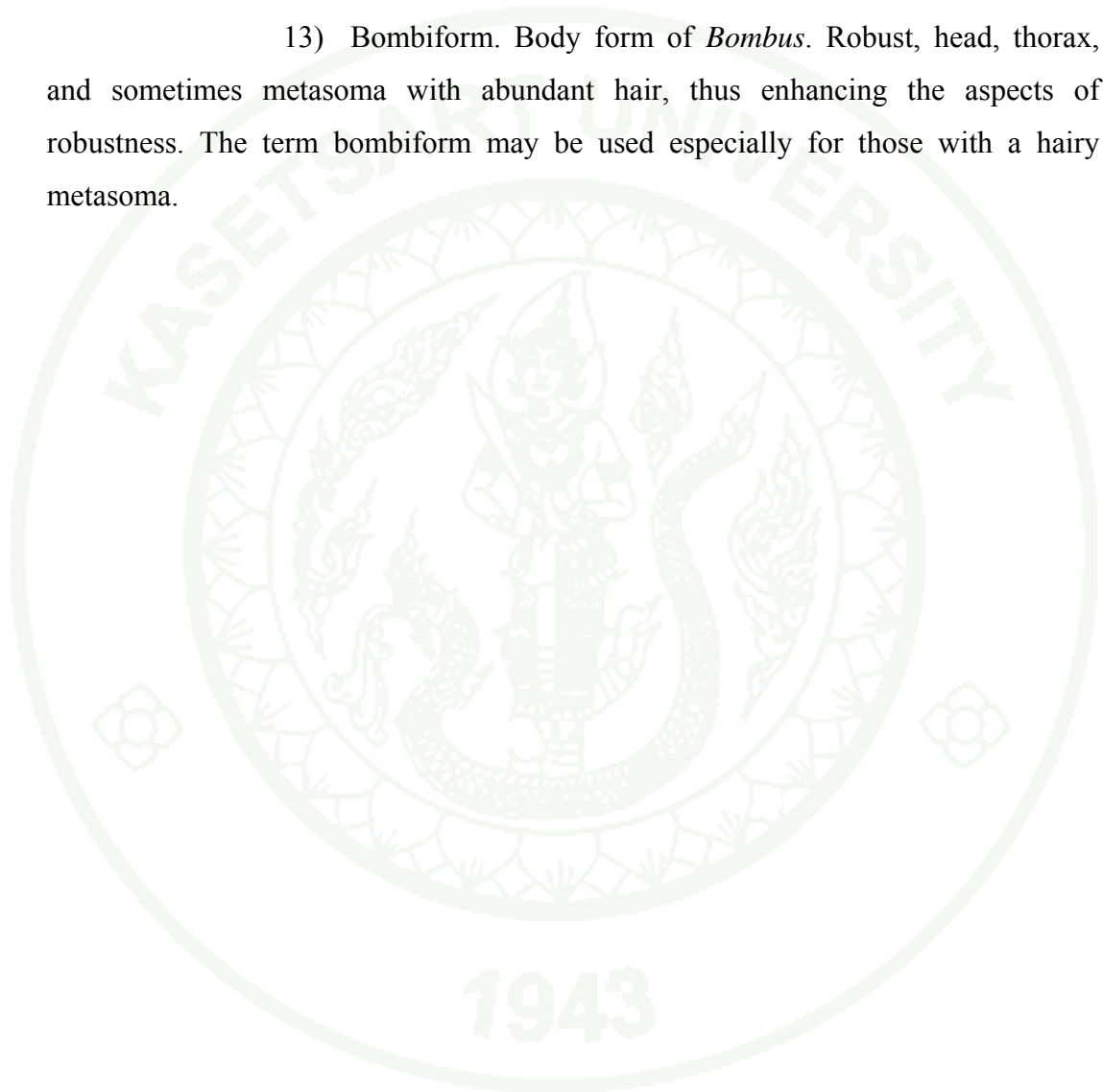
9) Trigoniform. Body form of *Trigona* and its relatives, e.g., of the genus *Partamona*. Metasoma small and robust to slender and parallel-sided; body not conspicuously hairy, i.e., hairs short, and metasoma usually shiny.

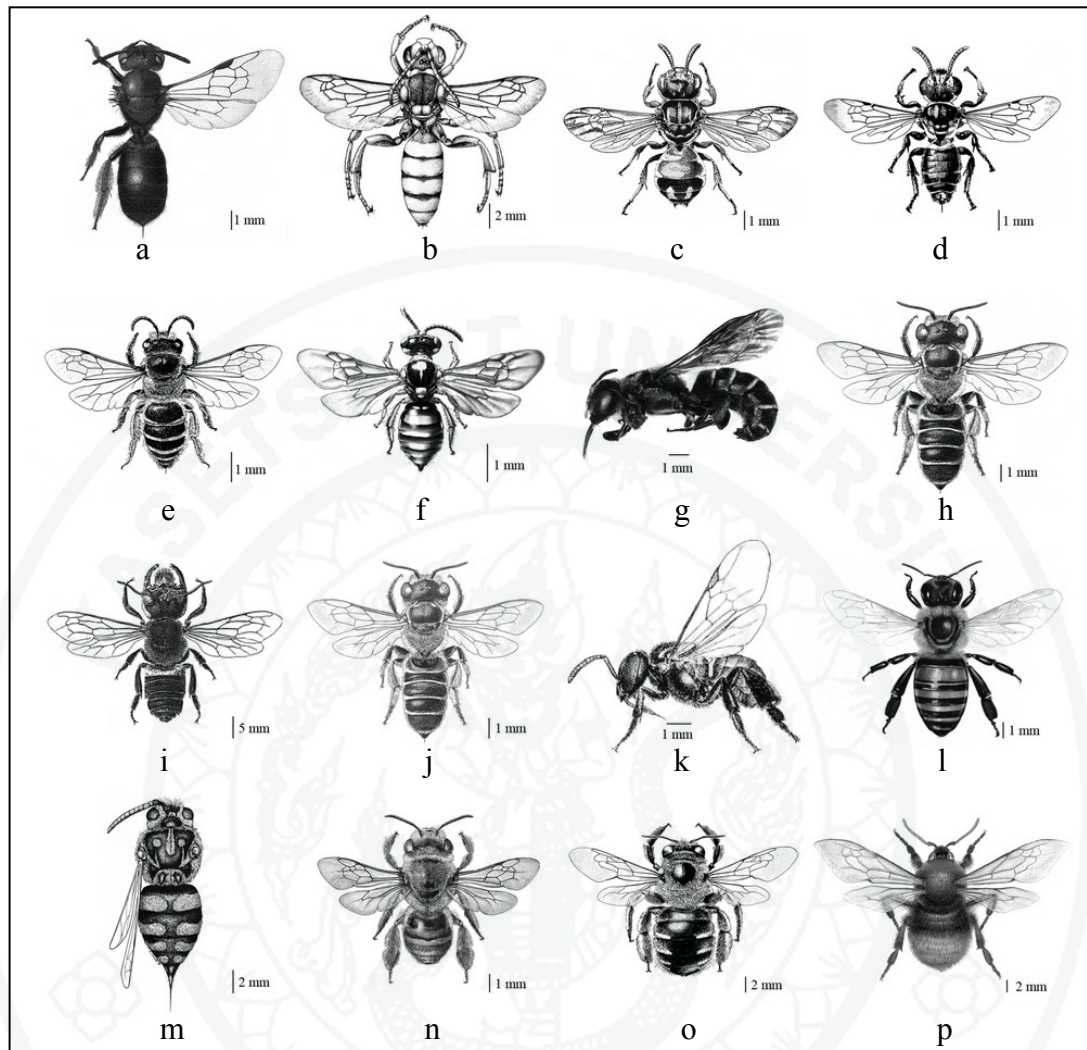
10) Apiform. Body form of workers of *Apis mellifera*, i.e., more robust than andreniform and more slender than euceriform.

11) Euceriform. Body form of *Eucera* or *Melissodes*. Similar to anthophoriform but somewhat less robust.

12) Anthophoriform. Body form of *Anthophora* or *Amegilla*.

13) Bombiform. Body form of *Bombus*. Robust, head, thorax, and sometimes metasoma with abundant hair, thus enhancing the aspects of robustness. The term bombiform may be used especially for those with a hairy metasoma.





**Figure 1** Bee forms of Apoidea

a = Hylaeiform, b = Nomadiform, c,d = Epeoliform,  
 e,f = Andreniform, g = Heriadiform, h = Hoplitiform, I= Chalicodomiform,  
 j = Megachiliform, k = Trigoniform, l = Apiform, m,n = Euceriform,  
 o = Anthophoriform, p = Bombiform.

**Source:** a-j and m-o: Michener (2007); k-p: Bakun (2011)

#### 2.2.4 Distribution

The present distribution of bee taxa depends on the climatic and vegetational factors and intercontinental and other barriers and the Tertiary and probably late Cretaceous continental movements. But present distribution also depends on bees' ability to disperse and to reach suitable areas under their own power. Because bees can fly well, one might think that they would be rather successful at crossing barriers, such as water or areas that are climatically or vegetationally inhospitable. The distribution of the various groups of bee (tribes to families) are indicated in Table 4 which is a modified version of a table presented by Michener (1979). The following is an explanation of the columns.

1) Aust. Australia, including Tasmania, New Guinea, the Bismarck Archipelago, and nearby islands such as the Solomons.

2) NZ. New Zealand.

3) Orient. The oriental faunal region, i.e., tropical Asia from Sri Lanka, India and Pakistan below the Himalayas, across southeastern Asia to Vietnam and southeastern China, also Taiwan, the Philippines, and western Indonesia. (Most of China and Japan are in the eastern part of the palearctic region, not in the oriental region. Likewise, the mountainous parts of northern India and of Pakistan and its western area in Baluchistan are palearctic).

4) Madag. Madagascar.

5) Afr. Sub-Saharan Africa (Africa north of the Sahara is palearctic).

6) Palear. The palearctic faunal region, including northern Africa, Turkey and the Middle East, northern India and Pakistan is oriental, the western Baluchistan area is palearctic.

7) Nearct. The nearctic faunal region, including the Mexican plateau and surrounding mountains. Seemingly nearctic areas on the mountains of Chiapas, Mexico, and Guatemala are explained separately in the text but are not included under the term “nearctic” for present purposes.

8) Neotr. The neotropical faunal region from tropical Mexico southward through South America, excluding region 9 and 10.

9) Antill. The Greater and Lesser Antilles, excluding Trinidad which is included in region 8.

10) Arauc. The Araucanian region, i.e., Chile and adjacent part of western and southern Argentina.

Areas (column headings) are explained in the text. Plus signs indicate presence, neither diversity nor abundance necessarily implied; “m” indicates marginal presence, i.e., the taxon enters the area only marginally, or one or a few of its species extend well into the area but not halfway across it. The second symbols in some of the notations are subjective indications of relative diversity of the taxon in those areas, “+” indicating more diverse, “-” less diverse, than indicated for the taxon by a simple “+” without a second symbol. Introductions by human agency are ignored.

**Table 4** Summary of distribution of families (-dae), subfamilies (-nae), and tribes (-ini) of bees

| Taxon             | Aust. | Nz. | Orient. | Madag. | Afr. | Palear. | Nearct. | Neotr. | Antill. | Arauc. |
|-------------------|-------|-----|---------|--------|------|---------|---------|--------|---------|--------|
| STENOTRITIDAE     | +     | --  | --      | --     | --   | --      | --      | --     | --      | --     |
| COLLETIDAE        |       |     |         |        |      |         |         |        |         |        |
| Colletinae        | ++    | +-  | m       | --     | +    | +       | +       | ++     | +-      | ++     |
| Caupolicanini     | --    | --  | --      | --     | --   | --      | +       | +      | +       | +      |
| Diphaglossini     | --    | --  | --      | --     | --   | --      | --      | +      | --      | +      |
| Dissoglottini     | ---   | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Chilicolini       | --    | --  | --      | --     | --   | --      | m       | +      | m       | +      |
| Xeromelissini     | --    | --  | --      | --     | --   | --      | --      | --     | --      | +      |
| Hylaeinae         | ++    | +-  | +       | +      | +    | +       | +       | +      | +       | -      |
| Euryglossinae     | +     | --  | --      | --     | --   | --      | --      | --     | --      | --     |
| ANDRENIDAE        |       |     |         |        |      |         |         |        |         |        |
| Alocandreninae    | --    | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Andreninae        | --    | --  | m       | --     | +    | ++      | ++      | +      | --      | +      |
| Protandrenini     | --    | --  | --      | --     | --   | --      | +       | +      | --      | +      |
| Panurgini         | --    | --  | --      | --     | --   | ++      | +       | --     | --      | --     |
| Melitturgini      | --    | --  | --      | --     | +    | +       | --      | --     | --      | --     |
| Protomelitturgini | --    | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Perditini         | --    | --  | --      | --     | --   | --      | +       | m      | m       | --     |
| Calliopsini       | --    | --  | --      | --     | --   | --      | +       | +      | --      | +      |
| Oxaeinae          | --    | --  | --      | --     | --   | --      | m       | +      | --      | --     |
| HALICTIDAE        |       |     |         |        |      |         |         |        |         |        |
| Rophitinae        | --    | --  | m       | --     | +    | ++      | ++      | +-     | --      | +-     |
| Nomiinae          | +     | --  | ++      | +      | ++   | +       | +       | m      | m       | --     |
| Nomioidinae       | +     | --  | +       | +      | +    | +       | --      | --     | --      | --     |
| Halictini         | +     | +-  | +       | +      | +    | +       | +       | +      | +       | +      |
| Augochlorini      | --    | --  | --      | --     | --   | --      | +       | ++     | +       | +      |

**Table 4** (Continued)

| Taxon               | Aust. | Nz. | Orient. | Madag. | Afr. | Palear. | Nearct. | Neotr. | Antill. | Arauc. |
|---------------------|-------|-----|---------|--------|------|---------|---------|--------|---------|--------|
| <b>MELITTIDAE</b>   |       |     |         |        |      |         |         |        |         |        |
| Dasypodini          | --    | --  | m       | --     | +    | +       | +       | --     | --      | --     |
| Promelittini        | --    | --  | --      | --     | --   | +       | --      | --     | --      | --     |
| Sambini             | --    | --  | --      | --     | +    | --      | --      | --     | --      | --     |
| Meganomiinae        | --    | --  | --      | +      | ++   | m       | --      | --     | --      | --     |
| Melittinae          | --    | --  | m       | --     | +    | +       | +       | --     | --      | --     |
| <b>MEGACHILIDAE</b> |       |     |         |        |      |         |         |        |         |        |
| Pararhophitini      | --    | --  | --      | --     | --   | +       | --      | --     | --      | --     |
| Fideliini           | --    | --  | --      | --     | +    | m       | --      | --     | --      | +      |
| Lithurgini          | +     | --  | +       | +      | +    | +       | +       | ++     | +       | +      |
| Osmini              | --    | --  | +/-     | +/-    | +    | ++      | +       | m      | m       | --     |
| Anthidiini          | +/-   | --  | +       | +/-    | +    | +       | +       | +      | --      | +/-    |
| Dioxyini            | --    | --  | m       | --     | +/-  | ++      | +       | --     | --      | --     |
| Megachilini         | +     | --  | +       | +      | +    | +       | +       | +      | +       | +      |
| <b>APIDAE</b>       |       |     |         |        |      |         |         |        |         |        |
| <b>Xylocopinae</b>  |       |     |         |        |      |         |         |        |         |        |
| Manueliini          | --    | --  | --      | --     | --   | --      | --      | --     | --      | +      |
| Xylocopini          | +     | --  | ++      | +      | ++   | +       | +       | ++     | +       | --     |
| Ceratinini          | +     | --  | +       | +/-    | +    | +       | +       | +      | +       | --     |
| Allodapini          | +     | --  | +       | +      | +    | m       | --      | --     | --      | --     |
| <b>Nomadinae</b>    |       |     |         |        |      |         |         |        |         |        |
| Hexepeolini         | --    | --  | --      | --     | --   | --      | +       | --     | --      | --     |
| Brachynomadini      | --    | --  | --      | --     | --   | --      | +       | +      | --      | +      |
| Nomadini            | m     | --  | +       | --     | +    | ++      | ++      | ++     | +       | --     |
| Epeolini            | --    | --  | --      | --     | +    | ++      | ++      | ++     | +       | +      |
| Ammobatoidini       | --    | --  | --      | --     | +    | +       | +       | --     | --      | --     |
| Biastrini           | --    | --  | --      | --     | --   | +       | +       | --     | --      | --     |

**Table 4** (Continued)

| Taxon            | Aust. | Nz. | Orient. | Madag. | Afr. | Palear. | Nearct. | Neotr. | Antill. | Arauc. |
|------------------|-------|-----|---------|--------|------|---------|---------|--------|---------|--------|
| APIDAE           |       |     |         |        |      |         |         |        |         |        |
| Nomadinae        |       |     |         |        |      |         |         |        |         |        |
| Townsendiellini  | --    | --  | --      | --     | --   | --      | +       | --     | --      | --     |
| Ammobatini       | --    | --  | --      | +      | ++   | +       | +       | --     | --      | --     |
| Caenoprosopidini | --    | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Apinae           |       |     |         |        |      |         |         |        |         |        |
| Isepeolini       | --    | --  | --      | --     | --   | --      | --      | +      | --      | +      |
| Osirini          | --    | --  | --      | --     | --   | +-      | +-      | +      | --      | +      |
| Protepeolini     | --    | --  | --      | --     | --   | --      | m       | +      | --      | --     |
| Exomalopsini     | --    | --  | --      | --     | --   | --      | +       | ++     | +       | +      |
| Ancylini         | --    | --  | m       | --     | --   | +       | --      | --     | --      | --     |
| Tapinotaspidini  | --    | --  | --      | --     | --   | --      | --      | ++     | +       | +      |
| Tetrapediini     | --    | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Ctenoplectrini   | m     | --  | +       | --     | +    | m       | --      | --     | --      | --     |
| Emphorini        | --    | --  | --      | --     | --   | --      | +       | ++     | --      | +      |
| Eucerini         | --    | --  | +-      | +-     | +    | +       | +       | +      | +-      | +      |
| Anthophorini     | +     | -   | +       | +      | +    | ++      | ++      | +      | +       | +      |
| Centridini       | --    | --  | --      | --     | --   | --      | +       | ++     | +       | +      |
| Rhathymini       | --    | --  | --      | --     | --   | --      | --      | +      | --      | --     |
| Ericrocidini     | --    | --  | --      | --     | --   | --      | +       | ++     | +       | +      |
| Melectini        | +-    | --  | +       | +-     | +    | ++      | +       | m      | +-      | --     |
| Euglossini       | --    | --  | --      | --     | --   | --      | --      | +      | m       | --     |
| Bombini          | --    | --  | +       | --     | --   | ++      | ++      | +      | --      | +-     |
| Meliponini       | +     | --  | +       | +-     | +    | --      | --      | ++     | m       | --     |
| Apini            | --    | --  | ++      | +      | +    | +       | --      | --     | --      | --     |
| Total higher     |       |     |         |        |      |         |         |        |         |        |
| Taxa present     | 18    | 3   | 26      | 18     | 31   | 36      | 41      | 43     | 24      | 28     |

**Source:** Michener (2007)

### 2.2.5 Social level of Apoidea

Many Apoidea are social to some degree, but some bees not social. Social type of bees can divide to eight levels.

1) Solitary: some females are solitary; they live in colonies. A solitary bee constructs her own nest and provides food for her off spring; she has no help from other bees and usually dies or leaves before the maturation of her offspring (Michener, 2007). The immature offspring of nest-building bees, like the offspring of many vertebrates, are helpless and rely entirely on adult carers to provide them with food. Unlike vertebrates, however, most bees are so-called “mass provisioners” each egg is sealed into a cell containing all the food required for maturation, so that within 1–2 days of oviposition the offspring is nutritionally independent of its mother.

2) Aggregations: many kinds of bees that nest in the ground construct numerous nests in limited areas; a patch of earth, a patch, or an earthen bank may be peppered with their holes. Each burrow may be made and inhabited by one female or may contain some sort of small colony. Some aggregations doubtless results from the availability of local patches of suitable soil, but often the bees choose to aggregate in only part of an extensive area that appears uniform. Sometimes, gregarious behavior seems to be a response to the presence of other bees or bee nest. For example *Andrena vaga* is aggregations, Mohra *et al.* (2004) supported that about 100 small tumuli of soil circa 5 cm in diameter can be seen, each representing the entrance of a single *A. vaga* female’s nest at nesting aggregation.

3) Subsocial: a female feeds and cares for her offspring rather than merely storing food for them, such as *Halictus*, *Lasioglossum*, *Euglossum*, *Allodapini*, *Ceratina*, *Pithitis*, and *Manuella*. A minority of bees exhibit a form of extended parental care known as “progressive provisioning.” In these, the mother provides only a small amount of food, or no food, before the egg hatches, and

provisions each developing larva gradually as it grows, over a period that can range from approximately 7-70 days. Mass provisioners include the vast majority of non social bees (Field, 2005).

4) Parasocial: a non committal umbrella term used for a colony whose member are of a single generation and interact in any of the three ways indicated or in some as yet unrecognized way.

4.1) Communal: two or more females use the same nest, but each makes and provisions her own cells and lays an egg in each of them. In most or all species that have communal colonies, other individuals in the same populations nest alone, and are truly solitary. Thus colonial life is facultative. A possible precursor of communal behavior arises when a nest burrow, abandoned by its original occupant, is then occupied by another bee of the same species. Such behavior is rarely reported, because without marked bees, one does not know of it. A condition that appears to promote communal behavior is very hard soil or other bees in a preexisting nest than to excavate a new nest starting at the surface.

4.2) Quasisocial: a few female occupying a nest cooperate in building and provisioning cells, but different individuals (as opposed to a single queen) lay eggs in cells as they are completed. That is, all the females have functional ovaries, mate, and can lay eggs. This may not be the terminal or most developed social state for any species of bees but at times some colonies exhibit this condition.

4.3) Semisocial: a colony of adults of the same generation usually sisters, some of which are incapable of laying fertilized eggs.

For example the parasocial members are various species of insects, such as *Hylaeus*, *Andrena*, *Pseudagapostemon*, *Ruizantheda*, *Lasioglossum*, *Augochloropsis*, *Augochlorella*, *Euglossa*, *Eulaema*, *Xylocopa*, *Ceratina*, *Pithitis*, *Manuelia*, *Exomalopsis*, *Eucera*, and *Chalicodoma*.

5) Primitive eusocial: most bumble bees (*Bombini*) and many sweat bees (*Halictinae*) and carpenter bees and their relatives (*Xylocopinae*) may live in small colonies, mostly started by single females working as solitary individuals performing all necessary functions of nest construction, foraging, provisioning cells or feeding larvae progressively, and laying eggs. Later, on the emergence of daughters, colonial life may arise, including division of labor between the nest foundress (queen) and workers. Queen and workers are essentially alike morphologically, although often differing in size; they differ more distinctly in physiology and behavior. Such colonies usually break down with production with production of reproductive; thus the colonies are obligately temporary rather than potentially permanent like those of highly social bees.

6) Advanced eusocial: bees are thought of as stinging, honey-producing social insects living in perennial colonies, each of which consists of a queen and her many daughter workers. This is indeed the way of life for the honey bees (genus *Apis*) and the stingless honey bees (*Trigona*, *Melipona*, etc.) of the tropics. Queen and workers in these cases are morphologically very different, and the queen is unable to live alone (e.g., she never forages); nor do workers alone form viable colonies (they cannot mate and therefore cannot produce female offspring). Such bees always live in colonies, and new colonies are established socially, by groups or swarms. Only two tribes, the Apini and the Meliponini (family *Apidae*), consist of such bees.

Bees are the world's most important pollinating agents. Bees are either beneficial or actually essential for the pollination, and therefore for the sexual reproduction, of much of the natural vegetation of the world, as well as for many agricultural crops. The pollinators are primarily female bees, which collect pollen as the principal protein source in their own food and especially to feed their larvae. Flowers produce not only nectar and sometimes oil but also excess pollen as bait or reward. The pollen that may fertilize ovules is that which bees lose inadvertently on floral stigmata as they go about collecting nectar, pollen, or other material. Male bees of nearly all species, as well as the females of parasitic species, take nectar from

flowers but carry only the pollen that happens to stick to them. All species of the Neotropical subtribes Stanhopeinae and Catasetinae (Orchidaceae) are pollinated exclusively by male euglossine bees which are attracted and collect the floral fragrances. The male bees are thought to use the collected floral fragrance compounds in their own reproductive biology, probably as precursors of their own sex pheromones (Norris and Whitten, 1983). They thus play a role in pollination, but a less important one than that of the females, which actively collect pollen and (as workers) in eusocial groups are vastly more numerous than males (Michener, 2000).

### **3. Nature and production of bees (Apoidea) attractants**

#### **3.1 Pollen as attractant**

Faegri and Pijl (1976) indicated pollen was the good reason to consider pollen the original attractant. The functioning as an attractant is generally well exposed and available also to visitors with a primitive organization. Pollen is a rich source of food, especially of proteins. Analyses give 16-30% protein, 1-7% starch, 0-15% sugars, 3-10% fat, and 1-9% ashes. Pollen is eaten directly (chewed) by beetles and primitive lepidoptera and in more indirect manner (digestion by diffusion) by other insects. Insects with brood management—bees, bumble bees—use great quantities of pollen for their larvae. Oligolectic bees have specific pollen preferences, possibly based on odour. The primitive Collectidae, dominant in Australia, seem to be connected with the dominance of Myrtaceae. Bees are unable to recognize the nutritive value of the grains they collect. Chang-wei *et al.* (2007a) reported that the floral syndrome of physic nut related to insect pollination, such as large pollen grains in diameter of 52-89  $\mu\text{m}$ , many verrucae on their exine with adhesion. Therefore, it is certain that the major pollination method of physic nut depended on entomophilous pollination that plays a critical role in its pollen flow.

### 3.2 Nectar as attractant

Nectar is the attractant having the greatest general appeal to all groups of animal—vertebrate and invertebrate—and any available source of nectar is likely to be utilized by any animal that can get at it. Generally the sugar concentration of nectar floral varies between 25-75% with varying proportions of glucose, fructose, and saccharose. The chemoreceptors of pollinating insects are sensitive to sugar, and generally the threshold value is lower than 10% (Faegri and Pijl, 1976).

Both types of physic nut flowers had nectar glands, which can pressurize nectar. The nectar dew in a bagged female flower could cover the ovary. Nectar could absorb ultraviolet rays, which could be sensitively observed by bees during its pollination (Chang-wei *et al.*, 2007a). Correspondingly, Bhattacharya *et al.* (2005) had been observed that the different grades of sucrose had an influence on insect behaviors, flower visits duration, pollen removal and deposition on stigmas by honey bees (*Apis* spp.). Highest number of pollen grains were transported and deposited on stigmas by *A. dorsata* when flowers were treated with 0.9 M sucrose, whereas, it was lowest by *A. florea* in 1.5 M sucrose treated flowers.

### 3.3 Other substances as attractant

A more important general attractant type in Scrophulariaceae, Malpighiaceae, and in *Oncidium* are species that secrete fatty oils from special “glands”, *elaiophors*, in or near the blossoms. The oil is collected by bees of the genus *Centris*. Its utilization is unknown as yet. This throws new light on an old observation of Fritz Muller’s that bees are attracted glands on the outside of the calyx of *Bunchosia quaidichaudiana*, getting dusted with pollen during their work on the glands, and carrying out pollination in the next flower. Calyx glands in many Malpighiaceae are oil glands, and it is of some interest to note the morphologic similarity between perianth members in these flowers and those of oil-producing orchids (Faegri and Pijl, 1976).

### 3.4 Sexual attractant

In many insect groups there is a difference in feeding habits between the sexes. The exclusive visit of one sex to a blossom does not therefore in itself constitute any proof that sexual attraction is involved. The use by male bees of “marcrotic” substances produced in the flowers of various orchids will be dealt with later, but a counterpart of the “pseudo-aggression” of *Centris* referred to above is found in some other orchids (*Brassia* and *Calochilus* spp.). One might call this a pseudo-parasitic attraction, belonging, of course, to the great group of deceptive devices. It is important in the evaluation of these syndromes that sexual attraction also activates for pollination a group of animals, viz. males which because of their weaker feeding instincts are less likely to be attracted by the regular attractants (Faegri and Pijl, 1976).

### 3.5 Odor as attractant

There are reason for believing that odor is an older secondary attractant than are the visual stimuli. Many hive bees and bumble bee being repelled by aminoid and skatoloid odors from fly blossoms and by the fatty acid smell from bat flowers. There is better evidence that hive-bees and also wild bees, like the genera *Euglossa* and *Xylocopa*, “label their empties” by leaving odor traces from the mandibular glands in blossoms visited. These odors marks are of short duration. The repellent effect of flower odors on ants still remains to be investigated more closely (Faegri and Pijl, 1976). The males of all five genera of Euglossinae (Hymenoptera, Apoidea, Apidae, Euglossinae), *Euglossa* (approx. 100 sp.), *Eufriesea* (52 spp.), *Eulaema* (13 spp.), *Exaerete* (5 spp.) and *Aglae* (monotypic) possess specific perfume storage organs on the tibiae of the hind legs. The females of the latter two genera are cleptoparasitic on other Euglossinae (Ackerman, 1989). Both female and male flowers of physic nut emit a slight fragrance, and females were more fragrant than males, which strengthen the attraction to pollinators (Chang-wei *et al.*, 2007a).

### 3.6 Visual attractant

The two means of visual attractions are color and shape—inclusive of size. In its function as an advertising organ, the larger the blossom is, and the more it contrasts with the surroundings, the more effective it will generally be. Generally, the perianth constitutes the advertising organ of the blossom. If it is differentiated, the inner members, the corolla, usually assume that function. Unlike blossoms of anemophilous plants, blossoms with visual advertising effects vary immensely in size (Faegri and Pijl, 1976).

As a rule colors through the range pink-red-violet-blue are due to anthocyanins, yellow-red-purple to carotinoids or flavones. White is due to multiple reflections in the intercellular spaces between uncolored cells, black to similar reflections between layers of complementary colors. Such reflections also contribute to the visibility of blossoms and to the saturation of colors; the appearance of a blossom from the intercellulars of which air has been driven out. Green is not considered a “color” in ecology. Chlorophyll is frequently present before anthesis, but is generally broken down. Very rarely green colors are due to substances other than chlorophyll (Faegri and Pijl, 1976).

Likewise, anthers of the male flowers of physic nut are bright yellow and look conspicuous. In this population, an inflorescence had 184 (88–238) male flowers, blooming 9.94 (0.21-21.57) flowers daily in average to display them more conspicuously, for big blooming it could attract more insects to visit (Chang-wei *et al.*, 2007a).

## 4. Commercial bees

Many insects visit flowers to collect pollen as food. As they do this they pollinate the flowers. Most flowers offer sugary liquid nectar as a reward for these pollinating insects. Bees are especially effective insect pollinators because they collected pollen and nectar almost exclusively, visit many flowers of the same

species during a single trip, and have hairy bodies that easily pick up pollen grains. There is a close association between flowering plants and bees. Bees pollinate over 16% of the world's flowering plants species (Buchmann and Nabhan, 1996) and nearly 400 of its agricultural plants (Crane and Walker, 1984). The pollen-collecting bees play an important role in pollinating plants. Some higher plants are self-pollinating, but a great many are cross-pollinated; that is, the pollen of one flower must be transferred to the stigma of another. The insects-pollinated plants include most orchard fruits, berries, many vegetables (particularly the cucurbits), field crops (such as the clovers, cotton, and tobacco), and flowers. Most pollination is done by bees-often chiefly honey bees (*Apis* spp.) and bumble bees, but a great deal of pollinating is done by solitary bees. Many growers, by bringing in hives of honey bees when the plants are in bloom, have been able to get greatly increased yields of orchard fruits, clover seed, and other crops that depend on bees for pollination (Triplehorn and Johnson, 2005).

Insect pollination is not only a critical ecosystem function but also an essential input in the production of a host of agricultural crops grown world-wide. Of the approximately 300 commercial crops about 84% are insect pollinated. Insects are responsible for 80–85% of all pollinated commercial hectares, with fruits, vegetables, oilseeds, legumes and fodders, representing approximately one-third of global food production, mostly pollinated by *Apis mellifera* L. (honey bees). However, the bulk of the world's staple foods are wind-pollinated, self-pollinated or propagated vegetative, and there is a bias where values ascribed to insect pollination come from high-value per unit crops (i.e. fruits, nuts, hybrid seed, and intermediate goods for the livestock and dairy industries). These authors therefore argue that global food security will not be threatened if insect pollinators decline or disappear, although this ignores the diverse (Allsopp *et.al*, 2008).

Modern commercial crop production is increasingly dependent on managed pollinators (e.g. the introduction of honey bee colonies into orchards or fields to improve crop production), and less on wild insects living on the periphery of crop fields. The honey bee is regarded as the most important commercial pollinator, and

although other bee species are also used for commercial pollination [alkali bees (*Nomia*); mason bees (*Osmia*); leafcutter bees (*Megachile*); bumble bees (*Bombus*)] at least 90% is performed by honey bees. Honey bees are excellent generalist pollinators, with commercial pollination being the most important derived value of commercial beekeeping worldwide (bees) (Allsopp *et.al*, 2008).

#### 4.1 Halictidae

The family Halictidae, the sweat bees, includes some of the common bees; in many temperate areas of the world, halictids dominate other bees in numbers of individuals, after *Apis* is ignored. They are mostly andreniform, although in the Nomiinae some Old World species are apiform, euceriform, or even more robust. Halictids agree with the characters of the short-tongued bees, some other characters of family: the body of the labrum, which is thick, elevated, and possibly homologous to the basal area of the labrum in Andrenidae, is much broader than long. In female an apical process, fringed with bristles, is often present, and sometimes is so long that the whole labrum is longer than broad. The one subantennal suture, or more accurately a feeble line, so that there may appear to be two subantennal sutures, as in the Andrenidae. The facial foveae, as they are found in female Andrenidae and many Colletidae, are absent or slightly evident in some Rophitinae and Nomiinae (Schuberth and Schönitzer, 1993), but even in Halictinae there may be comparable areas (Michener, 2007). Many species of Halictidae are important to pollination. Detail of dominant pollinators was described such as:

##### 4.1.1 *Nomia*

Alfalfa (=lucerne) flowers require visiting bees to trip the sexual column, thereby providing pollination and subsequent pod and seed set. Previous studies have compared the pollination values of different bee species solely by the speed with which they handle flowers and the proportion of visited flowers tripped. In this greenhouse study, five species of bees, including the three commercially managed alfalfa pollinators, are likewise compared for their floral tripping

frequencies. These bee species are also compared for the pod set and mature seed that results from their single visits to virgin flowers. Regardless of the identity of the pollinating bee, tripped flowers had the same probabilities of pod set and seed set. Thus, differences in the single-visit pollination efficiencies of the various bee species are entirely attributable to the proportion of visited flowers that they trip. Females of the alkali bee, *Nomia melanderi* Cockerell, tripped 81% of visited flowers. Males of these species are significantly less effective (61%) (Cane, 2002).

The world's only intensively managed ground-nesting bee, the alkali bee (*N. melanderi*), has been used for >50 years as an effective pollinator of alfalfa (*Medicago sativa* L.) grown for seed in the western USA. Across 240 km water shed in Washington, the 24 most populous of 56 nest sites found were annually surveyed for nesting bees for 8 years. Alkali bees multiplied females, the largest reported metapopulation of non-social bees. Several sites have remained populous for an unprecedented 50 years. The most populous nesting bed (1.5 ha) grew to 5.3 million nesting females (median=278 nests/m), the largest bee nesting aggregation ever recorded. This first-ever exhaustive landscape-level survey for any non-social bee reveals that even amid intensive conventional agriculture, a native bee can sustainably multiply to vast numbers, its nesting aggregations persisting for decades (Cane, 2008).

Similarly, Suthisut (2002) reported that *N. ridleyi* (Cockerell), a solitary bee, was found to be gregarious building nest in soil, revealed its life cycles as follows: egg, 2-3 days; larvae (5 instars), 6-7 days; pupa, 10-12 days; adult, 30-60 days. Sex ratio of male: female equaled 1:1. The experimental study during May-October, 2001 at Amphur Potharam, Rachaburi province in Thailand found the nomia bee to prefer nest building in the composed soil to bamboo root soil in baked-clay pot especially in the southern part of the pot. It was also observed the rate of nest building to be 1.78 times/month, 3 nests/pot (706 cm<sup>2</sup>), 8 cells/nest. Such activity stopped at the start of dry season. In addition, 14 species of insect pollinator visiting *Adenium* spp., *Xylocopa*, *Amegilla*, *Ceratina* and *Nomia* were observed in majority.

#### 4.1.2 *Osmia*

Use of osmia bees in crop pollination the relative facility with which osmias can be induced to nest in pre-prepared artificial nests and their strong gregarious nesting is the base of the management and multiplication of these insects in controlled pollination. The use of these bees in the pollination of open field cultivations has been very successful especially for early flowering fruit trees, and good results have recently been obtained also for the pollination of confined environment cultivations. Open field pollination since the bees collect pollen and nectar from flowers that are near the place where nests are placed and they will not fly more than 100 m away from the nest if it is not necessary, in order to achieve good pollination it becomes important to placed nests and cocoons in the orchard with spacing of about 20-50 m. As a general rule 2-5 females/fruit tree in blossom is sufficient to achieve 50-90 % of pollination depending on the orchard (Felicioli *et al.*, 2004). Similarly, the activity of 100 females/hectare of apricot plant cultivation is sufficient to increase eating by 30-50%. With oil-rape plant cultivation *Osmia* population could increase five times. Lucerne and white clover – *O. cornuta* and *O. rufa* in a density of 0.50 females/m has been utilized for pollination of both lucerne and white clover breeding material grown in isolation by mesh cages (10 m) in summer. For lucerne pollination *O. cornuta* gives a yield of 8.1 kg/ha while *O. rufa* gives a yield of 5.3 kg/ha. However, *O. cornuta* shows a great efficiency (175 kg/ha) in pollinating off-season generation in white clover grown in a growth cabinet during winter (24°C/18°C day/night and 16 hours photoperiod) if utilized in a density of 5 females/m (Felicioli *et al.*, 2004).

#### 4.2 Megachilidae

In most parts of the world the Megachilidae are among the more easily recognized families of bees; megachilids are long-tongued bees with a rectangular labrum that is longer than broad and broadly articulated to the clypeus. In recent decades, however, two taxa that do not, or do not always, agree with these megachilids characters have been shown to be relatives of the familiar megachilids.

These taxa in the Megachilidae make that family less easy to define than when it include only the taxa here placed in the subfamily Megachilinae. The characteristic thick-headed megachiline appearance is not shared by the Fideliinae, but recognition is a problem only in the limited areas where the Fideliinae occur, i.e., desertic areas in Morocco and Egypt eastward to the Punjab (India) and central Asia; also southern Africa and central Chile. Elsewhere, traditional characters for the Megachilidae will suffice (Michener, 2000).

Soroka (2001) studied in the field during 3 years, the activities of alfalfa leaf cutting bees (*Megachile rotundata* Fabricius) at varying stocking levels were monitored in shade cloth isolation tents containing male-sterile and male-fertile rows of oil-rape seed (*Brassica napus* L.) to determine optimum conditions for hybrid seed production. Leaf cutting bee activity, as measured by the number of female bees at the entrance to tunnels in the hive, at the first increased, and then decreased with increasing stocking rate. Duration of time spent visiting flowers did not vary with stocking rate, but was longer on male-fertile than on male-sterile flowers. Production of hybrid seed within tents varied with leaf cutting bee stocking rate, with the highest seed yield achieved at stocking rates equivalent to three charges at weekly intervals of 400,000 leaf cutting bees/hectare. At optimum leaf cutting bees stocking rates, the method described provided sufficient seed quantity for small-plot multi-location field evaluation of oil-rape seed hybrids.

### 4.3 Apidae

Apidae is one of the most diverse families of bees, containing more tribes than any other family. That Apidae includes all those long-tongued bees that are not Megachilidae, a statement suggesting that Apidae is a polyphyletic or paraphyletic unit. Although there are exceptions to all of the external characters of the Apidae, many and perhaps all such exceptions are derived. An example of a derived character that makes definition of the family difficult is reduction of the labial palpi, such that the first two segments are as short as the remaining segments. Thus the palpi are like those of short-tongued bees; and a major division of the bees

seems to have been broken down. This condition is found in some parasitic form of Allodapini; similar and also probably reduced palpi occur in the Ctenoplectrini and in the genus *Ancyla* (Ancylini). The biology of the Apidae is highly variable: from solitary to highly social behavior with dissimilar castes, from nest provisioners to social parasites and cleptoparasites, from mass provisioners to progressive provisioners of brood cells to forms that completely lack brood cells from nest excavators in soil or in wood to forms that occupy preexisting cavities or construct nests in the open, and so forth (Michener, 2007).

#### 4.3.1 Xylocopini

The Xylocopini are large, robust bees, 13-30 mm long, that typically have three submarginal cells, but the first and second are sometimes partly or wholly fused owing to the disappearance of the posterior part or the whole of the first submarginal crossvein (Michener, 2007). A feature of some Xylocopini is the pale, usually yellow or testaceous, coloration of males, in striking contrast to dark-colored females. In other species the sexes are similarly colored, usually largely dark. In most cases the pale coloration of males is a result of yellow or testaceous hairs, the integument being black or brownish black. In two groups, however, the integument of males is reddish brown (some species of the paleotropical subgenus *Koptortosoma*) or even testaceous and partly yellowish (the largely neotropical subgenus *Neoxylocopa*), and the pubescence is often even paler. It seems that such males attract females to their flight and perching places, which are independent of nest or foraging locations (Anzenberger, 1977; Alcock and Smith, 1987).

There are many accounts of nesting behavior by species of *Xylocopa*. Except for the subterranean nest of the subgenus *Proxylocopa*, and except when in stems or similarly restricted substrates, the burrows are in wood, often solid wood, and usually branch into parallel passages running with the grain of the wood, with barrel-shaped unlined cells in short series in the branches. Some of the smaller species of *Xylocopa*, such as *X. (Copoxylo) cyanescens* Brullé and *X. (Nanoxylocopa) ciliata* Burmeister, typically nest in dead stalks of large

herbaceous plants, in which there is no space for branch burrows. Some species of the subgenera *Biluna* and *Stenoxycopa*, and *X. (Schonnberria) bambusae* Schrottky, nest in bamboo stalks. The female must first cut hole through the hard wall of the bamboo, but then makes cells in the nearly empty cavity; thus the nest-making behavior is rather different from that of species that nest in wood or stems. Young adults are regularly fed by the mother, and daughters commonly remain in their mothers' nests with some social interactions. Watmough (1974) gave an account of ecology and behavior of various species. That each female lays about ten eggs; this low reproductive potential is, of course, associated with excellent maternal care, the mothers commonly being still active at the time of maturation of their offspring.

The successive study of large carpenter bees and their pollinators' role on yellow passion fruits was carried out at Surat Thani Province during June-August 2001. There were 19 species of insect visited passion fruit flower. The *Apis cerana indica* assumed the highest frequency visitation (44.93%) followed *Xylocopa latipes* whose frequency visitation accounted for 15.48%. The peak of visitation period was during 12.00-16.00 h for the pollinator group. The *X. latipes* visited passion fruit flowers nectar and pollen but *A. cerana indica* usually visited the flower for pollen. The comparative studies on percentage of fruit setting, fruit weight and the number of seeds per fruit resulted from various approaches in pollinating were conducted through eight treatments, as follows: 1) *X. latipes* as pollinator 2) hand-pollination crossing 3) emasculated and opened pollination 4) *A. cerana indica* as pollinator 5) opened-pollination 6) closed-pollination 7) hand-pollination selfing 8) emasculated and bagged. The result indicated that *X. latipes* was significant (with 99% reliability) the best pollinator which assumed highest efficiency in productivity (Boontop, 2002).

Many green house crops depend on bees for pollination. Global declines of honey bee populations, and their limited efficiency in pollinating some green house food-plants, motivate the search for additional pollinators. They evaluated the carpenter bee (*X. pubescens*), a local species to Israel, as a pollinator

of green house grown honeydew melons, in comparison to honey bees. They recorded the bees daily and seasonal activity patterns in relation to oral nectar levels, frequencies and durations of flower visits, and fruit quantity and quality. The bees daily foraging schedule on melon did not correlate with nectar yield and nectar production patterns by the flowers. Visit durations per flower were shorter for *X. pubescens* than for honey bees. Pollination by both bees resulted in similar fruit mass and seed numbers, but *X. pubescens* pollination increased fruit set three fold as compared to honey bee pollination. They conclude that *X. pubescens* can effectively pollinate melons in enclosures (Sadeh *et al.*, 2007).

#### 4.3.2 Bombini

Bumble bees are important pollinators in mountainous regions. The highland region of Sichuan and Chongqing (together, Sichuan in the former broad sense) includes part of the greatest hotspot of bumble bee diversity worldwide, with half of the species of China and more than a fifth of the world's species. Six species were recorded from the Sichuan-Chongqing region for the first time (*Bombus avanus*, *B. branickii*, *B. difficillimus*, *B. humilis*, *B. norvegicus*, *B. tibetanus*), of which *B. avanus* was only the second published record worldwide. One species, *B. braccatus*, was endemic to Sichuan and was confirmed as extant in 2005. They provide diagnoses and keys to species for both sexes. Color variation was described, distributions within the Sichuan-Chongqing region were mapped, altitudinal and seasonal activity were plotted, and the authors' records of food plants were listed (Williams *et al.*, 2009).

As stated before, *B. terrestris* colonies are used in the Asian market (Japan, Korea). These colonies are mainly imported from Europe. Forced by the discussions about the possible ecological impact of the use of *B. terrestris* colonies, bumble bee breeders have decided to start breeding a native species. The species chosen is *B. ignitus*. Although this species has turned out to be the most suitable one, there are some clear disadvantages. In comparison to *B. terrestris*, the full-grown *B. ignitus* colonies are much smaller having about half the number of workers, and

the breeding success rate is much lower. Therefore, using *B. ignitus* colonies costs at least twice as much as using *B. terrestris* colonies. The Japanese growers are not yet willing to pay that price, a condition which strongly hampers the breakthrough of this species. For experimentation purposes small numbers of *B. ignitus* colonies became available on the market since 1999. In 2003, some 2,500 *B. ignitus* colonies were used in a total Japanese market of 70,000 colonies. In Korea the use of bumble bees (*B. terrestris* only) is estimated to involve 9,000 colonies (Velthuis and Doorn, 2004).

Sabir *et al.* (2011) reported that in agricultural habitat 10 bumble bee species were recorded; 68.62% of which were dominated by five species viz., *B. asiaticus*, *B. melanurus*, *B. rufofasciatus*, *B. semenovianus* and *B. tunicatus*. However, in non-agricultural habitat 13 bumble bee species were recorded and more than 50% bumble bees were dominated by four species viz., *B. asiaticus*, *B. avinoviellus*, *B. biroi* and *B. haemorrhoidalis*. Significant variations were observed in the species richness at non-agricultural habitat but none in case of abundance at habitat level.

#### 4.3.3 Apini

The genus *Apis* is found in the palearctic region north to southern Norway and the Pacific maritime provinces of Russia and in the entire African and Oriental regions. *A. mellifera* L. has been distributed worldwide by human activity. The vary many specific and subspecific name have been reduce to about 11 species. The members of bees construct combs in cavities; hence construction of combs in the open, as in the other two groups must be ancestral. The species in the three groups are as follows: 1) small species with single exposed combs; dances on expanded horizontal base of comb: *A. florea* Fabricius, *A. andreniformis* Smith, 2) Large species with single exposed combs; dances on vertical curtains of bees or on comb: *A. dorsata* Fabricius, *A. laboriosa* Smith, *A. binghami* Cockerell, *A. breviligula* Maa. The last two are probably allopatric segregates of *A. dorsata* and may not represent distinct species, and 3) Middle-sized species with multiple combs in cavities; dances

on vertical surfaces of combs in the dark: *A. mellifera* Linnaeus, *A. cerana* Fabricius, *A. koshevnikovi* Buttel-Reepen, *A. nigrocincta* Smith, *A. nuluensis* Tinget, and Koeniger. The last two are only recently recognized as specifically distinct from *A. cerana* (Michener, 2007).

Pollinators play an important role in poverty alleviation and food security by pollinating crops and other plants. But, in recent years the nature is experiencing a serious decline in pollinator populations and diversity, the impact of which is clearly visible in several areas as the agricultural productivity is seriously declining as a result of inadequate pollination due to the lack of sufficient numbers of pollinators in these agricultural landscapes. This decline in pollinators is attributed to several factors such as habitat loss, land-use changes, monoculture-dominated agriculture, and excessive and indiscriminate use of agricultural chemicals and pesticides (Partap, 2004). In addition, honey bees (*A. mellifera*) can be attacked by many eukaryotic parasites, and bacterial as well as viral pathogens. Especially in combination with the ectoparasitic mite *Varroa destructor*, viral honey bee diseases are becoming a major problem in apiculture, causing economic losses worldwide. Several horizontal transmission routes are described for some honey bee viruses. Here, the first time the detection of viral sequences in semen of honey bee drones suggesting mating as another horizontal and/or vertical route of virus transmission. Since artificial insemination and controlled mating is widely used in honey bee breeding, the impact of our findings for disease transmission is discussed (Yue *et al.*, 2006).

The degree to which a particular crop needs bee pollination depends on the flower morphology, level of self-fertility exhibited by the plant, and arrangement of flowers on the plant or on neighboring plants. It is thus possible to categorize crops according to their degree of dependence on bee pollination, and it follows that the economic value of bee pollination is highest in those crops most dependent on bee pollination (Delaplane and Mayer, 2000). Many researches were studied pollination by honey bees in the world, but this sample were represented to study of honey bee pollination in Thailand.

For example, Kongpitak (1987) reported that the percent of seed set of various sunflower varieties which were pollinated by *A. mellifera* in the cage and the opened-plot is significantly higher than that of self and hand pollinated plants. It was found that when using honey bees as the pollinator, the yield of sunflower increased around 33-300%. And Boongird (1985) stated that the dwarf honey bee (*A. florea*) was most consistent visitors to the mango cv. Nam Dokmai, made up almost three-fourths (74.04%) of the total number of visiting insects through the blooming period. *A. mellifera* visited the mango flowers cv. Nam Dokmai for collecting nectar or pollen, mango pollen pellets from *A. mellifera* colony contained mean of 14.18% of the total pollen pellets were collected. The visiting insect speeds on the mango flower of the *A. cerana* was the highest speed from flower to another within one minute covered 17.16±5.01 flowers, 8.88±2.52, 7.60±2.47 and 1.80±1.16 flowers for the speed of *A. mellifera*, *A. florea* and *Chrysomyia* sp. respectively. All honey bees prefer staminate flower to perfect flower. The highest peak of *A. florea* visitation of mango flower cv. Nam Dokmai was found 256.29 bees/trees on the 17<sup>th</sup> day of blooming, 47.52 bees on the 13<sup>th</sup> day for *A. cerana*, 30.05 bees on the 11<sup>th</sup> day for *A. mellifera*.

#### 4.3.4 Meliponii

Stingless honey bees are a group of small-to medium-sized bees, with vestigial stings, found in tropical and many subtropical parts of the world. They are the major visitors of many flowering plants in the tropics. Colonies are perennial and usually consist of hundreds or thousands of workers. The estimated several hundred species of stingless honey bees are arranged into 21 genera. The most important genera are *Melipona* and *Trigona*. *Melipona* consists of 50 species, and *Trigona* is the largest and most widely distributed genus, with 130 species in 10 subgenera. In additional, stingless honey bees are divided to minor genera e.g. *Hypotrigona*, *Liotrigona*, *Lisotrigona*, *Pariotrigona*, *Trigonisca*, *Austroplebeia*, *Meliponula*, *Nannotrigona*, *Paratrigona*, *Plebeia*, *Plebeina*, *Cleptotrigona*, *Lestrimelitta*, *Partamona*, *Scaptotrigona*, *Trigonas*, and *Geotrigona*.

- *Melipona* (neotropical) and *Meliponulas*. str. (Africa). Robust, thorax and head densely hairy, integument dull, basal propodeal area hairy, dorsal vessel arched between longitudinal indirect muscles of flight. The arch of the dorsal vessel, characteristic of many large, fast-flying bees, may be related to the robust body and fast flight of *Melipona* and *Meliponulas*. str.; the form of the dorsal vessel was documented for various taxa. In spite of these similarities, the hidden characters of the worker stings place these genera far apart.

- *Trigona* (neotropical; Asia to Australia) and *Dactylurina* (Africa), typically rather elongate and long-legged, although in some American forms (like the subgenus *Geotrigona* and some species of *Trigonas* str.) the metasoma is short and broad. The inner surface of the hind tibia of workers has a longitudinal band of keirotrichia on an elevated ridge, usually little if any wider than the depressed, shining upper zone of the tibia. The upper fringe of the hind tibia of workers includes plumose hairs except in some small subgenera of *Trigona*. In spite of the similarities of the two genera listed above, the worker sting rudiments differ greatly, those of *Dactylurina* resembling those of various other African genera.

- *Hypotrigona* (Africa), *Liotrigona* (Africa), *Lisotrigona* (Asia), *Pariotrigona* (Asia), and *Trigonisca* (neotropical). Minute, sparsely haired, the pterostigma relatively large, wing venational characters as listed in the first alternative of couplet 1 of each key to genera, below. All except possibly the two little-known Asiatic taxa are attracted perspiration. At least *Hypotrigona*, *Liotrigona*, and *Trigonisca* are quite unrelated to one another to judge by the sting and male genitalic characters, although superficially almost indistinguishable. *Cleptotrigona* (Africa) also falls in this group, being a relative of *Liotrigona*, but has the special features of robbers.

- *Austroplebeia* (Australia), *Meliponula* (except s. str.) (Africa), *Nannotrigona*, *Paratrigona*, and *Plebeia* (neotropical), and *Plebeina* (Africa). Mostly small, robust bees of superficially similar aspect, often with restricted dull yellowish (or bright yellow in *Paratrigona*) markings on the head and thorax. The

upper margin of the inner surface of the hind tibia is commonly shiny, often depressed, but keirotrichia sometimes reach the margin.

- *Cleptotrigona* (Africa), *Lestrimelitta* (neotropical). Robber bees with shiny, sparsely haired bodies. The following features are presumably somehow related to robbing behavior: vertex and genal areas broad; proboscis fossa greatly narrowed posteriorly; eyes small; clypeus small; labrum concave between lateral prominences; corbicula hairs. That in spite of their similarities, these genera are not closely related, as shown, for example, by the male genitalia.

- *Partamona*, *Scaptotrigona*, and *Trigona* str. (*spinipes* group), and *T. (Geotrigona)* (all neotropical). Robust, often black bees with short metasomas, superficially similar in form and color, but well differentiated by the generic characters (Michener, 2007).

Stingless honey bees are common visitors to flowering plants in the tropics. They are known to visit the flowers of 90 crop species. They were confirmed to be effective and important pollinators of nine species. They may make a contribution to the pollination of 60 other species. They have been recorded from another 20 crops. The strengths and limitations of stingless honey bees as crop pollinators are discussed. Aspects of their biology that impact on their potential for crop pollination are reviewed, including generalized flower visiting behavior of colonies, floral constancy of individual bees, flight range, and the importance of natural vegetation for maintaining local populations (Heard, 1999).

In Northeastern Region of Brazil, in order to investigate the use of stingless honey bee (*Melipona subnitida* Ducke) in the pollination of greenhouse sweet pepper (*Capsicum annuum* L.). Results showed that despite sweet pepper flowers are considered autogamous, this crop benefits from pollination by *M. subnitida*, producing fruits significantly heavier and wider, containing a greater number of seeds and of better quality (lower percentage of malformed fruits) than self-pollinated sweet pepper. Thus, *M. subnitida* can be considered an efficient

pollinator of greenhouse sweet pepper (Darci, 2005). Similarly, Dos Santos (2009) reported that the pollination effectiveness of the stingless honey bee (*M. quadrifasciata*) and the honey bee (*Apis mellifera*) was tested in tomato plots. Results showed the heaviest and largest tomatoes, and the ones with the most seed were collected from the greenhouse with stingless honey bees. The stingless honey bee, (*M. quadrifasciata*) was significantly more efficient than honey bees in pollinating greenhouse tomatoes.

Pollination of stingless honey bees were studied in Thailand, that mention about *Trigona* spp. on various plants such as durian (*Durio zibethinus*: Bombacaceae), rambutan (*Nephelium lappaceum* L.: Sapindaceae), lychee (*Lichi chinensis* Sonn.: Sapindaceae), and dragon fruit (*Hylocereus undatus*: Cactaceae).

Thawiphon (2003) reported that the highest major population on durian flowers was observed during 08.00-09.00 h such as *T. laeviceps* (52 bees/flower), *A. dorsata* (48 bees/flower), *T. terminata* (35 bees/flower), *A. cerana indica* (23 bees/flower), and *A. florea* (13 bees/flower), but those bees showed behavior for the side visit whereas the structure of the flower was not suitable for the bees to pollinate. The highest nectar concentration of durian cultivar Chanee was 17.5 % Brix during 21.00-23.00 h. Likewise, Jinarite (2006) reported that the stingless honey bees to prefer visiting lychee inflorescences on the east side of four years old of lychee tree from morning till noon, followed by the south side and the west side, the lowest foraging was found on the north side. From the afternoon to evening, the bees of nearly equal numbers dispersed to visit the inflorescences in all directions. This might be due to the inflorescence of the sun pathway which tilted to the south in this season, thus affecting the bee behavior. Placing one nest in each corner of 400x500 m<sup>2</sup> plot was found to give the most efficiency in pollination of the stingless honey bees as well as increasing yields of lychee. The next best thing was to place four nests in the same long row in the middle of the plot. Arranging four nests in group in the middle of the plot gave the least result. When the four nests of each *T. apicalis* and *T. collina* were placed close to lychee plot and most bees (110 bees/tree) foraged away from the nests at the distance of

5 m in lychee plantation was found to give the greatest yield percentage of 82, followed by foragers (54, 25, 13 and 5 bees/each tree) at the distances of 10, 15, 20 and 25 m to give yields of 45, 25, 17 and 5%, respectively. Similarly, Namvong (2003) reported that the *T. laeviceps* was introduced into the experimental site, suggested that *T. laeviceps* was the most effective pollinator. It was observed that on *Hylocereus undatus* was self-compatible, while *H. costaricensis* was self-incompatible, the percentage pollinator yielded were 51.47, 49.96 and 43.44, respectively. For *H. costaricensis*, the highest population was observed during 07.30-08.30 h with approximately 41 bees/flower, while *H. undatus* were visited by bees during 06.30-07.30 h with 8 and 6 bees/flower, respectively.

## MATERIALS AND METHODS

### 1. Species diversity

#### 1.1 Study sites

Surveys of insect pollinators were undertaken at physic nut (*Jatropha curcas* L.) plantations in 20 provinces, in five regions as follows: Region 1 (north): Chiang Mai, Chiang Rai, Lampang, Lamphun, Nan and Phayao; Region 2 (northeast): Kalasin, Khon Kaen, Maha Sarakham, Nakhon Ratchasima, Sakon Nakhon and Udon Thani; Region 3 (central): Chai Nat, Kamphaeng Phet, Nakhon Pathom and Suphan Buri; Region 4 (east): Chon Buri and Rayong; Region 5 (south): Chumphon and Phangnga. At each site, at least an area of 100 m<sup>2</sup> was sampled.

#### 1.2 Sampling methods

The sampling method followed Malaipan *et al.* (2007) and Kwaiser and Hendrix (2008). Twenty quadrants (each of 100 m<sup>2</sup>) were selected from the large area in each province. Wherever physic nut flowers were observed, pollinators were caught by sweep-net during the period 08.00-12.00 h, from April to August 2009. All specimens were preserved and maintained in the laboratory of Kasetsart University, Bangkok.

#### 1.3 Identification and grouping of insect pollinators

All insect specimens were identified into family or species as possible, and these specimens were divided into two groups. Identifications were performed by employing the taxonomic keys from Triplehorn and Johnson (2005), Michener (2000), Sakagami *et al.* (1985), Schwarz (1937, 1939), Osamu and Tasen (2009), Ekamnuay (2002), Insect Museum of Entomology Department, Kasetsart University (2006), Beaver *et.al.* (2009) and Neal and Patrick (2002). The specimens were grouped by their colonizing behavior and the frequency of occurrence.

## 1.4 Data analysis

Data were compared among 20 provinces in Thailand. Species diversity of insect pollinators was analyzed using the Shannon-Weiner diversity index ( $H'$ ) from the statistical package (Species Diversity and Richness) while diversity percentages were calculated using MS Excel. The frequency of occurrence bees (*Apis* spp. and *Trigona* spp.) used to examine bee distributions and the high frequency of occurrence bees were grouped in the major group.

## 2. Variation of physic nuts

### 2.1 Study sites

Botanical characteristics of physic nut such as flower sex ratio, size of plant canopy and number of branches, fruit set and yield, character of plantation and type of irrigation, and pollen grades were observed and recorded at physic nut plantation in 20 provinces of Thailand.

### 2.2 Sampling methods and observations

After the insects on physic nut flowers were observed and caught by sweep-net during 08.00-12.00 h, botanical characteristics of physic nut were observed and recorded.

#### 2.2.1 Flower sex ratios

Physic nut flowers in each province were randomly counted for number and classified types of flowers (female, male and perfect flower). At each site, at least 500 flowers were observed. At least an area of 100 m<sup>2</sup> was made for sampling. About 10 plants in each 100 m<sup>2</sup> were counted for number of branches and inflorescences per branch in each plant. On 10 plants at each site were counted for number of fruit set in each inflorescence on branch. These data were calculated for

a yield per plant and area (1 rai=1,600m<sup>2</sup>) in annual. Physic nut plantations in 20 provinces were observed on the character of plantation (mono-cultivated, multi-plantation, and fencing), and type of irrigation (sprinkler, dripping, level furrow, and non-irrigated) and numbers of insect pollinators were observed on 100 flowers (in each female and male).

### 2.2.2 Amount of pollen grains on stigmas of physic nut flowers

After studied species diversity and population of insect pollinators on physic nut flowers in each province such as, the number of pollen grains, those covered on stigmas of the female flowers, were estimated amount of pollen grains grade on stigmas in the evening during 17.00-18.00 h. Amount of pollen grains were classified to the grade levels (A, B, C and F) A means very fulfillment of pollen grains (>1,500 pollen grains/stigma), B was covered 300-1,500 pollen grains/stigma, C was covered < 300 pollen grains/stigma, and F means very rare of pollen grain or almost none (Figure 2). Amount of pollen grains on stigma were counted and recorded by photos.



**Figure 2** Evaluation on amount of pollen grains grade on stigmas of *Jatropa curcas* L. flowers during 17.00-18.00 h

A-grade > 1,500 pollen grains/stigma

B-grade = 300-1,500 pollen grains/stigma

C-grade < 300 pollen grains/stigma

F-grade none pollen grain on stigma.

Stigma of physic nut flower has three lobes.

**Source:** Malaipan *et al.* (2007)

### 2.3 Data analysis

Data were compared among 20 provinces in Thailand. The differences in flower sex ratio, size of plant canopy and number of branches, fruit set and yield were tested by analysis of variance (ANOVA). Duncan's new multiple range test was used to determine these data in 20 provinces. The yield of physic nut/plant/rai (1 rai=1,600 m<sup>2</sup>) in the rainy season, these can be calculated by the number of branches, inflorescences/branch, and fruits/inflorescence. Characters of plantation and type of irrigation were recorded. In addition, density of insect pollinator related to the percentage amount of pollen grains was used to examine efficiency of pollinators in each province.

## 3. General botanical characteristic of physic nut (*Jatropha curcas* L.)

### 3.1 Study sites

#### 3.1.1 Kamphaeng Saen (KPS) location

This research had been done since 2008 to 2010. The study site was located on physic nut plantation at Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom Province (14°02'28" N, 99°97'59" E), with an elevation of 3 m, and annual air temperature of 28.4°C. Annual accumulative rainfall was 1,194.0 mm, annual average relative humidity (%RH) was 68.25%. Soil type is fine-silty, mixed, semiactive, isohypethermic, type Haplustalfs and pH 7.0-8.0 (Kamphaeng Saen series: Ks). The investigation was conducted in a single-plantation physic nut population, these were toxic variety (t), mono-clone (planted from stem cutting), 3x2 m in spacing, level furrow irrigation, 2 m in height and 3 years. The sample trees were promised adult ones with relative full blooming habit and a stem diameter at base of 8-12 cm (Figure 3).

### 3.1.2 Agriculture Research and Development Center (ARDC) location

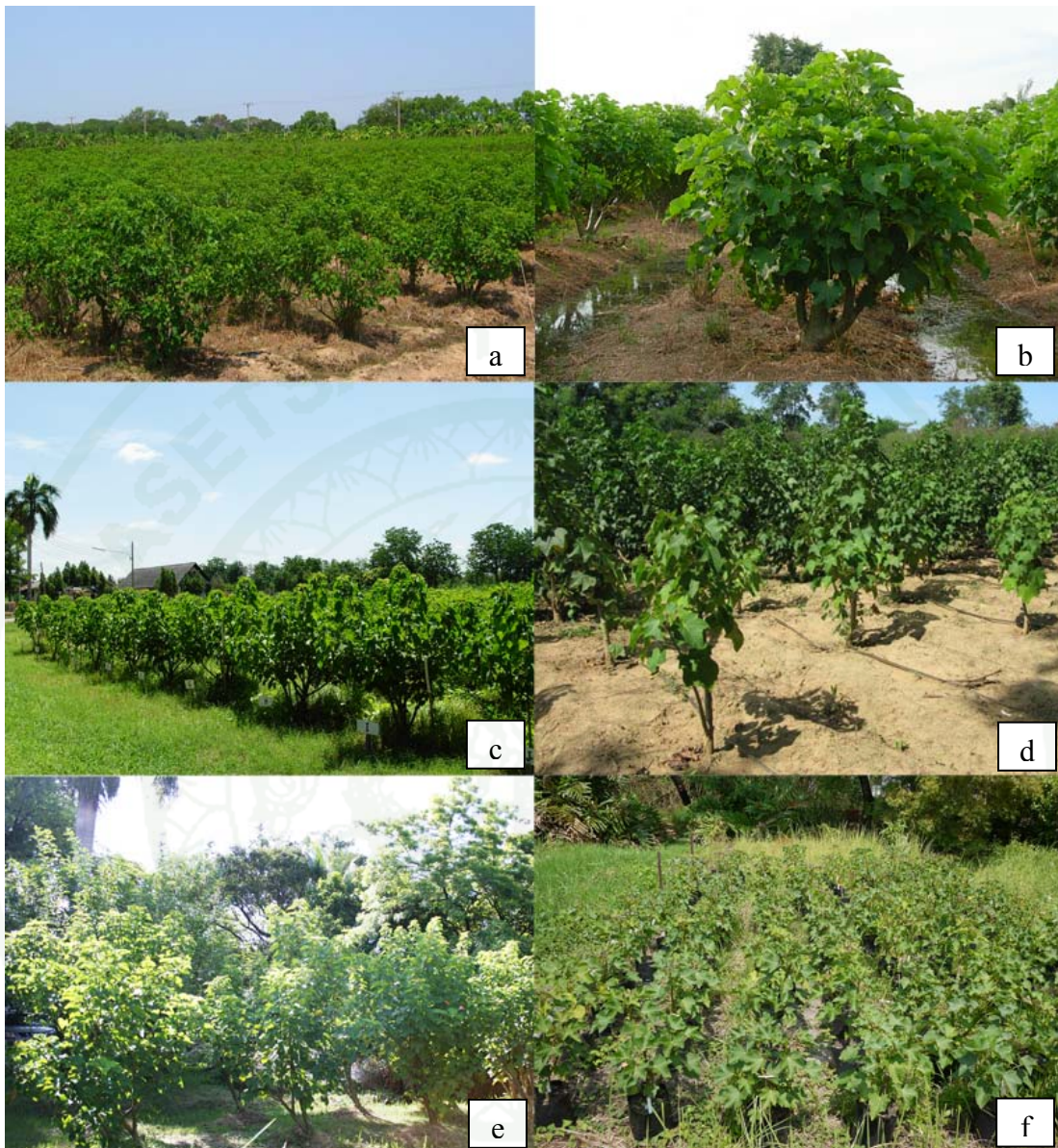
This experiment had been done in 2009, during June to August. The study site was located on physic nut plantation at Agriculture Research and Development Center, Ministry of Agriculture and Cooperatives, U Thong district, Suphan Buri Province (14°18'11" N, 99°51'63" E), with an elevation of 40 m, and annual air temperature of 27.5 °C. Annual accumulative rainfall was 874.5 mm, annual average relative humidity (%RH) was 75%. Soil type is Kamphaeng Saen series and pH 6.5-6.7. The investigation was conducted in a single-plantation physic nut population, these were non-toxic variety (nt), mixed clone (planted from seedling), 2x2 m in spacing, non irrigated crops, 2 m in height and 2 years. The sample trees were promised adult ones with relative full blooming habit and a stem diameter at base of 8-12 cm (Figure 3).

### 3.1.3 Aviation School (AS) location

This experiment had been done in 2009, during August to November. The study site is located on physic nut plantation at Aviation School, Kamphaeng Saen district, Nakhon Pathom Province (14°09'27" N, 99°93'34" E), with an elevation of 19 m, and annual air temperature of 28.0°C. Annual accumulative rainfall was 1,152.5 mm. Annual average relative humidity (%RH) was 67.35%. Soil type is Kamphaeng Saen series, and pH 7.0-8.0. The investigation was conducted in a single-plantation physic nut population these were non-toxic variety (nt), mono-clone (planted from stem cutting), 2x3 m in spacing, dripping system irrigation, 2 m in height and 3 years. The sample trees were promised adult ones with relative full blooming habit and a stem diameter at base of 8-12 cm (Figure 3).

### 3.1.4 Bangkhen (BK) location

This study had been done in 2011. The study site was located on physic nut plantation at Bangkhen Campus, Kasetsart University, Bangkok (14°02'28" N, 99°97'59" E), with an elevation of 3 m, and annual air temperature of 29.2 °C. Annual accumulative rainfall was 2,240.2 mm, annual average relative humidity (% RH) was 74%. The investigation was conducted in a multi-cultivated physic nut population, the mixed varieties of physic nut from the plot-A; toxic variety at Nong Yai (NYt), were planted mixed varieties of non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunphiboon (SSnt) on the same plants and the different plants with cleft grafting method, these root stocks were the stem cutting plantation. The root stocks of plot-A were planted 3x2 m in spacing, 2 m in height and 3 years. The plot-B (four original sources such as toxic variety at Kamphaeng Saen (KPSt), non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunphiboon (SSnt) were planted in the pots with stem cutting method. The stem cutting were planted in 10 inches of diameter in 100 pots, these were laid pots in Latin Square sampling; four original sources of physic nut, four replications and four repetitions. After, plants were planted about 4-5 months; these were adult ones with relative full blooming habit (Figure 3).

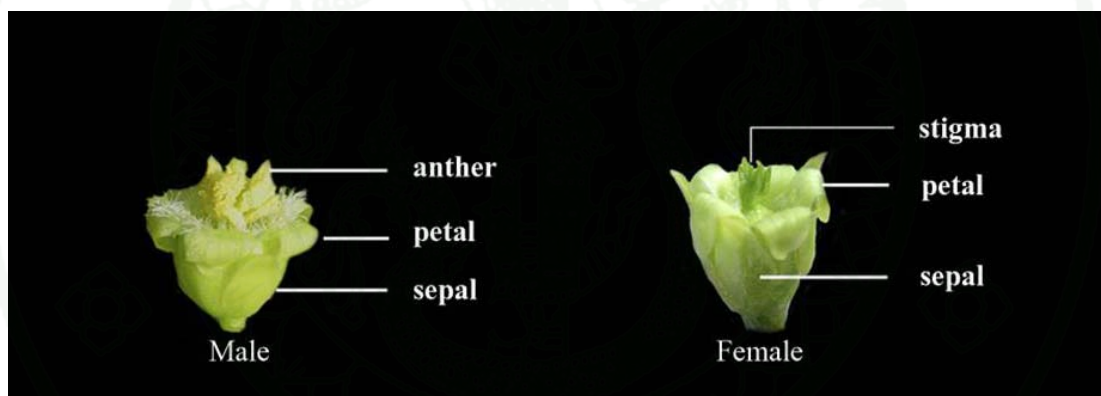


**Figure 3** Study sites

- a, b = Kamphaeng Saen (KPS) location
- c = Agriculture Research and Development Center (ARDC) location
- d = Aviation School (AS) location
- e = Bangkhen (BK) location; Plot-A
- f = Bangkhen (BK) location; Plot-B

### 3.2 Type of flowers, flower sex ratio and flowering of physic nut

A sample of 10 plants was selected, and 30 inflorescences were marked (three inflorescences/plant, each inflorescence from five direction of plant canopy (the north, south, central, east and west)), which were the first inflorescences of branch. The numbers of blooming female and male flowers were counted every day until ended blooming on inflorescence. Types of flowers and flower sex ratios were compared among four locations. In addition, flowering of physic nut were compared among 20 provinces in the rainy season (April-August, 2009), among three seasons in the year (2008) at KPS location, between non-toxic variety from ARCD location and AS location in the rainy season (2009), and among four original sources in the rainy season (2011) at BK. Characteristics of flowers were shown in Figure 4.



**Figure 4** Male and female flowers of *Jatropha curcas* L. (toxic variety)

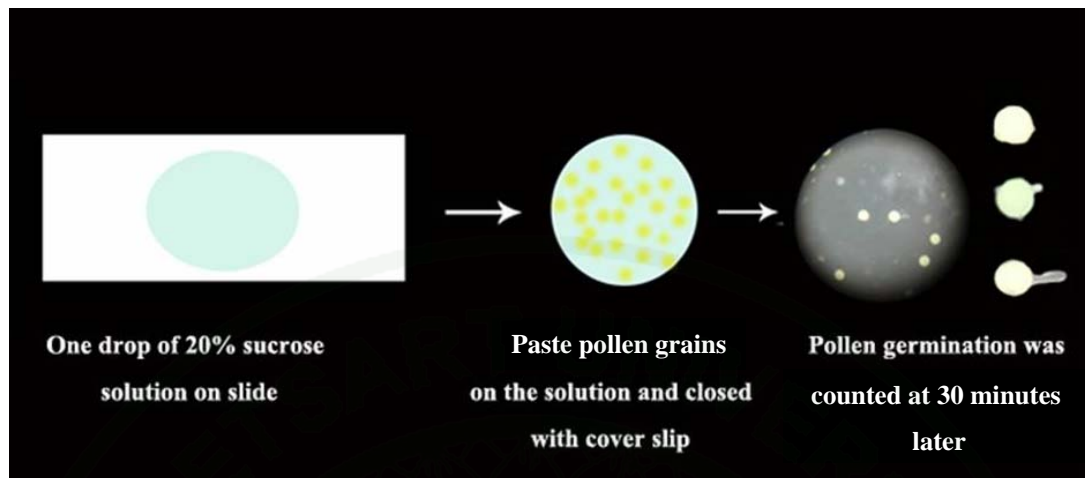
**Source:** Malaipan *et al.* (2007)

### 3.3 Anthesis and receptive time

At KPS location on April 16<sup>th</sup> and December 27<sup>th</sup>, 2009; flower anthesis was noticed by tagging 30 male flowers from the first inflorescences of branch, on the east of plant canopy and 10 different plants. In addition, these flowers were observed anthesis and receptive time during the morning (07.00-11.00 h), and recorded time, air temperature and relative humidity percentage (%RH) from Nakhon Pathom Meteorological Station. Anthesis and receptive time were studied in the dry season (December) and the wet season (April). Likewise, flower receptive time was observed on female flowers similar to anthesis period.

### 3.4 Pollen germination

The selecting method was employed following Malaipan *et al.* (2007), 20 of the blooming male flowers of physic nut were selected during 10.00-12.00 h, selecting one from each plant at the east of plant canopy and the first inflorescence of branch. After that kept only pollen grains from 20 flowers in 20% sucrose solution on 10 pieces of slide and closed with cover slits about 30 minutes. Some pollen grains will germinate, which had long pollen tubes, these pollen grains were counted by microscope and calculated the percentage of pollen germination (Figure 5). The vary percentage of pollen germinations were compared between intra-variety (toxic) in different season of year at KPS location in 2009, inter-variety (toxic and non-toxic) in the dry season at BK location in 2011, and pollen grain from male and perfect flowers at AS location in 2009. In addition, pollen grains from body of bee pollinators (except pollen grains from pollen corbicular) were studied pollen germination at KPS location in 2009.



**Figure 5** Processing of pollen germination

**Source:** Malaipan *et al.* (2007)

### 3.5 Amount pollen grains

At KPS location in the summer season, 2009: amount of pollen grains on stigmas of physic nut flowers were the important factor for fruit setting. The effective bee (*Apis florea*) in the field was observed frequency of visiting on female flowers, these data connected to amount of pollen grains on stigmas during 17.00-18.00 h. Amount of pollen grains grade effective experiments were set in the physic nut plantation. Thirty female flowers were marked and observed in each type amount of pollen grains grade (A, B, and C), that pollen grains were classified to the grade levels (A, B, C and F), these means of each grade level followed Figure 2. These flowers were tagged and following observed the fruits after pollinated, finally yellow fruits were collected for weight of fresh fruits and dry seeds. Seeds were dried with 50-60°C about 48 hours, and extraction with hexane solution.

### 3.6 Fruits and fruit set

#### 3.6.1 Fruits from female and perfect flowers

At ARDC location in the rainy season (June 23<sup>rd</sup> to August 25<sup>th</sup>) 2009: fruit development from female and perfect flowers were studied by observation the 30 flowers, three female flowers/inflorescence on the east of plant canopy from 10 plants were randomly selected, tagged and following observed fruit development. In perfect flowers were selected, tagged and following observed, too.

#### 3.6.2 Frequency of occurrence of fruit setting per inflorescence

Randomized large numbers of fruits were observed in the various number of fruits per inflorescence of toxic variety, at KPS location on October, 2008.

### 3.6.3 Effect of fruit numbers per inflorescence

At KPS location in 2009 the fruit set experiments were observed, after female flowers were pollinated by various insect pollinators. Fruit numbers per inflorescence were selected made to five, 10, and 15 fruits/ inflorescence, at the east of plant canopy. Ten fruits in each treatment were tagged and containing observed. All yellow fruits were harvested, measured, weighted, and extracted oil content by hexane. Oil contents in kernel of seeds were compared among various pollination methods and insect pollinators. Three grams of kernel in each treatment had been extracted for oil by using hexane as dilution. Three replications were done.

## 4. Pollination factors

### 4.1 Varieties of physic nut (*Jatropha curcas* L.)

Physic nuts experiments at BK location, Bangkhen Campus, Kasetsart University, Bangkok in the rainy season, 2011 were observed, measured and recorded plant canopy diameter and plant height, leaf area of plants, growth development, flowers, fruits, weight, and oil content.

#### 4.1.1 Plant canopy diameter and plant height

The mixed varieties of physic nut from the plot-B four original sources i.e. toxic variety at Kamphaeng Saen (KPSt), non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunphiboon (SSnt) were planted in the pots with stem cutting method). Ninety days later, plant canopy diameter and plant height were measured.

#### 4.1.2 Leaf area of plants

The mixed varieties of physic nut from the plot-A; toxic variety at Nong Yai (NYt), were planted mixed varieties of ARDCnt, ASnt, and SSnt on the same plants and the different plants with cleft grafting method, and plot-B; four original sources i.e. KPSt, ARDCnt, ASnt, SSnt were planted in the pots with stem cutting method. The 10<sup>th</sup> of leaf on branches in each plant were direct measured leaf area (m<sup>2</sup>) with leaf photos on photoshop program.

#### 4.1.3 Growth development of physic nut

The mixed varieties of physic nut from the plot-B; vegetative period observation started the first day, when the stem cutting of physic nut had the first leaf until inflorescence start blooming, flowering, and fruit ripening were observed. Size and shape of flowers and fruits were measured.

#### 4.1.4 Weight and oil of physic nut

The mixed varieties of physic nuts from the plot-B were observed, after female flowers were pollinated by various insect pollinators. Fruit numbers/inflorescence was made to five, at the east of plant canopy. Ten fruits in each treatment were tagged and following observed. All yellow fruits were harvested, measured, weighted, and extracted for oil content by hexane.

#### 4.1.5 Diversity of insect pollinators

Physic nut flowers at BK location on plot-B in the rainy season, 2011 were studied diversity of insect pollinators in four original sources. Sampling flowers used method following (Malaipan *et al.*, 2007) the flower visitors observed to visit the physic nut flowers were caught and identified on self-expertise basis consulting with reference materials and compared them with the specimens in museums. Insect pollinators were watched during 08.00-12.00 h and recorded in

different original sources. Species list, species diversity index, and density of insect pollinators were compared among four original sources of physic nuts.

#### 4.2 Seasons

At KPS location, this large area (10 rais; 1 rai=1,600 m<sup>2</sup>) were randomly sampled following Malaipan *et al.* (2007) method. After the physic nut flowers were observed, the insect pollinators (especially Hymenoptera) were caught by sweep-net during 08.00-12.00 h, in the wet and the dry season 2008-2009. All specimens were identified, recorded and data analyzed to species diversity index ( $H'$ ).

#### 4.3 Times and directions of plant canopy, and positions of area effect on the position field

At KPS location on October 2008, sampling flowers used method following Malaipan *et al.* (2007), 75 inflorescences having both female and male flowers in five directions of plant canopy (the north, south, east, west, and central), in each tree of three different plants per position of area, which was a large area (10 rais, 1 rai=1,600 m<sup>2</sup>) of physic nut plantation, that were divided to five position of area i.e. the corner of plot in four directions (the northeast, southeast, northwest, southwest), and the central of plot. Flowers were targeted for determinations of during 30 minutes of visiting, number and species of pollinator every hour during 07.00-18.00 h by five researchers in the field. This data was compared among different times, directions of plant canopy, and positions of area in different parts of the field.

#### 4.4 Data analysis

Data were compared among various factors. Species diversity of insect pollinators was analyzed using the Shanon-Weiner diversity index ( $H'$ ) from the statistical software "Species Diversity and Richness". The differences in parameter

among various factors were tested by analysis of variance (ANOVA). Duncan's new multiple range test was used to determine various factors of pollination.

## 5. Effect of bees to pollination

### 5.1 Diversity of insect pollinators

Physic nut plantation at KPS location was established a transect grid covering an area of 10 rais during March to June 2007. Air temperature, accumulative rainfall (mm), and relative humidity (%RH) data were recorded for each month by The Meteorological Department at Kamphaeng Saen District, Nakhon Pathom Province. The insect pollinators on flowers were observed, caught, and identified. A total of censuses (1 minute/inflorescence) during 08.00-12.00 h that were made to record the population of pollinators, these were species list and abundance of insect pollinators. Diversity of ants during 10.00-13.00 h on December, 2008 was observed of species list, abundance, and behavior on the east of plant canopy in 400 m<sup>2</sup>.

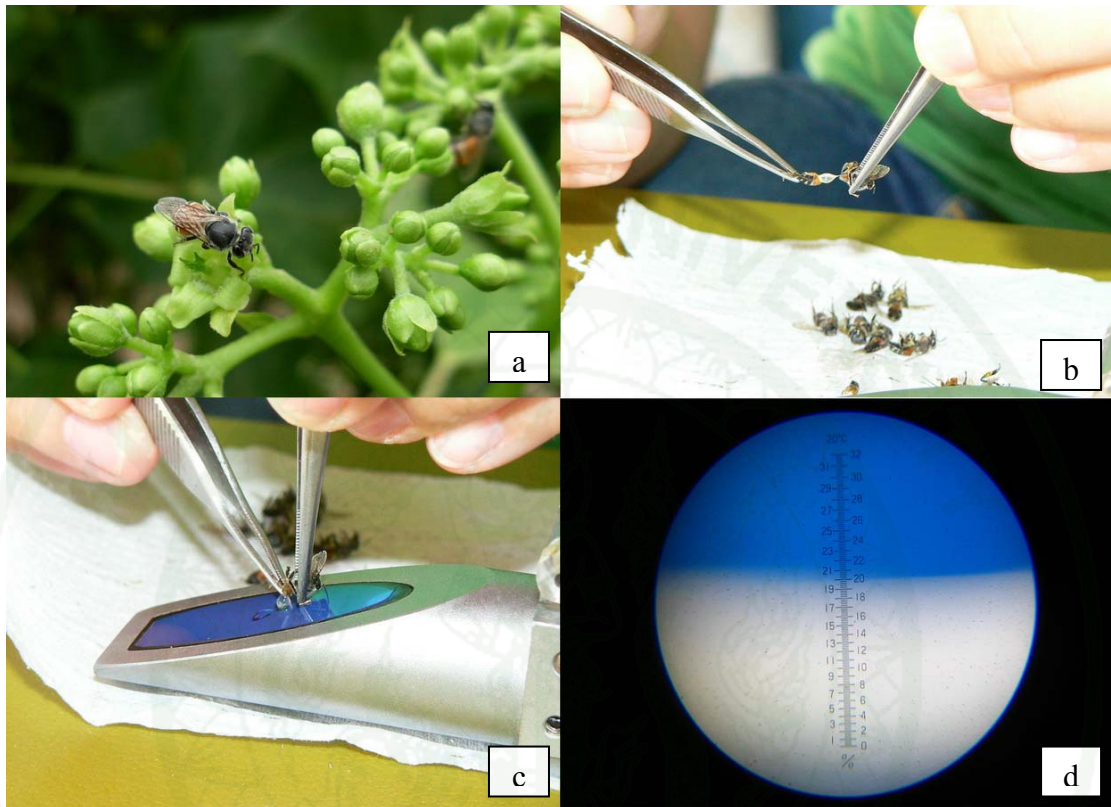
### 5.2 Bee behaviors

#### 5.2.1 Bee preferences

1) Preference of dwarf honey bee (*Apis florea*) was observed on visiting male or female physic nut flowers during 08.00-18.00 h on April 18<sup>th</sup>, 2007 at KPS location. Both female and male flowers, which were visited by dwarf honey bee that were counted number of flowers every hour, at the east of plant canopy from randomly 10 plants. Average of number flower visiting per hour can estimated the bee forager preference.

2) Effect of the nectar concentration to bee preference; nectar concentration in the honey stomach of dwarf honey bee visited on physic nut flowers during 07.00-17.00 h every two hours, on April 19<sup>th</sup>, 2007 at KPS location.

Average of nectar concentration (% Brix) from honey bee stomachs were measured with Refractometer or Brix Refractometer (Figure 6).



**Figure 6** Nectar concentration measuring procedure

a = *Apis florea* caught from *Jatropha curcas* L. flowers in the field.

b = Honey stomach of *Apis florea* extracted.

c = Dropping nectar on Refractometer.

d = Measuring nectar concentration.

3) Female and male flowers had effect on the bees preference at KPS location during 10.00-13.00 h on December, 2008: experimental trails were made in the 400 m<sup>2</sup> of physic nut plantation. Randomized large numbers of both female and male flowers were observed in the number of visitation of individual species of insect pollinators. The majority of pollinators in this site were ants (12 spp.), Indian honey bees (*A. cerana indica*), Italian honey bees (*A. mellifera ligustica*), and stingless honey bees (*Trigona pagdeni*, and *T. laeviceps*).

#### 5.2.2 Bees speed

Four species of bees visited on flowers were observed number of flowers in one minute during 10.00-13.00 h on February, 2009 at KPS location. These four bees were *A. cerana indica*, *A. florea*, *A. mellifera ligustica*, and *T. pagdeni* were recorded data from 10 individuals in each bee species.

#### 5.2.3 Bee foragers

Bee foragers of *A. cerana indica*, and *A. mellifera ligustica* were observed including the number of nectar and pollen foragers at the hive entrances every hour during 07.00-18.00 h on November 21<sup>st</sup>, 2008 at KPS location.

#### 5.2.4 Competitive plants

Competitive plants of two honey bee species (*A. cerana indica*, and *A. mellifera ligustica*) were surveyed during 08.30-16.30 h on November 19<sup>th</sup>, 2008 at KPS location, these competitive plants in a one kilometer radius around the bee hives were surveyed species of plant and bee visitors. While returning pollen foragers were captured at the hive entrances using an insect net and randomly capturing 10 foragers that had pollen loads. After removal of their pollen foragers were released. Pollen was collected every one hour during 08.30-16.30 h on November 22<sup>nd</sup>, 2008. These pollen grains were kept on slides and closed with cover

slips, and then the slides were immediately analyzed and pollen characteristics recorded.

### 5.3 Pollination experiments

The fruits number had been control five fruits/inflorescence and included yellow fruits in color stage for data analysis. To compare with the results of various treatments by fruit set (%), weight (g) of fruit, seed and kernel, and oil content (%).

#### 5.3.1 Comparisons among various botanical factors

At Agriculture Research and Development Center (ARDC) location during June to August in 2009, the experiments were performed respectively by bagging, emasculation of the inflorescences, conducting artificial pollination with female and perfect flowers, and bagging again with thin paper until fruit setting. Treatments for the target inflorescences were as follows:

1) Open-pollinated female flower, to check different color between black and yellow fruit effect to the oil content; BfOpFf (black fruit, open-pollination, on female flower), and YfOpFf (yellow fruit, open-pollination, on female flower).

2) Hand-pollination, with fulfillment amount of stigma pollen in A-grade, on female flower YfHpFf (yellow fruit, hand-pollination, stigma pollen A-grade, on female flower), to compare effect between full pollination by nature and YfOpFf (yellow fruit, open-pollination, on female flower).

3) Open-pollinated, on female and perfect flowers compare to the effect between two flower types; YfOpPf (yellow fruit, open-pollination, on perfect flower), and YfOpFf (yellow fruit, open-pollination, on female flower).

### 5.3.2 Comparisons between self- and cross-pollinations

The experiment was performed respectively like to the comparisons between self-pollination in non-toxic variety, and cross-pollination between toxic and non-toxic variety during November 2009 to January 2010 at Aviation School (AS) location. Treatments for the target inflorescences were as follows:

1) Control: to check pollination of female flowers under the natural conditions un-bagging, un-emasculature, and open pollination; OpFf (open-pollination, on female flower).

2) Hand xenogamy: to detect whether out-crossing was compatible, in the condition of emasculature and bagging the female flowers, and artificial pollination with male flowers from different plant; SHpOFF (self-hand-pollination, outside plant, on female flower).

3) Hand geitonogamy: to check whether this measure would lead to fertilization and bearing fruits in the condition of emasculature and bagging the female flowers, and artificial pollination with male flowers from the same plant; self-hand-pollination, inside plant, on female flower; SHpIFf (self-hand-pollination, inside plant, on female flower).

4) Cross-pollination: to check whether this measure would lead to fertilization and bearing fruits in the condition of emasculature and bagging the female flowers and artificial pollination with male flowers from toxic (male) at Kamphaeng Saen (KPS) location; CrHpFf (cross-hand-pollination, on female flower).

5) On perfect flowers: to check pollination under the natural conditions un-bagging, un-emasculature, and open-pollination; OpPf (open-pollination, on perfect flower).

### 5.3.3 Comparisons among ants and four introduced bees

At Kamphaeng Saen (KPS) location during November to December in 2008, the nests of different kinds of pollinators such as two species of the honey bees (*Apis cerana indica*, *A. mellifera ligustica*) and stingless honey bees (*Trigona laeviceps*, *T. pagdini*) were introduced three hives/species of bees to the sites of physic nut plantations, in each species of introduced bees per week at 400 m<sup>2</sup> of this area.

1) Amount of pollen grains covered on one day old of the female flowers had been classified to grade levels (A, B, C and F) (Figure 2). At least randomized 50 female flowers during 17.00-18.30 h of the experimental day in each insect species of the foraging area had been recorded and classified by insect pollinator and amount of pollen grains on stigmas.

2) Fruit set: a large number of female flowers that were visited by various insect pollinators had been closed after visited, tagged and following observed. All fruits were harvested on 50 days old.

3) Dry seeds were weighted, and three grams of kernel in each treatment had been extracted for oil by using hexane as dilution. Three replications were done

### 5.3.4 Comparisons among experiments with artificial pollination

At KPS location during September to November in 2009, the experiments were performed by stingless honey bee (*T. pagdeni*) hives introducing in this crop. Male flowers on inflorescences were emasculated and bagged with thin paper in the evening (before female flowers open in the morning on the next day). Inflorescences were opened, that female flowers were artificial pollinated with three visitors' species (ants, *A. florea* and *T. pagdeni*) these 10 individuals of bees pollen frager were caught and treated on physic nut inflorescence within net during

10.00-11.00 h, that is the artificial full pollination. Inflorescences, which were visited by ants, were selected and emasculated. And then all inflorescences were bagged again with thin paper until fruit setting and yellow fruits, for fruits prevention from *Chrysocoris stollii* (Scutelleridae). Treatments for the target inflorescences were as follows:

- 1) Three experiments of full pollination: to check the effect of ants, dwarf honey bee (*A. florea*), and stingless honey bee (*T. pagdeni*).
- 2) One time visited with fulfillment of pollination by the dwarf honey bees, compared effect to the dwarf honey bees.
- 3) Hand-pollinated (A-grade): to detect whether out-crossing was compatible, in the condition of emasculation and bagging the female flowers, and pollen A-grade artificial pollination with male flowers from different plants.
- 4) Open-pollination: to check pollination under the natural conditions un-bagging, un-emasculation, and free pollination or open-pollinated.

#### 5.3.5 Fruit setting

Fruit set experiments had been done during project in each location. Female flowers were treated with experiments that had been closed after pollinated with thin paper, tagged and following observed. All fruits were harvested on 50-60 days old (yellow fruit), similar to Dhillon *et al.* (2006) stated that period of fruit development and maturity ranged from 55-61 days from date of first fruit initiation. Fresh yellow fruits were measured size and weight. In each treatment was controlled five fruits number per inflorescence.

### 5.3.6 Seeds

Physic nut seeds were harvested at physiological maturity, which color of fruits turn yellow was proof to be the best seed germination and seedling vigor comparing to the seeds of immature green fruits and dark brown fruits, which passed through timing of physiological maturity (Tongpumnug *et al.*, 2007). Also, yellow fruits were extracted the seeds, they were weighted fresh and dry seeds. Dried process of seeds were executed respectively by let to dry during two days and put to the oven at 50°C during 24 hours.

### 5.3.7 Oil content

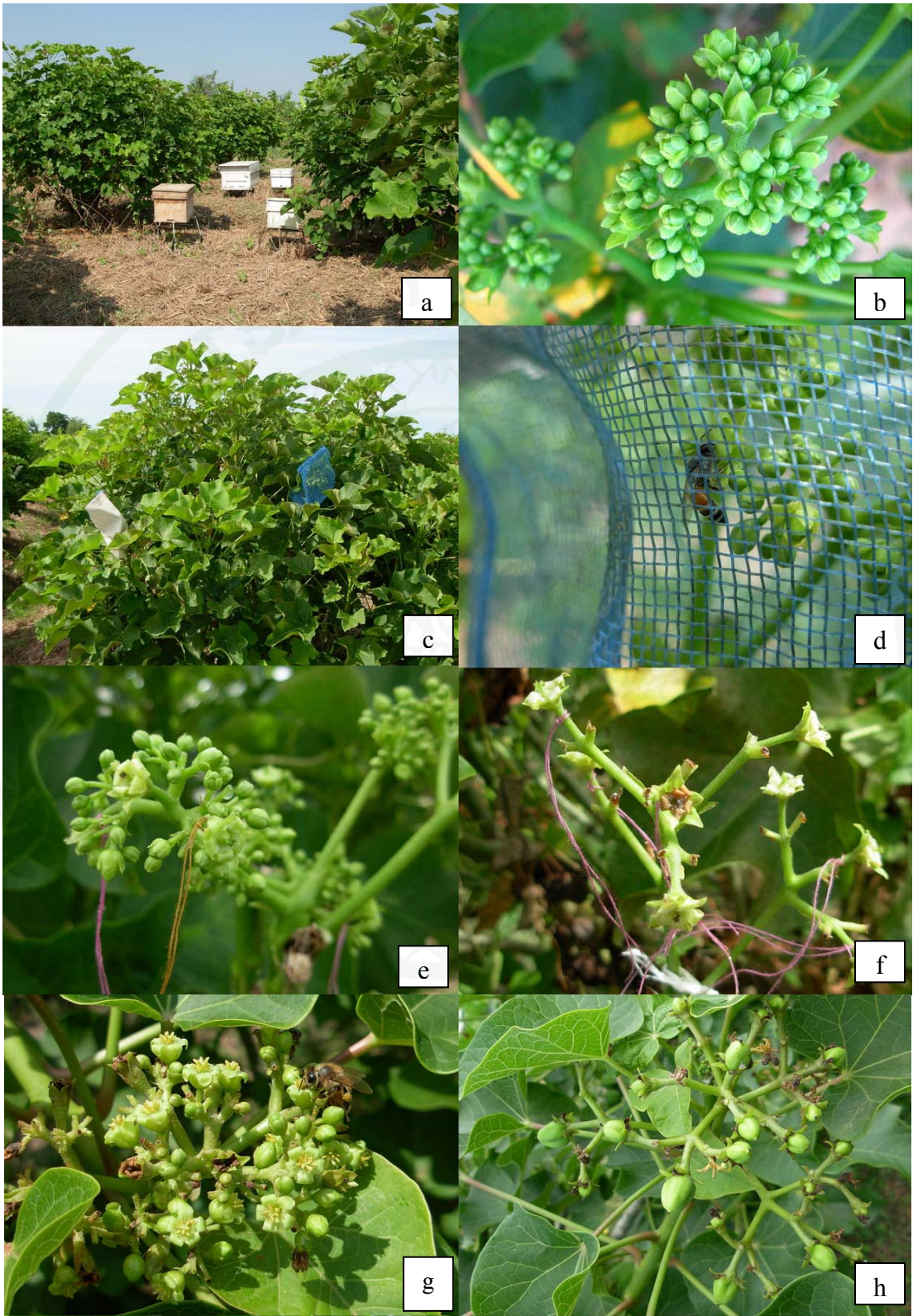
The kernel of seeds were extracted, oil content in kernel of seeds from various treatments of pollinations and insect visitation. Three grams of kernel in each treatment had been extracted for oil by using hexane as dilution. Three replications were done.

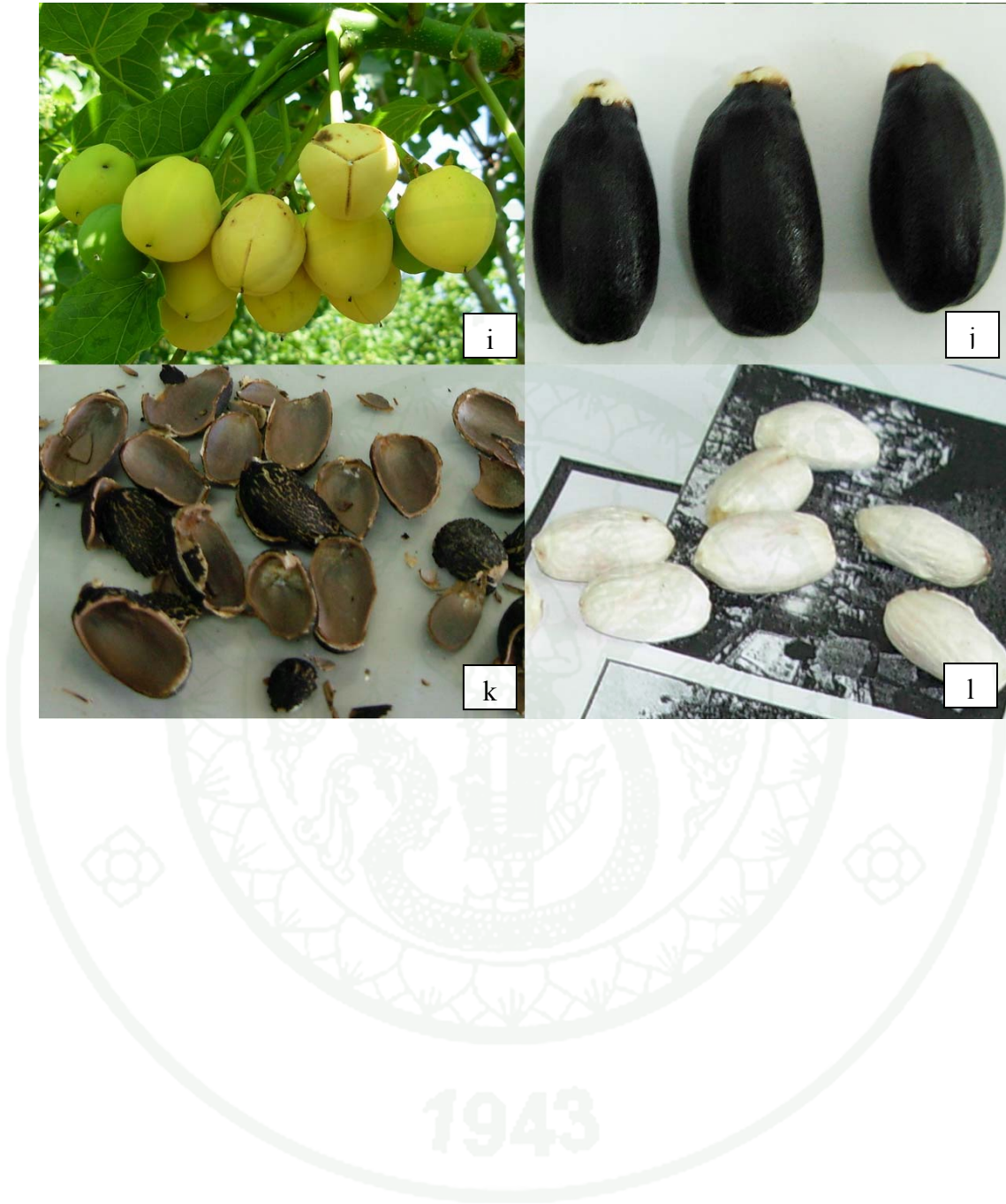
### 5.3.8 Data analysis

Data were compared among experiments. Weight and oil content were calculated by simple excel program. The weight of fruit, seed, and kernel, and oil content were tested by analysis of variance (ANOVA). Duncan's new multiple range test was used to compare different parameter among various treatments.

**Figure 7** Procedure of pollination experiments in *Jatropha curcas* L. plantation

- a = Efficient bees hives were introduced into physic nut plantation.
- b = Female or perfect flowers were chosen for test, which flowers will open in the next morning.
- c = Inflorescence of physic nuts were bagged with thin paper.
- d = *Apis florea* from another plants were treated on physic nut flowers within the net.
- e = Female or perfect flowers were tagged after pollinated by the visitors.
- f = All male flowers were emasculated.
- g = Flowers and pollinators on inflorescences were observed and recorded.
- h = Fruit set numbers of *Jatropha curcas* L. were counted.
- i = Mature or yellow fruits of physic nuts were collected.
- j = Seeds of physic nuts
- k = Seed coats were peeled.
- l = Kernels of physic nuts





## RESULTS AND DISCUSSION

### 1. Insect Pollinators on physic nuts (*Jatropha curcas* L.) flowers

#### 1.1 Diversity of insect pollinators

A total of 762 insect specimens were collected in 20 provinces of Thailand from April to August 2009, representing 311 species, 138 genera and 64 families, and eight orders, namely Hymenoptera, Lepidoptera, Diptera, Coleoptera, Hemiptera, Orthoptera, Mantodea and Blattodea (Table 5). Base on the number of species Hymenoptera (with 45.02% of the species) exhibited the highest diversity followed by Lepidoptera (20.58%), Diptera (15.43%), Coleoptera (9.97%), Hemiptera (7.40%), Mantodea and Orthoptera (0.64%) while the lowest diversity percentage was found in Blattodea (0.32%) (Table 6).

#### 1.2 Species diversity indices

The species diversity indices ( $H'$ ) were different among 20 provinces. The highest value was observed in Chiang Rai (4.5), followed by Chiang Mai (4.3), Lampang (4.1), Lamphun, Nan and Phayao (3.8), Chai Nat and Kamphaeng Phet (3.4), Rayong (3.3), Nakhon Pathom (3.2), Kalasin (3.1), Chon Buri (3.0), Nakhon Ratchasima, Suphan Buri and Phangnga (2.9), Sakon Nakhon and Udon Thani (2.8). The lowest values were found in Khon Kaen, Maha Sarakham and Chumphon (2.5) (Table 7 and Figure 8). Species diversity were different among 20 provinces; species list, and frequency of occurrence on pollinators were shown in Appendix Table 1 and 2.

Diversities of insect pollinators on physic nut flowers were investigated in 20 provinces of Thailand. The study yielded 311 species, a much larger tally than six species recorded on physic nut flowers in India by Bhattacharya *et al.* (2005), 13 species recorded also in India by Raju and Ezradanam (2002), and the 50 species recorded in Thailand by Malaipan *et al.* (2007). This might be because the

present study was undertaken during the rainy season (April to August) when insect pollinators in general tend to be abundant and diverse on flowers and because a large number of sites were surveyed; all previous studies were performed during the dry season and included only a few sampling sites. Thysanoptera were not recorded in the present study but thrips were found on physic nut flowers in previous surveys (Raju and Ezradanam, 2002; Malaipan *et al.*, 2007). The large number of species and the abundance of some in the present study suggested that physic nut flowers were attractive insects and provided suitable nectar and/or pollen.

All insect pollinators (311 species) at the sampling sites in the 20 provinces of Thailand were clearly different from that found at sites in National Botanic Garden, Lucknow (Bhattacharya *et al.* (2005), the Eastern Ghats in India (Raju and Ezradanam, 2002) and previously surveyed site in Thailand (Malaipan *et al.*, 2007). Very rare insect pollinators were observed on physic nut flowers at the Lop Buri Campus, of Kasetsart University (Malaipan *et al.*, 2007). Perhaps, the Lop Buri site was too dry, lacked of alternative host plants for insect pollinators or was too windy for insect pollinators. In the absence of pollination physic nut flowers failed to set fruit.

**Table 5** Species list of insect pollinators found on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Orders         | Families      | Scientific Name  |   |
|----------------|---------------|--|---|
| Blattodea      | Blattellidae  | Unidentified (1 sp.)   |   |
| Coleoptera     | Brentidae     | <i>Eubactrus</i> sp.   |   |
|                | Bruchidae     | Unidentified (2 spp.)  |   |
|                | Cerambycidae  | <i>Chlorophorus annularis</i> Fabricius, <i>Polyzonus obtusus</i> Bates, <i>Polyzonus</i> sp.  |   |
|                | Chrysomelidae | <i>Aulacophora</i> sp., <i>Chrysochus</i> sp., <i>Donacia aenaria</i> Baly, <i>Galerupipla</i> sp., <i>Luperomorpha</i> sp.  |   |
|                | Cleridae      | Unidentified (1 sp.)   |   |
|                | Curculionidae | <i>Ectatorhinus</i> sp., <i>Episomus</i> sp.   |   |
|                | Elateridae    | <i>Alaus</i> sp., <i>Diploconus</i> spp.(2 spp.)   |   |
|                | Lycidae       | <i>Lycostomus</i> spp. (4 spp.)  |   |
|                | Cantharidae   | Unidentified (1 sp.)   |   |
|                | Nitidulidae   | Unidentified (2 spp.)  |   |
|                | Scarabaeidae  | <i>Gametis histrio</i> Olivier, <i>Glycyphana nicobarica</i> Janson, <i>Glycyphana horsfieldi</i> Hope, <i>Glycyphana quadricolor quadricolor</i> Wiedemann, <i>Ixorida mouhotii</i> Wallace, Unidentified (1 sp.) |   |
|                |               | Staphylinidae  | Unidentified (1 sp.)                            |
|                | Diptera       | Asilidae   | <i>Proctacantella</i> sp., <i>Promachus</i> sp. |
|                |               | Bombyliidae  | <i>Systropus</i> spp. (3 spp.)                  |
| Calliphoridae  |               | <i>Chrysomyia megacephala</i> Fabricius, <i>Chrysomyia</i> spp. (2 spp.), <i>Hypopygropsis</i> sp., Unidentified (1 sp.)   |   |
| Dolichopodidae |               | <i>Chrysosoma</i> sp.  |   |
| Drosophilidae  |               | <i>Drosophila</i> sp., Unidentified (1 sp.)  |   |
|                | Empididae     | <i>Hilara</i> sp.  |   |

**Table 5** (Continued)

| Orders        | Families      | Scientific Name  |  |
|---------------|---------------|--|--|
| Diptera       | Muscidae      | <i>Musca</i> spp. (7 spp.)   |  |
|               | Sarcophagidae | <i>Parasarcophaga</i> sp.  |  |
|               | Stratiomyidae | <i>Hermetia</i> sp., <i>Ptecticus</i> sp., <i>Stratiomys</i> sp.,<br>Unidentified (1 sp.)  |  |
|               | Syrphidae     | <i>Eristalis arvorum</i> (Fabricius), <i>E. obscuritarsis</i><br>Meijere, <i>Helophilus bengalensis</i> Wiedemann,<br><i>Helophilus</i> spp. (2 spp.), <i>Megapis</i> sp.,<br><i>Physocephala</i> sp., <i>Rhingia</i> spp. (4 spp.),<br><i>Syrphus</i> spp. (2 spp.), Unidentified (1 sp.) |  |
|               | Tabanidae     | <i>Chrysops dispar</i> (Fabricius),<br><i>C. fasciata</i> Wiedemann  |  |
|               | Tachinidae    | <i>Drino</i> spp. (3 spp.)   |  |
|               | Tephritidae   | <i>Bactrocera</i> sp.  |  |
|               | Therevidae    | Unidentified (1 sp.)   |  |
|               | Tipulidae     | <i>Tipula</i> sp.  |  |
|               | Hemiptera     | Coreidae   | <i>Clavigralla</i> sp., <i>Riptortus linearis</i> Fabricius,<br><i>Serinetha abdominalis</i> Fabricius,<br>Unidentified (3 spp.) |
|               |               | Lygaeidae  | <i>Geocoris</i> sp., <i>Graptostethus servus</i> Fabricius,<br>Unidentified (2 spp.)   |
| Miridae       |               | Unidentified (1 sp.)   |  |
| Pentatomidae  |               | <i>Eocanthecona furcellata</i> (Wolff), <i>Erothesima</i><br><i>fullo</i> Thunberg, <i>Eusarcocoris guttiger</i> Thunberg  |  |
| Reduviidae    |               | <i>Chitapa</i> sp., <i>Ectomocoris</i> sp., <i>Rhynocoris</i> spp.<br>(2 spp.), <i>Sycanus collaris</i> Fabricius,<br>Unidentified (1 sp.)   |  |
| Scutelleridae |               | <i>Callidea</i> sp., <i>Chrysocoris grandis</i> Thunberg,<br><i>C. stollii</i> Wolff   |  |

Table 5 (Continued)

| Orders      | Families     | Scientific Name   |
|-------------|--------------|---|
| Hymenoptera | Apidae       | <i>Amegilla</i> sp., <i>Apis andreniformis</i> Smith, <i>A. cerana indica</i> Fabricius, <i>A. dorsata</i> Fabricius, <i>A. florea</i> Fabricius, <i>A. mellifera ligustica</i> Linnaeus, <i>Ceratina</i> spp. (3 spp.), <i>Pithitis smaragdula</i> Fabricius, <i>Podalirius crocea</i> Bingham, <i>Thyreus</i> sp., <i>Trigona collina</i> Smith, <i>T. laeviceps</i> Smith, <i>T. melanoleuca</i> Cockerell, <i>T. pagdeni</i> Schwarz, <i>T. ventralis</i> Smith, <i>Trigona</i> spp. (4 spp.), <i>Xylocopa aestuans</i> (Linnaeus), <i>X. collaris</i> Cockerell, <i>X. latipes</i> (Drury) |
|             | Chrysididae  | <i>Stilbum cyanarum</i> (Förster), <i>Stilbum</i> sp.   |
|             | Evaniidae    | <i>Evania</i> sp.   |
|             | Formicidae   | <i>Anoplolepis gracilipes</i> (Smith), <i>Camponotus</i> spp. (4 spp.), <i>Iridomyrmex</i> sp., <i>Meranoplus</i> sp., <i>Monomorium</i> spp. (2 spp.), <i>Ochetellus</i> spp. (2 spp.), <i>Oecophylla smaragdina</i> Fabricius, <i>Paratrechina</i> spp. (3 spp.), <i>Solenopsis geminata</i> (Fabricius), <i>Tetraopnura rufonigra</i> (Jerdon), Unidentified (1 sp.)   |
|             | Halictidae   | <i>Halictus</i> spp. (3 spp.), <i>Lasioglossum</i> spp. (4 spp.), <i>Nomia albofasciata</i> Smith, <i>Nomia</i> spp. (5 spp.), Unidentified (1 sp.)   |
|             | Megachilidae | <i>Coelioxys</i> sp., <i>Euaspis</i> spp. (2 spp.), <i>Lithurge</i> sp., <i>Megachile hera</i> Bingham, <i>M. disjuncta</i> (Fabricius), <i>M. ampulata</i> Smith, <i>Megachile</i> spp. (15 spp.), Unidentified (1 sp.)  |
|             | Mutillidae   | <i>Trogaspidia</i> sp.  |
|             | Pompilidae   | <i>Pompilus</i> spp. (2 spp.)   |

Table 5 (Continued)

| Orders      | Families    | Scientific Name   |
|-------------|-------------|---|
| Hymenoptera | Scoliidae   | <i>Camsomeris collaris 4-fasciata</i> Fabricius,<br><i>Camsomeris phalerata</i> Saussure, <i>Liacos</i> sp.,<br><i>Megascolia azurea rubiginosa</i> Fabricius, <i>Scolia</i><br><i>quadripustulata humeralis</i> Saussure,<br><i>Scolia</i> spp. (4 spp.), Unidentified (5 spp.)  |
|             | Sphecidae   | <i>Chalybion bengalense</i> (Dahlbom), <i>Chlorion</i><br><i>lobatum</i> (Fabricius), <i>Chlorion</i> spp. (2 spp.),<br><i>Episylon</i> sp., <i>Liris</i> sp., <i>Sceliphron javanum</i><br>(Lepeletier), <i>Sphex argentatus</i> Fabricius,<br><i>Sphex sericeus lineolus</i> Lepeletier,<br><i>Sphex viduatus</i> Christ, <i>Sphex</i> spp. (2 spp.)  |
|             | Vespididae  | <i>Apodynerus</i> sp., <i>Auterhynchium</i> sp., <i>Delta</i><br><i>esuriens</i> Fabricius, <i>Delta</i> spp. (5 spp.), <i>Eumenes</i><br><i>conica</i> Fabricius, <i>Eumenes</i> spp. (3 spp.),<br><i>Phimenes</i> spp. (2 spp.), <i>Polistes stigma</i><br>(Fabricius), <i>Polistes</i> spp. (5 spp.), <i>Rhynchium</i><br><i>haemorrhoidala</i> (Fabricius), <i>R. quinquecinctum</i><br>(Fabricius), <i>Vespa affinis</i> (Linnaeus),<br><i>Vespa</i> spp. (6 spp.) |
|             |             |   |
| Lepidoptera | Acraeidae   | <i>Acraea violae</i> Fabricius  |
|             | Arctiidae   | <i>Amata sperbius</i> Fabricius, <i>Amata</i> sp., <i>Argina</i> sp.,<br><i>Euchromia elegantissima</i> Wallgram,<br>Unidentified (2 spp.)  |
|             | Danaidae    | <i>Danaus chrysippus chrysippus</i> (Linnaeus),<br><i>D. genutia genutia</i> (Cramer), <i>Euploea aglae</i><br><i>limborgii</i> Moore, <i>E. core godartii</i> (Lucas),<br><i>E. klugii erichsonii</i> Felder, <i>Euploea</i> sp.,<br><i>Ideopsis</i> sp.   |
|             | Gelechiidae | Unidentified (1 sp.)  |

**Table 5** (Continued)

| Orders      | Families     | Scientific Name  |
|-------------|--------------|--|
| Lepidoptera | Geometridae  | Unidentified (1 sp.)   |
|             | Hesperiidae  | <i>Caltoris bromus bromus</i> Leech, <i>Spialia galba</i> (Fabricius), <i>Telicota linna</i> Evans, Unidentified (4 spp.)  |
|             | Lycaenidae   | <i>Amblypodia anita anita</i> Hewitson, <i>Cyclosia panthona</i> Cramer, <i>Everes lacturnus rileyi</i> Godfrey, <i>Loxura atymnus continentalis</i> Fruhstofer, <i>Rapala pheretima petosiris</i> (Hewitson), <i>Spindasis syama terana</i> (Fruhstorfer), <i>Surendra quercetorum quercetorum</i> (Moore), <i>Zizina otis sangra</i> (Moore), Unidentified (1 sp.)   |
|             | Noctuidae    | Unidentified (1 sp.)   |
|             | Nymphalidae  | <i>Cethosia cyane euanthus</i> Fruhstorfer, <i>Cirrochoa tyche mithila</i> Moore, <i>Junonia</i> sp., <i>Neptis hylas kamarupa</i> Moore, <i>Tanaecia</i> sp., Unidentified (1 sp.)  |
|             | Papilionidae | <i>Chilasa clytia clytia</i> (Evans), <i>Graphium agamemnon agamemnon</i> Linnaeus, <i>G. doson axion</i> (Felder), <i>Lamproptera meges virescens</i> (Butler), <i>Pachliopta aristolochiae goniopeltis</i> (Rothschild), <i>Papilio demoleus malayanus</i> Wallace, <i>Papilio memnon agenor</i> Linnaeus, <i>Papilio polytes romulus</i> Cramer, <i>Pathysa antiphates pompilius</i> (Fabricius), <i>Troides aeacus aeacus</i> Felder |

**Table 5** (Continued)

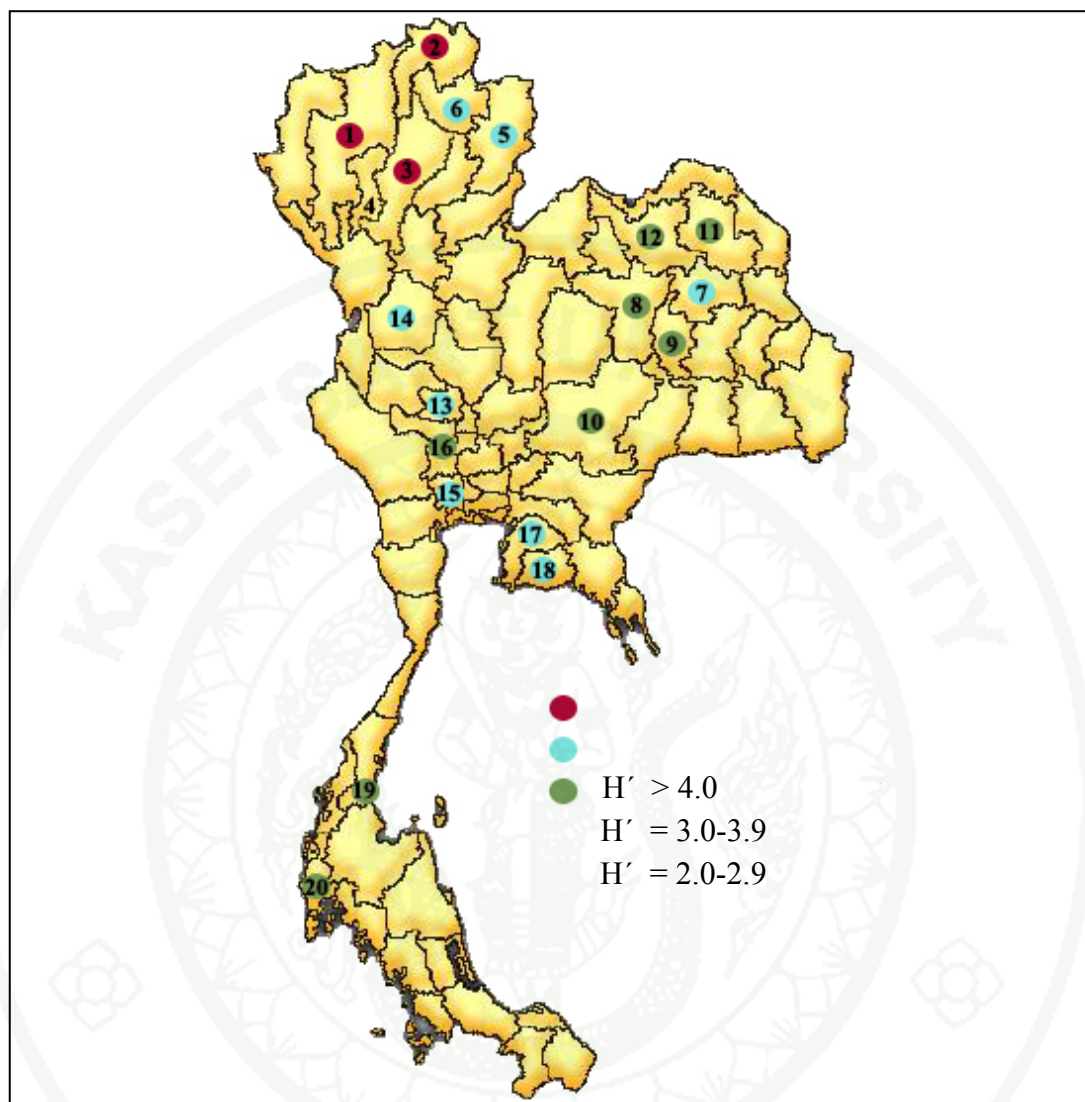
| Orders      | Families       | Scientific Name   |  |
|-------------|----------------|---|--|
| Lepidoptera | Pieridae       | <i>Appias albina darada</i> (Felder), <i>A. olferna olferna</i> Fruhstorfer, <i>Catopsilia pomona pomona</i> (Fabricius), <i>Eurema</i> sp., <i>Ixias pyrene yunnanensis</i> (Druce), <i>Leptosia nina nina</i> (Fabricius) |  |
|             |                | Pyrilidae   | Unidentified (2 spp.)                                  |
|             |                | Satyridae   | <i>Mycalasis</i> sp., <i>Ypthima</i> sp.               |
|             |                | Sessidae  | <i>Melitta</i> spp. (3 spp.)                           |
|             | Sphingidae     | <i>Cephonodes hylas hylas</i> (Linnaeus)  |  |
|             | Tortricidae    | Unidentified (1 sp.)  |  |
|             | Mantodea       | Mantidae  | <i>Mantis religiosa</i> Linnaeus, Unidentified (1 sp.) |
| Orthoptera  | Acrididae      | Unidentified (1 sp.)  |  |
|             | Tettrigoniidae | Unidentified (1 sp.)  |  |

**Table 6** Number of families, genera, species and diversity percentage of insect pollinators found on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Orders      | Families | Genera | Species | Diversity (%) |
|-------------|----------|--------|---------|---------------|
| Blattodea   | 1        | 1      | 1       | 0.32          |
| Coleoptera  | 12       | 15     | 31      | 9.97          |
| Diptera     | 15       | 19     | 48      | 15.43         |
| Hemiptera   | 6        | 14     | 23      | 7.40          |
| Hymenoptera | 11       | 47     | 140     | 45.02         |
| Lepidoptera | 16       | 39     | 64      | 20.58         |
| Mantodea    | 1        | 1      | 2       | 0.64          |
| Orthoptera  | 2        | 2      | 2       | 0.64          |
| Total       | 64       | 138    | 311     | 100           |

**Table 7** Species diversity indices ( $H'$ ) of insect pollinators on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Thailand Regions | Provinces         | Species diversity indices ( $H'$ ) |
|------------------|-------------------|------------------------------------|
| North            | Chiang Mai        | 4.3                                |
|                  | Chiang Rai        | 4.5                                |
|                  | Lampang           | 4.1                                |
|                  | Lamphun           | 3.8                                |
|                  | Nan               | 3.8                                |
|                  | Phayao            | 3.8                                |
| Northeast        | Kalasin           | 3.1                                |
|                  | Khon Kaen         | 2.5                                |
|                  | Maha Sarakham     | 2.5                                |
|                  | Nakhon Ratchasima | 2.9                                |
|                  | Sakon Nakhon      | 2.8                                |
|                  | Udon Thani        | 2.8                                |
| Central          | Chai Nat          | 3.4                                |
|                  | Kamphaeng Phet    | 3.4                                |
|                  | Nakhon Pathom     | 3.2                                |
|                  | Suphan Buri       | 2.9                                |
| East             | Chon Buri         | 3.0                                |
|                  | Rayong            | 3.3                                |
| South            | Chumphon          | 2.5                                |
|                  | Phangnga          | 2.9                                |



**Figure 8** Species diversity indices ( $H'$ ) of insect pollinators on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

1 = Chiang Mai, 2 = Chiang Rai, 3 = Lampang, 4 = Lamphun,  
 5 = Nan, 6 = Phayao, 7 = Kalasin, 8 = Khon Kaen, 9 = Maha Sarakham,  
 10 = Nakhon Ratchasima, 11 = Sakon Nakhon, 12 = Udon Thani,  
 13 = Chai Nat, 14 = Kamphaeng Phet, 15 = Nakhon Pathom,  
 16 = Suphan Buri, 17 = Chon Buri, 18 = Rayong, 19 = Chumphon,  
 and 20 = Phangnga

### 1.3 Grouping of insect pollinators

The insect pollinators were divided by their behavior and the frequency of their occurrence on flowers (Appendix Table 3). The first major group belonged to the colonized or socialized bees, honey bees and stingless honey bees (*Apis* spp. and *Trigona* spp.: Apidae, Hymenoptera). They and their broods depended on both nectar and pollen (Figure 9). Some flies in the order Diptera, such as blow fly (*Chrysomya megacephala*: Calliphoridae) and syrphid flies (*Eristalis arvorum*, *E. obscuritarsis*, *Helophilus bengaliensis* and *Rhingia* spp.: Syrphidae) were observed to gather only nectar but some fed on both nectar and pollen. These insects in this group were found as the highest population in physic nut plantation (Figure 10). The second minor group, which was not so often encountered on the physic nut flowers were other Apidae, Chrysididae, Evaniidae, Formicidae, Halictidae, Megachilidae, Pompilidae, Scoliidae, Sphecidae and Vespidae (Figure 11). Other Blattodea, Coleoptera, Diptera, Hemiptera, Lepidoptera and Orthoptera were found positively gathering in both nectar and pollen (Figure 12-15). Some insects of the minor group were insect pests (*Callidea* sp., *Chrysocoris grandis* and *C. stollii*) of physic nut (Figure 16). Within this minor group there were also predators of the insect pollinators, such as some insects in the families of Reduviidae and Mantidae, and spiders (Figure 17).

The insect pollinators recorded during the present study can be divided into “major” and “minor” groups as in Malaipan *et al.* (2007) they reported that some social bees such as *Apis* spp. and *Trigona* spp. and large populations of Diptera such as Calliphoridae (*C. megacephala*) and Syrphidae (*E. arvorum*, *E. obscuritarsis*, *H. bengaliensis* and *Rhingia* spp.) were found in major group. The minor group consisted of some insect pollinators belonging to the Coleoptera, other Diptera, Hemiptera, other Hymenoptera, Lepidoptera, and Thysanoptera. Raju and Ezradanam (2002) reported that bees were the most abundant pollinators. When floral structure and the adaptive features of the insects are considered, honey bees (*Apis dorsata*, *A. florea* and *A. mellifera*) are considered to be the most effective pollinators. Species of *Eumenes*, *Vespa*, and the beetles are not considered as

effective pollinators since the pollination syndromes of these visitors do not match with physic nut flowers. The bees encountered in their study mostly collected floral rewards on different, conspecific plants and thus promoted cross- (or xenogamous) pollination, over pollination within the same, individual plant (geitonogamous pollination).



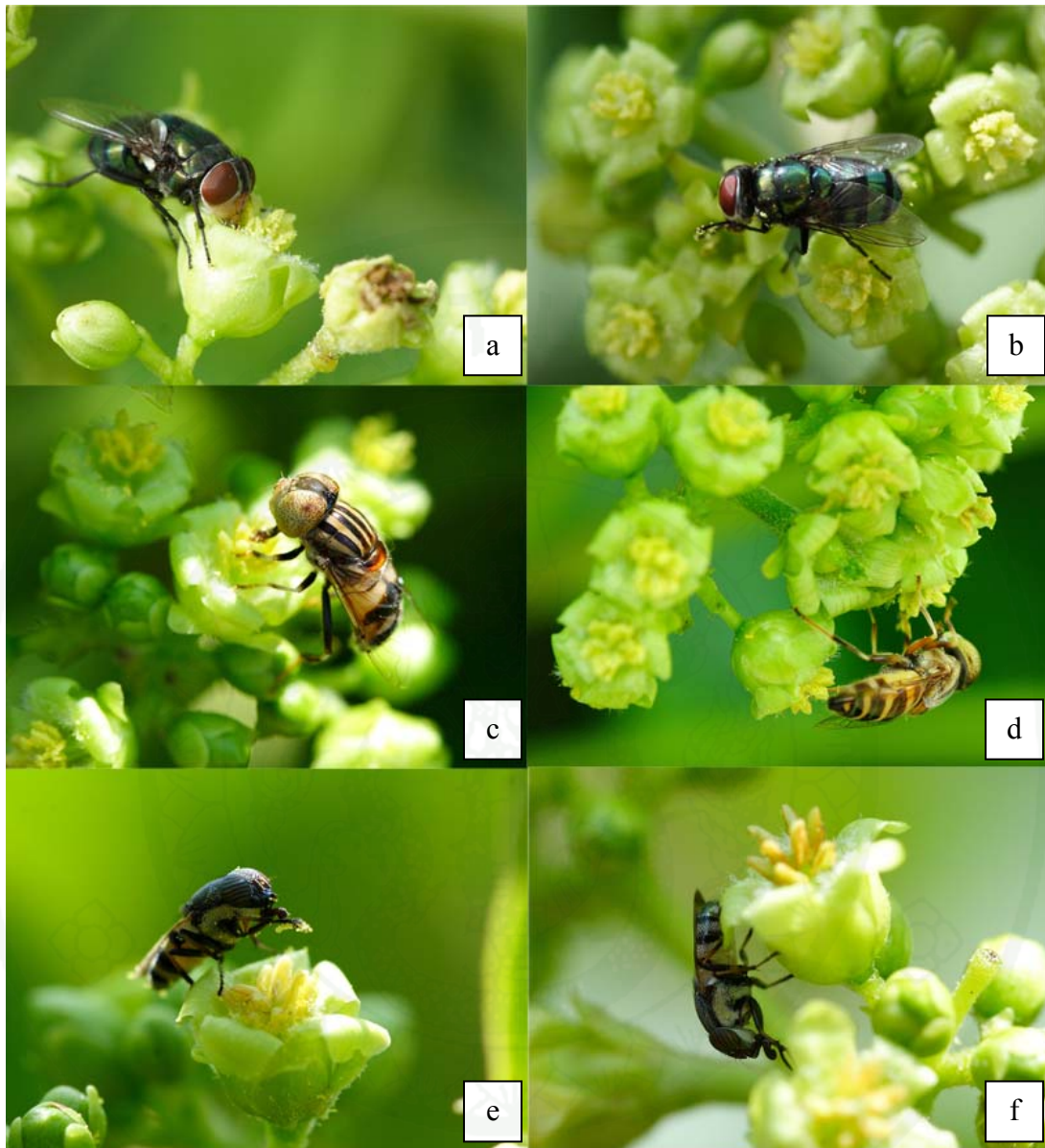


**Figure 9** Major group: *Apis* spp. and *Trigona* spp. visited on *Jatropa curcas* L. flowers in 20 provinces from April to August 2009

Apidae; a = *Apis cerana indica*, b = *A. dorsata*, c = *A. florea*,  
 d = *A. mellifera ligustica*, e = *Trigona pagdeni* on female flower,  
 f = *T. pagdeni* on male flower

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

Diameter of physic nut female flower is  $0.9 \pm 0.1$  cm



**Figure 10** Major group: Calliphoridae and Syrphidae visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Calliphoridae; a,b = *Chrysomya megacephala*

Syrphidae; c,d = *Eristalis* spp., e,f = *Rhingia* sp.

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

**Figure 11** Minor group: other Hymenoptera visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Apidae; a, b = *Ceratina* spp., c = *Phithitis smaragdula*,  
d = *Trigona collina*

Chrysididae; e = *Stilbum cyanarum*

Formicidae; f = *Solenopsis geminata*

Halictidae; g = *Nomia albofasciata*, h = *Nomia* sp.

Megachilidae; i = *Megachile ampulata*

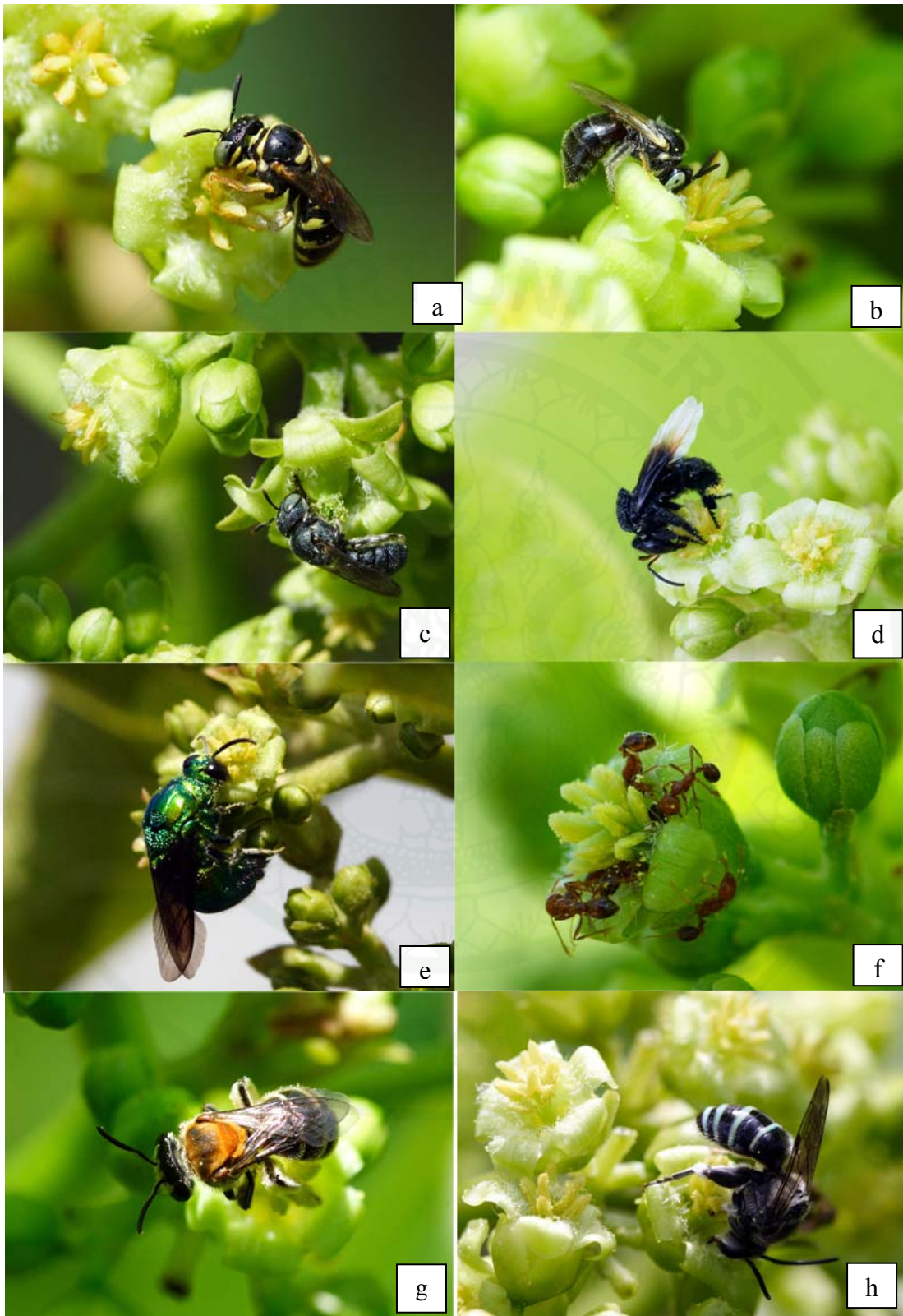
Scoliidae; j = *Camsomeris collaris 4-fasciata*

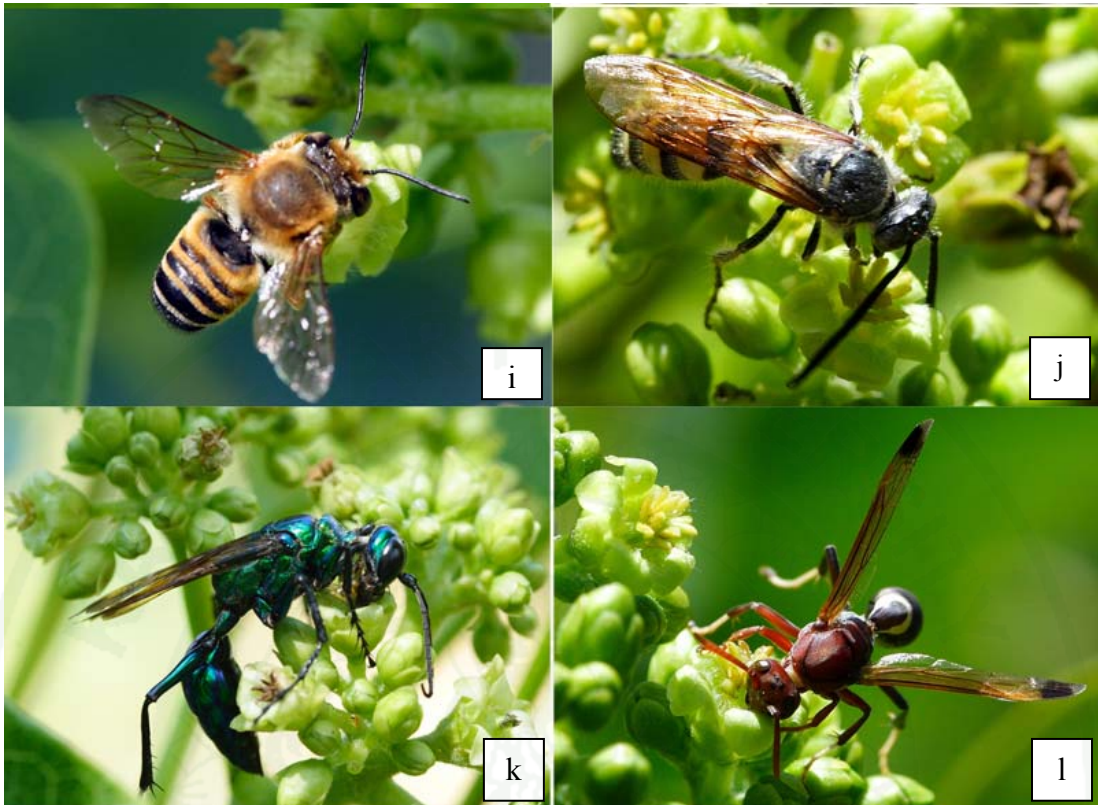
Sphecidae; k = *Chlorion lobatum*,

Vespidae; l = *Polistes stigma*

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

Diameter of physic nut female flower is  $0.9 \pm 0.1$  cm







**Figure 12** Minor group: Coleoptera visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Brentidae; a = *Eubactus* sp.

Cerambycidae; b = *Chlorophorus annularis*, and c = *Polyzonus* sp.

Chrysomelidae; d = *Aulacophora* sp.

Elateridae; e = *Diploconus* sp.

Scarabaeidae; f = *Ixorida mouhotii*

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm



**Figure 13** Minor group: other Diptera visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Calliphoridae; a = *Hypopygropsis* sp.

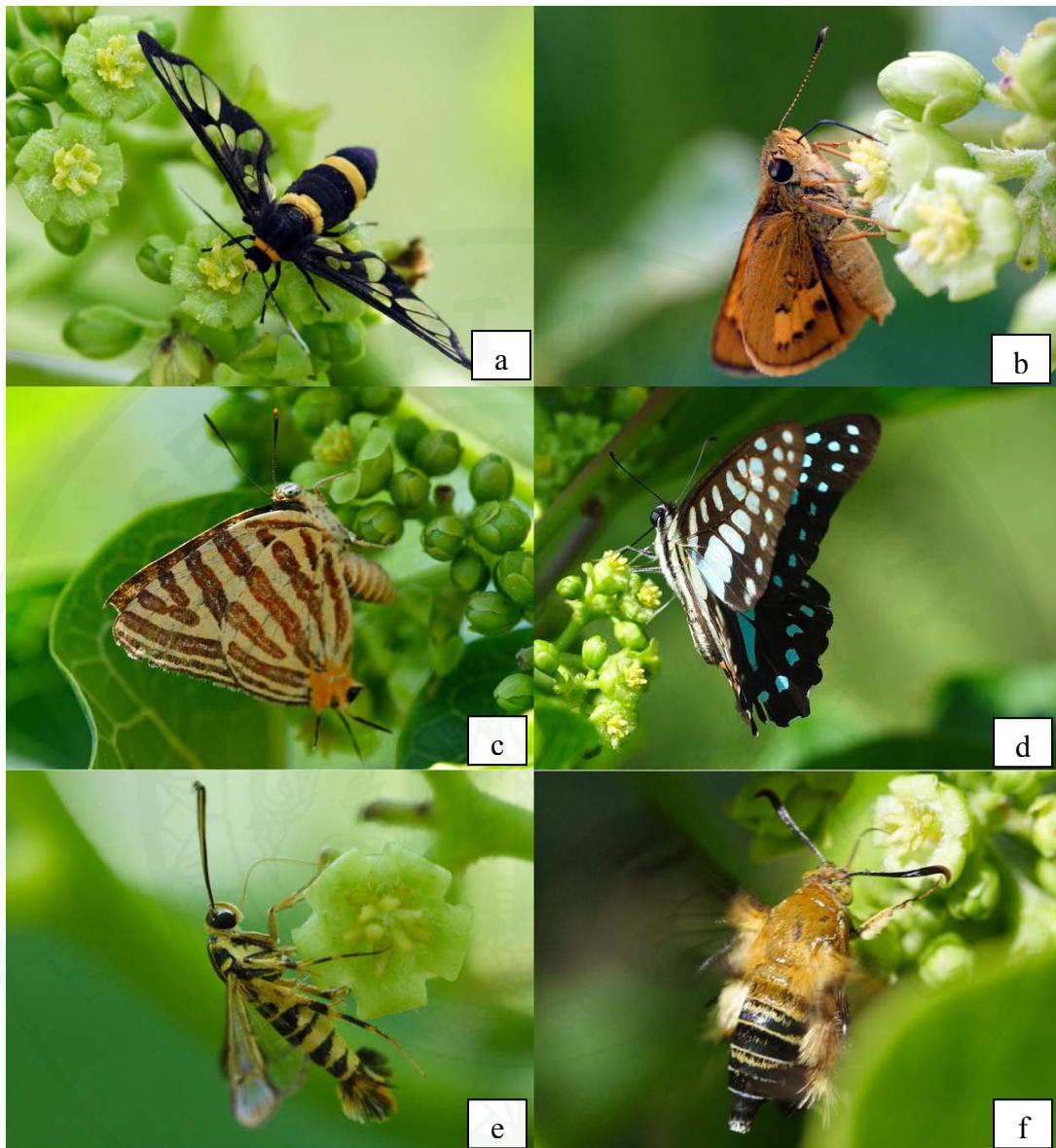
Empididae; b = *Hilara* sp.

Syrphidae; c = *Helophilus bengalensis*

Tabanidae; d = *Chrysops dispar*, and e = *C. fasciata*

Tachinidae; f = *Drino* sp.

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm



**Figure 14** Minor group: Lepidoptera visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Arctiidae; a = *Amata sperbius*,

Hesperiidae; b = *Telicota linna*

Lycaenidae; c = *Spindasis syama terana*

Papilionidae; d = *Graphium doson axion*

Sessidae; e = *Melitta* sp., Sphingidae; f = *Cephonodes hylas hylas*

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm



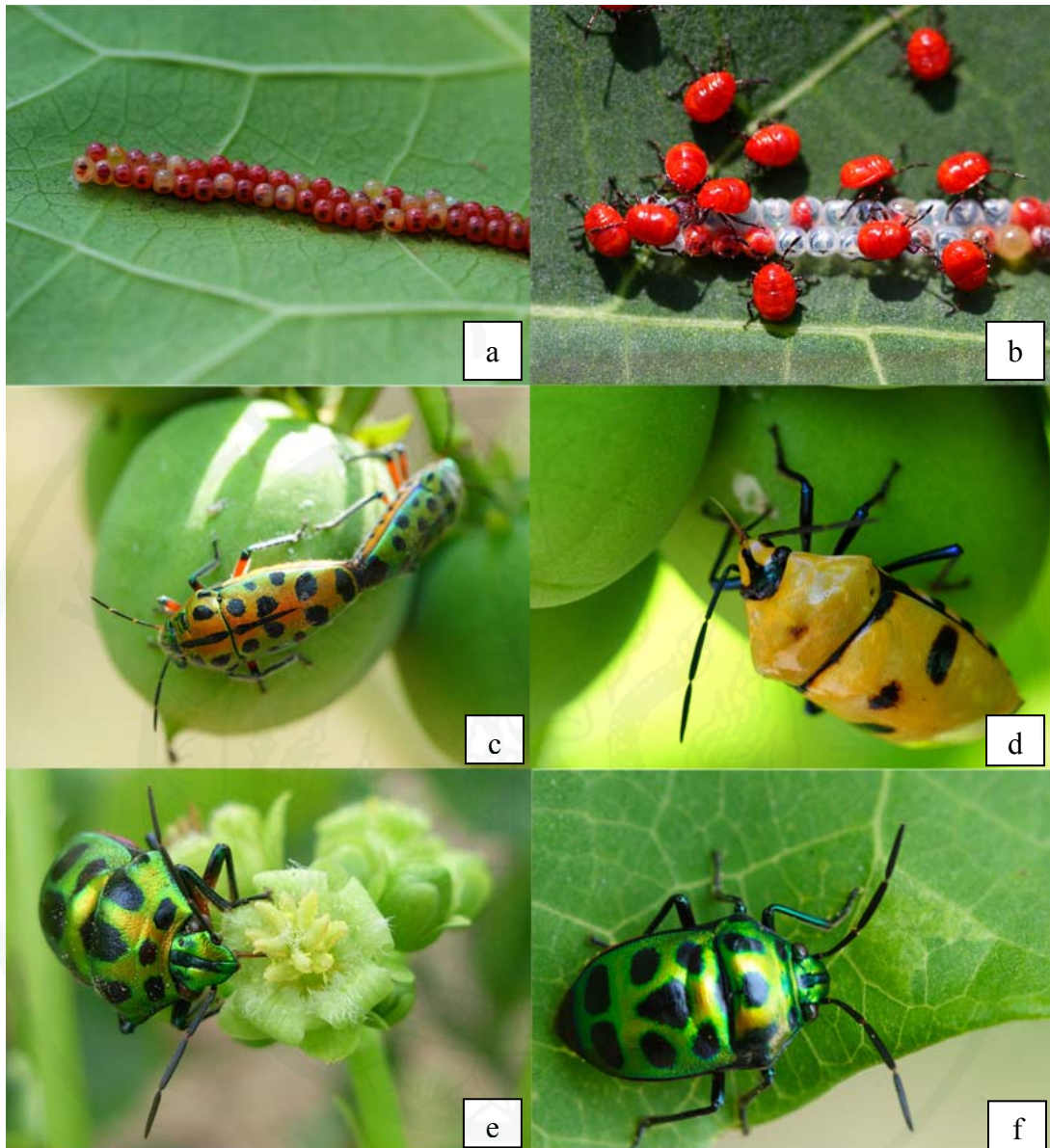
**Figure 15** Minor group: Hemiptera, Blattodea and Orthoptera visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Blatodea; a = Blattellidae

Hemiptera; b = *Riptortus linearis*, and c = *Serinetha abdominalis*  
(Coreidae)

Orthoptera; d = Acrididae

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm



**Figure 16** Minor group: pests of physic nut visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

a = eggs, b = nymphs, c = mating adults of *Callidea* sp.,  
 d = *Chrysocoris grandis*, and e, f = *C. stollii* (Scutelleridae)  
 Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

**Figure 17** Minor group: predators visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

Hemiptera; a = *Sycanus collaris* (Reduviidae),

b = *S. collaris* is eating *Polyzonus* sp. (Cerambycidae),

Mantodea; c = Mantidae is eating *Chrysomyia megacephala* (Calliphoridae),

d = *Mantis religiosa* (Mantidae) is eating *Chalybion benjalense* (Sphecidae), and e, f = spiders

g = spider is eating *Apis cerana indica*,

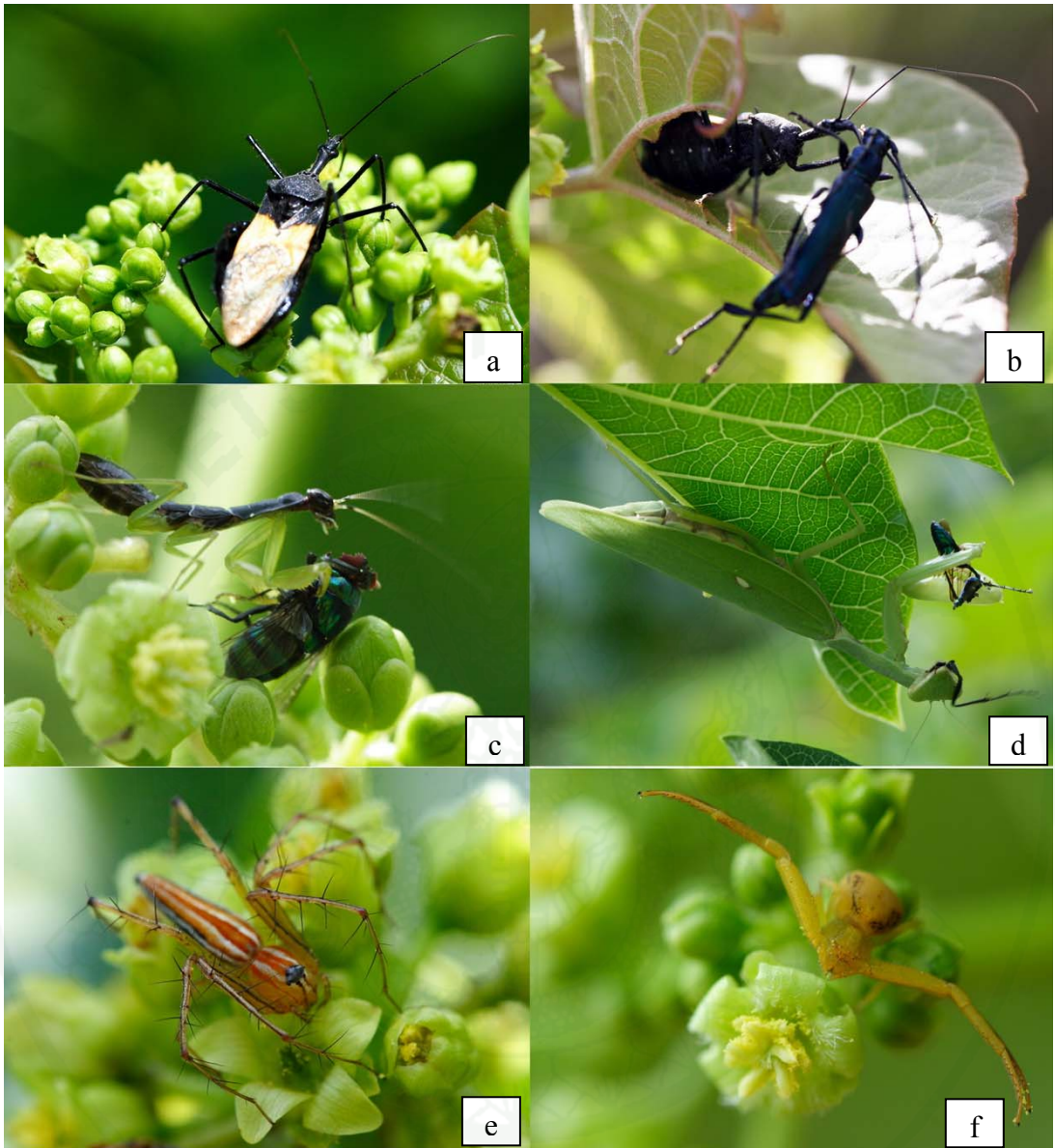
h = spider is eating *A. florea*,

i = spider is eating *A. cerana indica*,

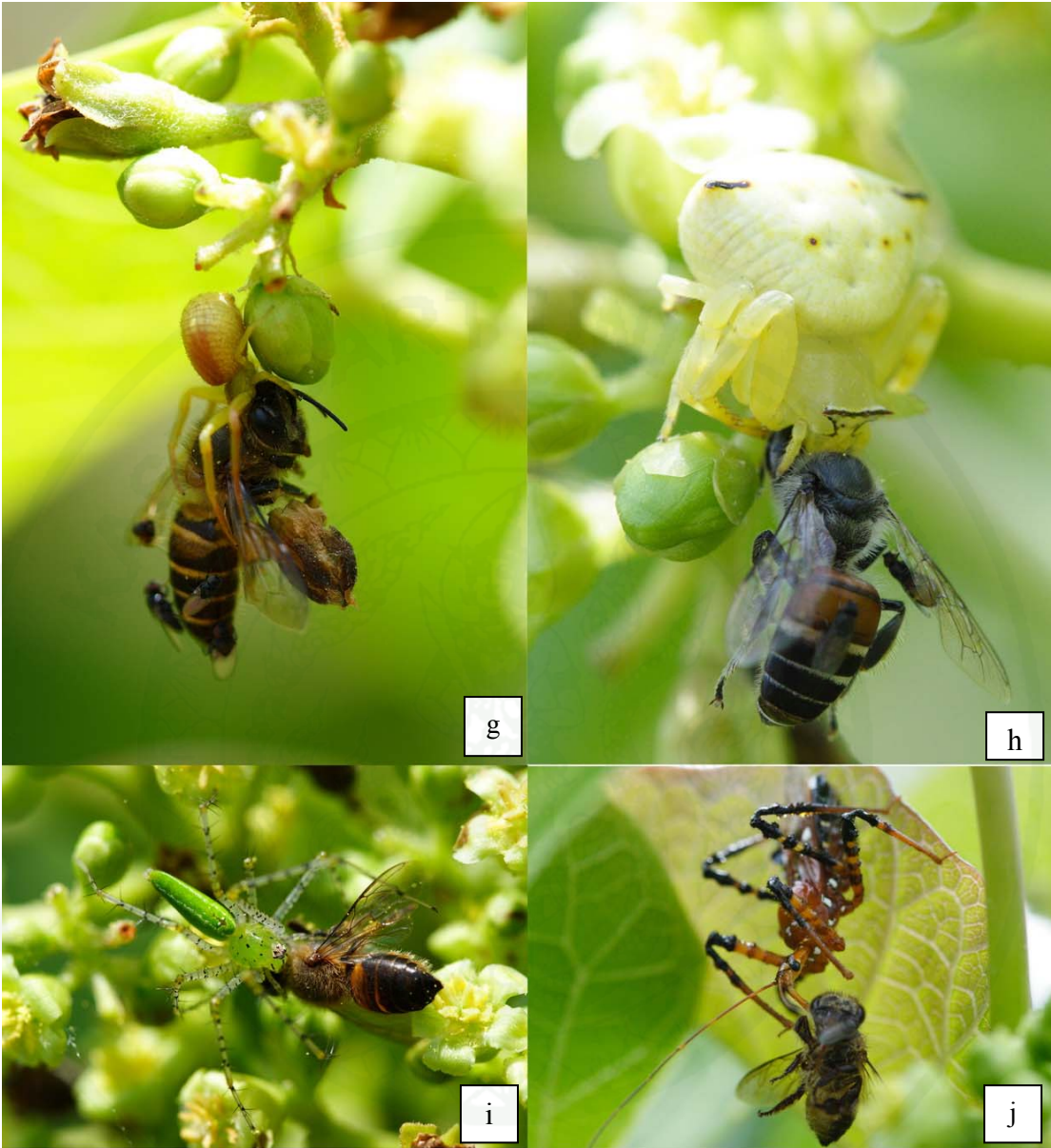
j = *Rhynocoris* sp. (Reduviidae) is eating *A. cerana indica*

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

Diameter of physic nut female flower is  $0.9 \pm 0.1$  cm



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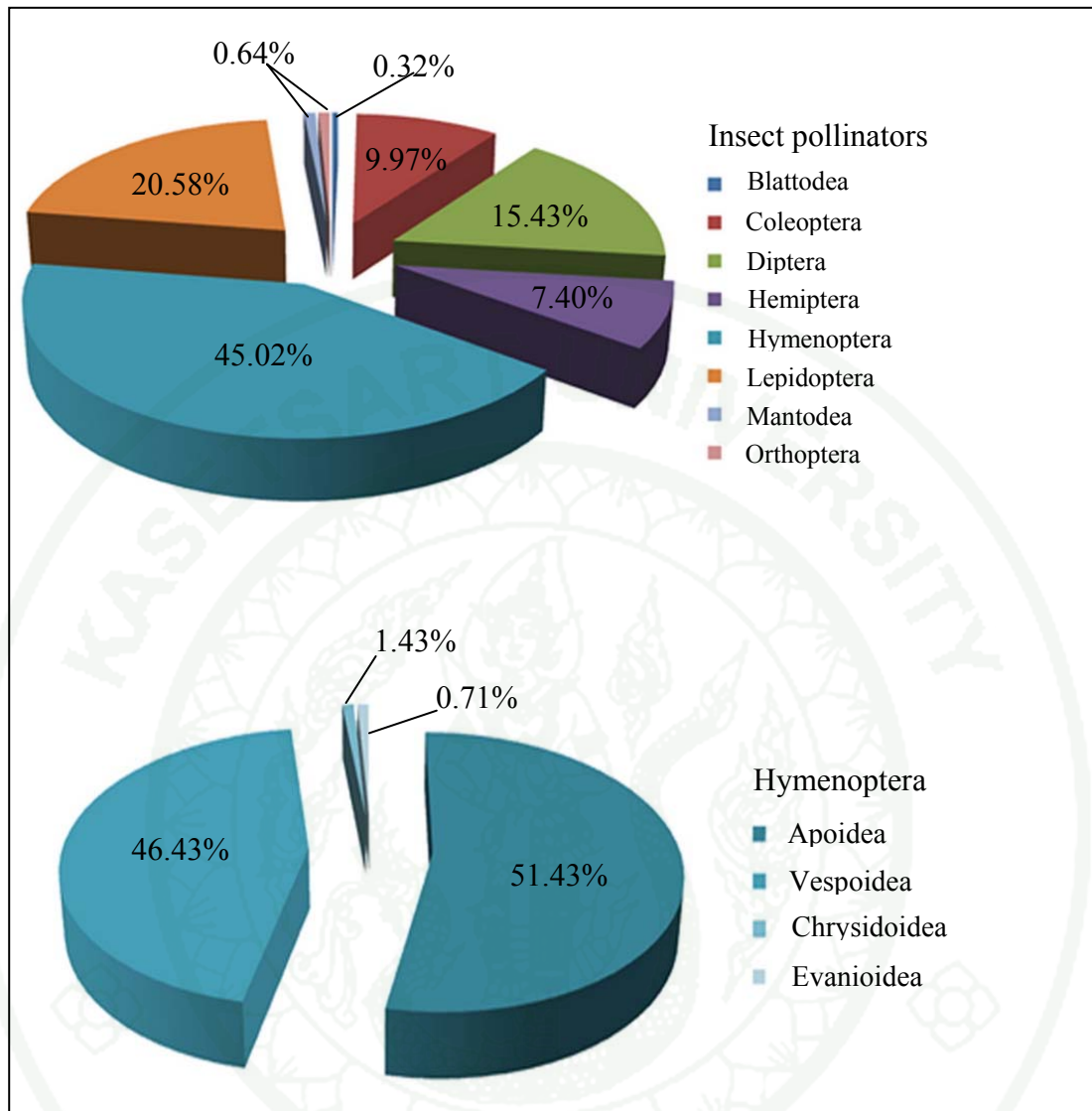


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#### 1.4 Diversity of Apoidea

This study focused to Apoidea, because of this superfamily was found in the highest number of species and the highest of frequency of occurrence in 20 provinces, that bees had advantage as alert activity, cross-pollination, effective pollinators, on the major group such as *Apis* spp., and *Trigona* spp. (Apidae: Hymenoptera) and on some minor group i.e. other Hymenoptera. These Hymenoptera were divided into four superfamily such as Apoidea (51.43%), Vespoidea (46.43%), Chrysidoidea (1.43%) and Evanoidea (0.71%) (Figure 18, Appendix Table 4).

Apoidea were divided into Apiformes (60 spp.) and Spheciformes (12 spp.). Apiformes were chosen for pollination topic, while Spheciformes in Sphecidae such as *Chalybion benjalense*, *Chlorion lobatum*, *Chlorion* spp. (2 spp.), *Episylon* sp., *Liris* sp., *Sceliphron javanum*, *Sphex argentatus*, *S. sericeus lineolus*, *S. viduatus*, *Sphex* spp. (2 spp.) (Table 5) were ignored. Because of Sphecidae differ from Apiformes. Even though, adults sphecids feed at flowers and extra-floral nectarines but the larvae are exclusively carnivorous and the females of the various species provide their larvae with a wide range of insects and spiders as prey (Gauld and Bolton, 1988).



**Figure 18** Proportionality of eight orders and four superfamilies of Hymenoptera, that visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

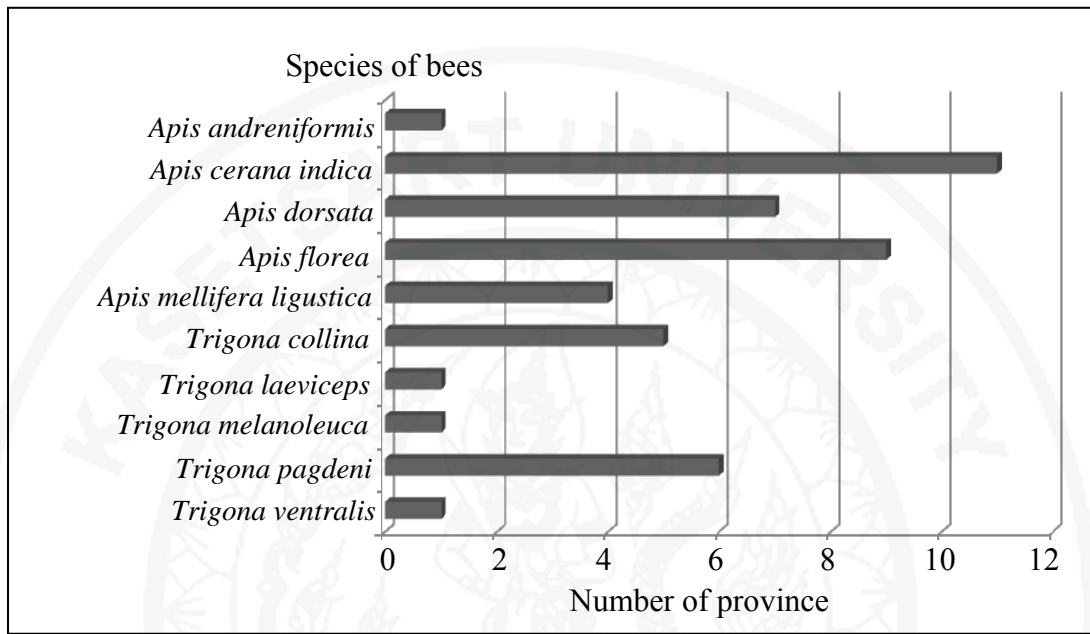
### 1.5 Distribution of Apoidea

Sixty species in Apoidea were different distribution among 20 provinces (Appendix Table 1 and 2). The highest frequency of occurrence (11 provinces) was Indian honey bees (*Apis cerana indica*) and small carpenter bees (*Ceratina* sp.1). Indian honey bees were found in Chiang Mai (CM), Chiang Rai (CR), Lampang (LP), Lamphun (LPH), Phayao (PHY), Kalasin (KS), Nakhon Pathom (NP), Suphan Buri (SB), Rayong (RY), Chumphon (CP), and Phangnga (PNG). Whereas small carpenter bees were found in Chiang Mai (CM), Chiang Rai (CR), Lampang (LP), Phayao (PHY), Kalasin (KS), Maha Sarakham (MS), Nakhon Ratchasima (NR), Sakon Nakhon (SN), Suphan Buri (SB), and Rayong (RY).

Three hundred and 11 species of insect visitors were collected from 20 provinces in Thailand. Ten efficient bees i.e. *A. andreniformis*, *A. cerana indica*, *A. dorsata*, *A. florea*, *A. mellifera ligustica*, *Trigona collina*, *T. laeviceps*, *T. melanoleuca*, *T. pagdeni*, and *T. ventralis*, were chosen for study pollinators belonging to the colonization or socialization bees. Indian honey bee (*A. cerana indica*) was found to be the highest frequency of occurrence (11 provinces) followed by dwarf honey bee (*A. florea*) (9 provinces), giant honey bee (*A. dorsata*) (7 provinces), and stingless honey bee (*T. pagdeni*) (6 provinces). The other honey bees were found in some provinces (1-5 provinces) (Figure 19 and Appendix Table 2).

The relatively widespread occurrence on physic nut of several species of social bees (Figure 19) is consistent with Bhattacharya *et al.*(2005) who suggested that, *A. dorsata*, *A. florea* and *A. mellifera* were the most common and effective pollinators. The present survey found 60 species of Apiformes in the superfamily Apoidea on physic nut. Of these, *A. cerana indica*, *A. florea*, *A. mellifera ligustica* and *T. pagdeni* would be the most appropriate subjects for more detailed studies to determine efficiency pollination in physic nut plantations. The present study revealed that most physic nut plantations in each province have a high diversity of insect pollinators and there are marked differences among 20 provinces.

This indicates that an intensive, various provinces investigation is needed to confirm the hypothesis that insect pollinators are an important factor for the successful pollination of physic nut.



**Figure 19** Frequencies of bee occurrence in visiting *Jatropa curcas* L. flowers in 20 provinces from April to August 2009

## 2. Variation of physic nuts (*Jatropha curcas* L.)

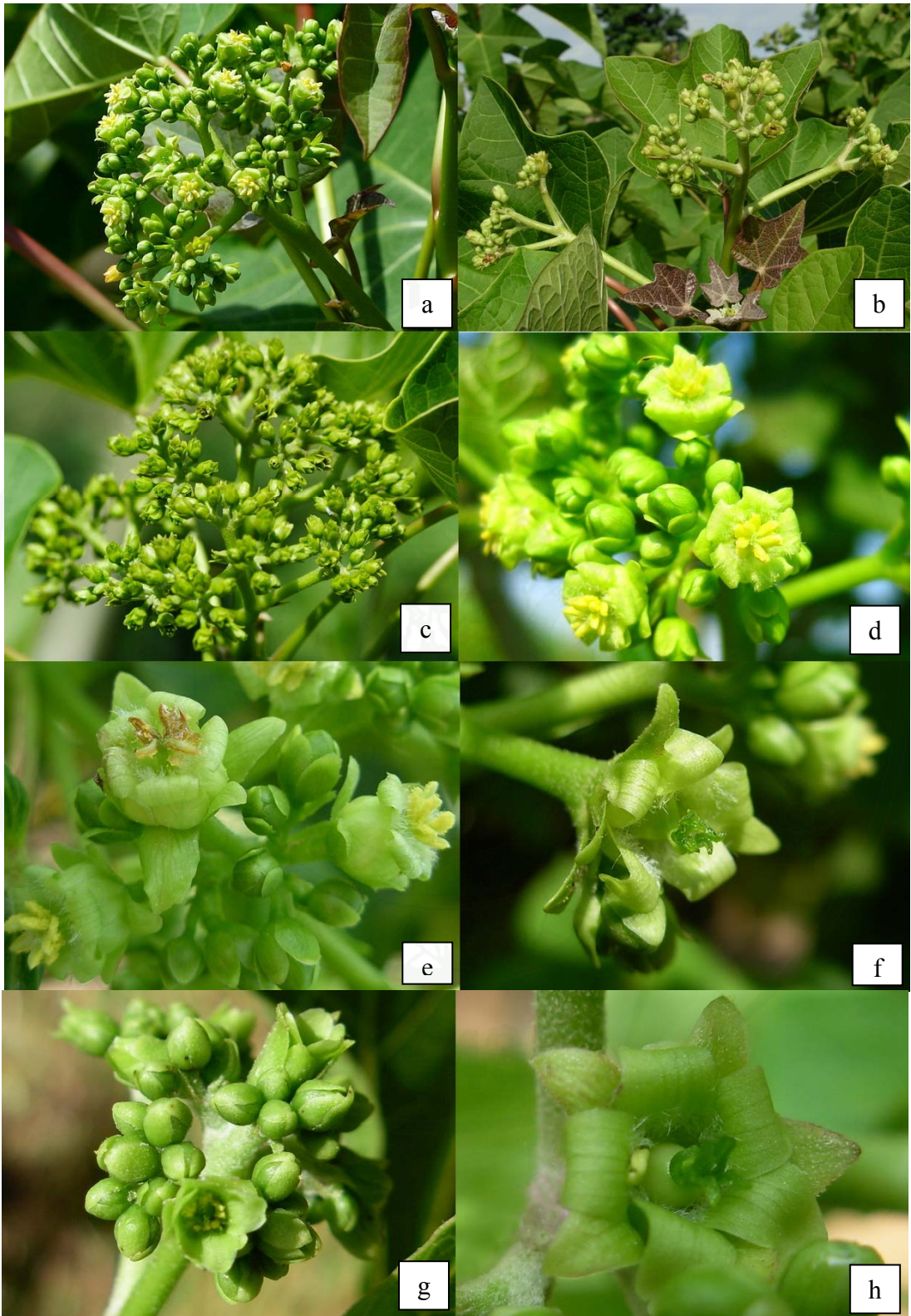
### 2.1 Flower types of physic nuts

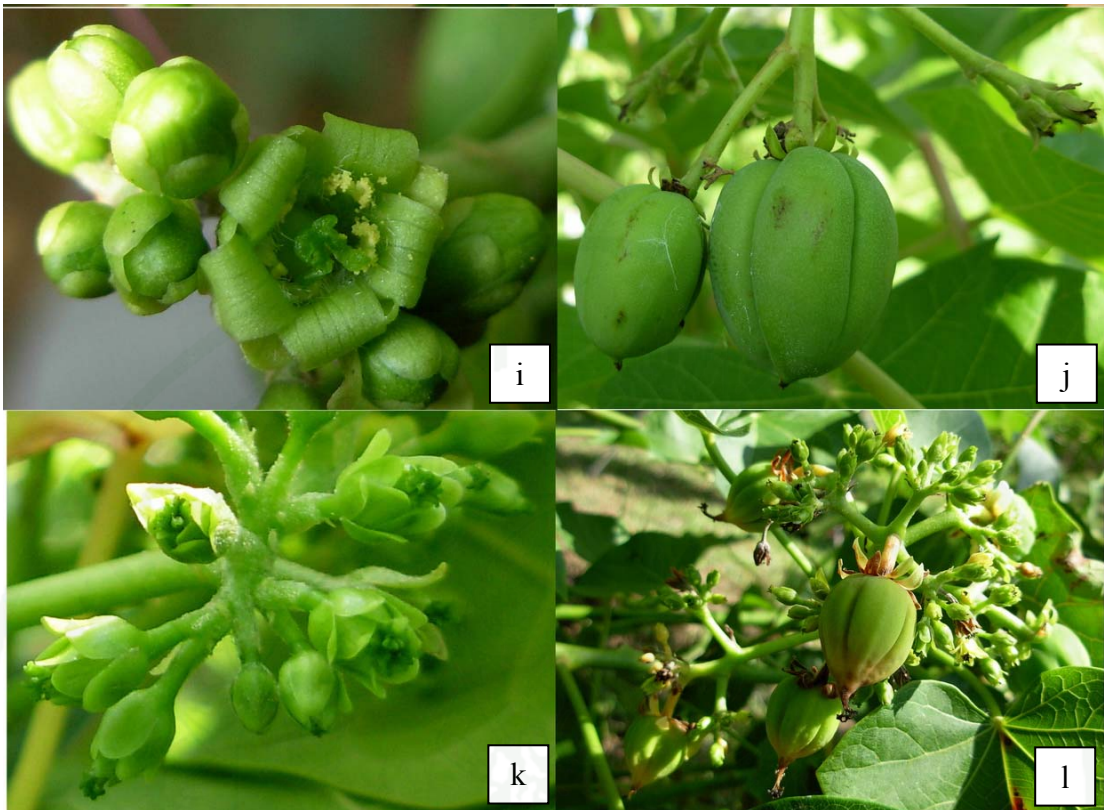
Flowers of all sites about 10,000 flowers (female and male) were classified to be three types i.e. androgynous, gynoecious, and polygamous of plant in both toxic and non-toxic variety. These plants were found to be androgynous or monoecious—with female and male flowers on the same plant and in the same inflorescence, polygamous—with female, male, and perfect (hermaphrodite) flowers in the same inflorescence, and gynoecious plant. This later type was found only in one plant at Nan (NA) province, that plant produced only female flowers in every inflorescence. Flowers of gynoecious plant are mutation, and without visiting by insect pollinators, always non-fertilization occurred or no pollen on stigma usually in F-grade. Most fruits from pistillate flowers are not completed development. The others of physic nut in this place were monoecious plants. In Suphan Buri (SB) experimental site found polygamous, and perfect flowers in the high rate 9:23:7 (female:male:perfect flowers) of sex ratio and some abnormal fruits (Figure 20).

These results argue that reports of Raju and Ezradanam (2002); Bhattacharya *et al.* (2005); Chang-wei *et al.* (2007a) that physic nut is monoecious—with female and male flowers on the same plant and in the same inflorescence (androgynous). These observations indicated that most physic nuts are monoecious, but some physic nuts are polygamous—female, male, and hermaphrodite floral morphogenesis all appear on the same plant and only plant was found producing female flowers only, this was gynoecious plant.

**Figure 20** Type of flowers and infloresces in *Jatropha curcas* L.

- a = Androgynous plants: female and male flowers in the same inflorescence
- b = Polygamous plants: female, male and perfect (hermaphrodite) flowers in the same inflorescence
- c = Gynoecious plants: producing only pistillate flowers.
- d = Staminate flowers, diameter of male flowers is  $0.7\pm 0.1$  cm
- e = Larger male flower at the female flowers position in inflorescence
- f = General female flower, diameter of female flowers is  $0.9\pm 0.1$  cm
- g, h, i = Various of anther number on perfect flowers
- j = Abnormal fruit from perfect flowers
- k = Female flowers from gynoecious plant in Nan (NA) province
- l = Abnormal fruits from gynoecious plant





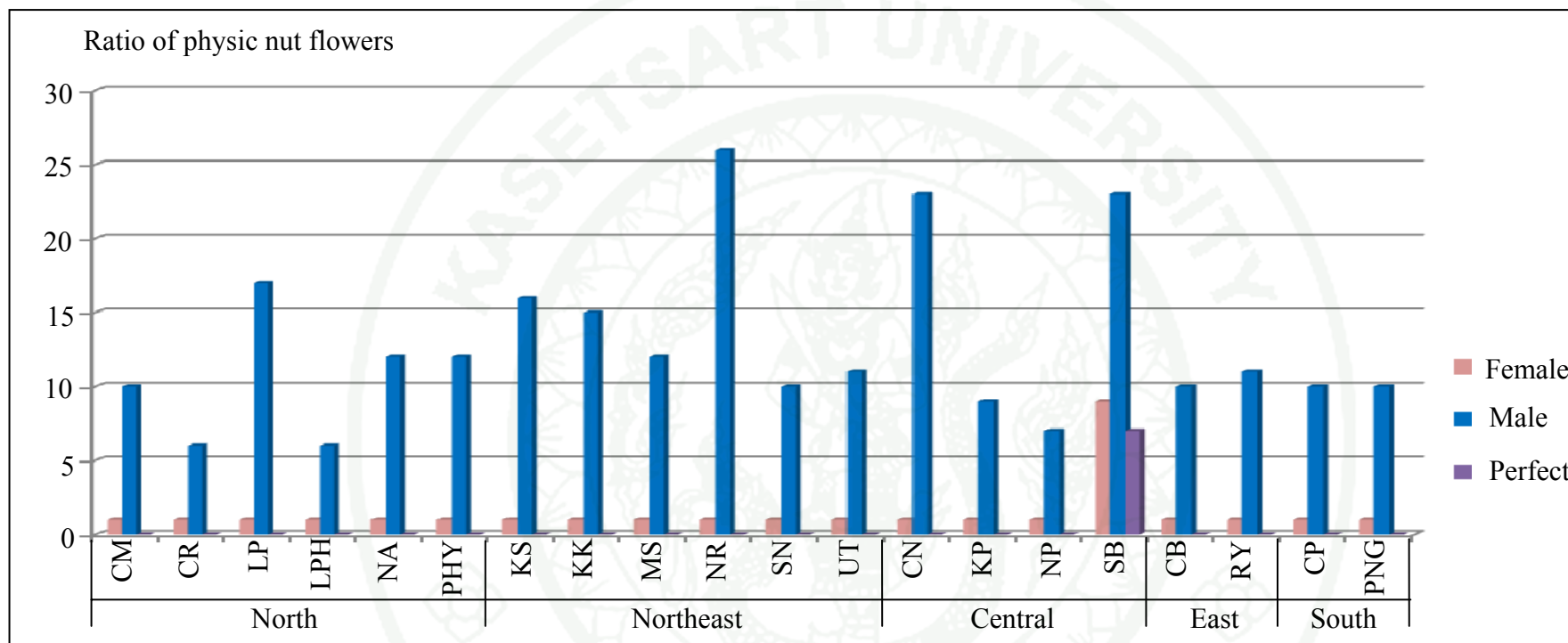
## 2.2 Flower sex ratios of physic nut

About 10,000 flowers of all sites were classified to be three types of flowers i.e. female, male and perfect flowers. The number of female and male flowers per inflorescence varies a lot among 20 provinces in the rainy season. Flowers sex ratios in each province revealed slightly different ratio (female:male). Figure 21 and Appendix Table 5 showed such flower ratio of 20 provinces, these ratios (female:male) were sorted from the highest to the lowest ratio; Chiang Rai (CR), and Lamphun (LPH) (1:6), Nakhon Pathom (NP) (1:7), Kamphaeng Phet (KP) (1:9), Chiang Mai (CM), Chumphon (CP), Sakon Nakhon (SN), Chon Buri (CB) and Phangnga (PNG) (1:10), Udon Thani (UT) and Rayong (RY), (1:11), Nan (NA), Phayao (PHY) and Maha Sarakham (MS) (1:12), Khon Kaen (KK) (1:15), Kalasin (KS) (1:16), Lampang (LP) (1:17), Chai Nat (CN) (1:23), and Nakhon Ratchasima (NR) (1:26). Except sex ratio in Suphan Buri (SB) there was 9:23:7 (female:male:perfect).

However, Dehgan and Webster (1979); Kumar and Sharma (2008) are similar in that a perfect or hermaphrodite flowers occasionally and rarely occurred in the hot seasons (unknown variety). Likewise, perfect flowers very rare occurred in toxic variety, but in non-toxic variety at Suphan Buri (SB) province usually found perfect flowers all year. The sex ratios are similar to Raju and Ezradanam (2002), and Chang-wei *et al.* (2007a), they recorded number of female and male flowers per inflorescence varies a lot among the observations (1-19 female flowers, and 25-238 male flowers), and the female to male flower ratios were reported it was similar (2:49 and 1:29). In contrast, sex ratios of physic nut in these observations are higher than previously studies. It might be possible that were observed in the dry season, but these studies were observed only in the rainy season. Various flower sex ratios were found in different seasons. Likewise, Dhillon *et al.* (2006) observed flowering at the terminal end of branches after the rainy season in India. However, some plants flower even in the spring season (March-April). The average female to male (F/M) flower ratio was 1:20, which changes drastically (1:108) with the fall in temperature. Usual peak period of flowering varies from 3-20 days. In additional,

Prakash *et al.* (2007) reported variation in the F/M ratio during the first year with average F/M ratio of 1:25, and it increased to 1:13 in the second year indicating a positive trend towards productivity.





**Figure 21** Types and sex ratios of *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

CM = Chiang Mai, CR = Chiang Rai, LP = Lampang, LPH = Lamphun, NA = Nan, PHY = Phayao, KS = Kalasin, KK = Khon Kaen, MS = Maha Sarakham, NR = Nakhon Ratchasima, SN = Sakon Nakhon, UT = Udon Thani, CN = Chai Nat, KP = Kamphaeng Phet, NP = Nakhon Pathom, SB = Suphan Buri, CB = Chon Buri, RY = Rayong, CP = Chumphon, and PNG = Phangnga

### 2.3 Effective factors to the yield of physic nut plantations

Table 8 summarized various factors such as number of fruits per plant, fruits per inflorescence, number of plants per rai and spacing, plants propagation and pruning, plantation and irrigation, age variation had effect on yield per rai (1 rai=1,600 m<sup>2</sup>) that important yields were dry seeds and oil of physic nut.

#### 2.3.1 Number of inflorescences per plant

Number of inflorescences per plant were different among 20 provinces, the highest number of inflorescence were observed in Chiang Rai (CR) (504 inflorescences/plant) followed by Chai Nat (CN) (270 inflorescences/plant), Nakhon Ratchasima (NR) (198 inflorescences/plant), Phayao (PHY) (185 inflorescences/plant), Lampang (LP) (175 inflorescences/plant), Nakhon Pathom (NP) (170 inflorescences/plant), Nan (NA) (120 inflorescences/plant), Rayong (RY) (100 inflorescences/plant), Kamphaeng Phet (KP) (108 inflorescences/plant), Chiang Mai (CM) (96 inflorescences/plant), Sakon Nakhon (SN) (60 inflorescences/plant), Udon Thani (UT) (48 inflorescences/plant), Suphan Buri (SB) (45 inflorescences/plant), Phangnga (PNG) (36 inflorescences/plant), Kalasin (KS) and Chon Buri (CB) (30 inflorescences/plant), Maha Sarakham (MS) (25 inflorescences/plant), Chumphon (CP) (12 inflorescences/plant) and Lamphun (LPH) (10 inflorescences/plant), while the lowest value were found in Khon Kaen (KK) (6 inflorescences/plant).

#### 2.3.2 Fruits per inflorescence

Number of fruits per inflorescence were different among 20 provinces, the highest value were observed in Kalasin (KS) (14 fruits/inflorescence) followed by Nakhon Pathom (NP) (10 fruits/inflorescence), Khon Kaen (KK) (9 fruits/inflorescence), Chiang Rai (CR), Udon Thani (UT), Kamphaeng Phet (KP), Suphan Buri (SB) and Rayong (RY) (8 fruits/inflorescence), Lampang (LP), Lamphun (LPH), Nan (NA) and Nakhon Ratchasima (NR) (7 fruits/inflorescence),

Chai Nat (CN) and Chon Buri (CB) (6 fruits/inflorescence), Chiang Mai (CM), Phayao (PHY), Chumphon (CP) and Phangnga (PNG) (5 fruits/inflorescence), and Maha Sarakham (MS) (3 fruits/inflorescence), while the lowest value were found in Sakon Nakhon (SN) (2 fruits/inflorescence).

### 2.3.3 Number of plants per rai and spacing

Number of plants per rai and spacing were different among 20 provinces, the highest number of plants per rai were observed in Maha Sarakham (MS) (800 plants/rai, spacing=2x1 m) followed by Khon Kaen (KK) and Nakhon Ratchasima (NR) (533 plants/rai, spacing=2x1.5 m), Sakon Nakhon (SN), Udon Thani (UT), Suphan Buri (SB) and Phangnga (PNG) (400 plants/rai, spacing=2x2 m), Chai Nat (CN) and Kamphaeng Phet (KP) (289 plants/rai, spacing=2.5x3 m), Chiang Mai (CM), Lampang (LP), Phayao (PHY), Kalasin (KS), Nakhon, Pathom (NP) and Chon Buri (CB) ( 266 plants/rai, spacing=2x3 m), and Nan (NA) (177 plants/rai, spacing=3x3 m), while the lowest value were found in Chiang Rai (CR), Rayong (RY) and Chumphon (CP) (85 plants/rai, spacing=2.5x7.5 m). In additional, in Lamphun (LPH) had 600 plants/row, that physic nut were planted in 0.2 m of spacing, and 120 m per row.

### 2.3.4 Plants propagation and pruning

Plants propagation and pruning were different among 20 provinces; most physic nut plants propagation used stem cutting (sc) except Suphan Buri (SB) planted with seedling. In additional, branches of physic nut plants were pruned in Lamphun (LPH), Kalasin (KS), Nakhon Pathom (NP), and physic nut plants in other provinces were never pruned.

### 2.3.5 Plantation and irrigation

Plantation and irrigation were different among 20 provinces; multi-plantations were observed in Chiang Rai (CR); mixed among physic nut, lychee (*Lichi chinensis* Sonn.), and para rubber (*Hevea brasiliensis* Mull. Arg.: Euphorbiaceae), Kamphaeng Phet (KP); mixed among physic nut, mangos (*Mangifera indica* Linn.: Anacardiaceae), bananas (*Musa acuminata* Colla: Musaceae), lime (*Citrus aurantifolia* Swing: Rutaceae), burimese grape (*Baccaurea ramiflora*: Phyllanthaceae), guava (*Psidium guajava* L.: Myrtaceae), and *Stoma collinsae* Craibr.: Stemmonaceae, Rayong (RY); mixed between physic nut and para rubber (*Hevea brasiliensis* Mull. Arg.: Euphorbiaceae), and Chumphon (CP); mixed among rambutan (*Nephelium lappaceum* L.: Sapindaceae), mangosteen; (*Garcinia mangostana*: Guttiferae), durian (*Durio zibethinus* L.: Bombacaceae), and longkong (*Lansium domesticum*: Miliaceae). Whereas physic nut in Lamphun (LPH) province was planted for fencing in the sampling field. In other sites were single-plantation. In additional, Nakhon Pathom (NP) physic nut plantation was level furrow irrigated into area, while other areas were non irrigated (Figure 22).

### 2.3.6 Age variation

Age of physic nut plants was different among 20 provinces. The oldest plants were found in Chiang Rai (CR) (5 years) followed by Rayong (RY) (4 years), Lampang (LP), Nan (NA), Kalasin (KS), Sakon Nakhon (SN), Chai Nat (CN), Kamphaeng Phet (KP), Nakhon Pathom (NP) and Chon Buri (CB) (3 years), and Chiang Mai (CM), Lamphun (LPH), Phayao (PHY), Khon Kaen (KK), Maha Sarakham (MS), Nakhon Ratchasima (NR), Suphan Buri (SB) and Phangnga (PNG) (2 years), while the youngest plants were observed in Udon Thani (UT) and Chumphon (CP) (1 year).

### 2.3.7 Dry seeds and oil of physic nut

Yields (dry seeds and oil) were calculated from number of inflorescences per plant, fruits per inflorescence, plants per rai, average dry seeds (1.89 g/ 3seeds) and 25% of oil content. Weight of dry seeds and oil of physic nut were different among 20 provinces. The highest weight per rai and oil (litre/rai) were harvested in Nakhon Ratchasima (NR) (1,396 kg/rai, 349 litres/rai) followed by Chai Nat (CN) (885 kg/rai, 221 litres/rai), Nakhon Pathom (NP) (855 kg/rai, 214 litres/rai), Chiang Rai (CR) (684 kg/rai, 162 litres/rai), Lampang (LP) (616 kg/rai, 154 litres/rai), Kamphaeng Phet (KP) (472 kg/rai, 118 litres/rai), Phayao (PHY) (465 kg/rai, 116 litres/rai), Udon Thani (UT) (290 kg/rai, 73 litres/rai), Nan (NA) (281 kg/rai, 70 litres/rai), Suphan Buri (SB) (272 kg/rai, 68 litres/rai), Chiang Mai (CM) (241 kg/rai, 60 litres/rai), Kalasin (KS) (211 kg/rai, 53 litres/rai), Phangnga (PNG) (136 kg/rai, 34 litres/rai), Rayong (RY) (129 kg/rai, 32 litres/rai), Maha Sarakham (MS) (113 kg/rai, 28 litres/rai), Sakon Nakhon (SN) (91 kg/rai, 23 litres/rai), Chon Buri (CB) (90 kg/rai, 23 litres/rai), Lamphun (LPH) (79 kg/rai, 20 litres/rai), and Khon Kaen (KK) (54 kg/rai, 14 litres/rai), while the lowest value were found in Chumphon (CP) (20 kg/rai, 2 litres/rai).

KU Biodiesel (2010) in Thailand reported various yield of physic nut in 1-3 years that showed seeds product 200-700 kg/rai/year. Report of the Committee on Development of Biofuel (2003) in India stated that physic nut given 0.4-12 tons/hectare/year (64-1,920 kg/rai/year). In the same way, this study showed seeds product 10-1,396 kg/rai/the rainy season.

Different age of plants and locations had effect on various yield in this investigation. In additional, number of inflorescences per plant, fruits per inflorescence, plants per rai, spacing, pruning, plantation, and irrigation of physic nut seems to influence to seeds production and oil. Previously research stated that there were various methods to cultivate physic nuts, which vary from region to region and also on climatic conditions. These were direct seeding, pre-cultivation of seedlings (nursery raising), transplanting of spontaneous wild plants and direct planting of

cuttings. Wider spacing (3x3m) was reported to give larger yields of fruit, at least in early years (Heller, 1996). In different countries and regions the seed yields of physic nut may range from 0.1-15 tonnes/ha/year (16-2,400 kg/rai/year) (Kumar and Sharma, 2008).

Rao *et al.* (2008) said that physic nut has adapted itself to a wide range of edaphic and ecological conditions, suggested that there exists considerable amount of genetic variability to be exploited for potential realization. Similarly, Jongschaap *et al.* (2007) concluded that provenance trails with local physic nut accessions were reported for India, and for various accessions, mainly from Africa. They reveal that the genetic base of physic nut provenances from India was quite small, that significant differences in plant morphological aspects (plants height, leaf area index, stem girth, number of primary-secondary-tertiary branches) and yield contributing factors (fruits per plant, seeds per plant, seed weight, and percentage of oil) could be distinguished between provenances. Apart from age, vigour, crown exposure and genotype of mother tree, soil and climate of the place of seed original were important factors affecting the seed traits. In additional, Abdelgadir *et al.* (2008) indicated that low numbers of female flowers, limited branching and inadequate pollination are the major factors that limit seed production and thus oil yield of physic nut. To summarize, high variable yield is caused by different location (soil, climate, and environments), age, cultivation (spacing, pruning, plant propagation, plantation, and irrigation), female sex ratio, and inadequate pollination.

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**Table 8** Various number of inflorescence per plant, fruits per inflorescence, plants per rai, spacing (m x m), age, pruning, plantation, irrigation, dry seeds (kg/rai), and oil (Litres/rai) of *Jatropha curcas* L. in 20 provinces

| Region of Thailand | Province <sup>1</sup> | H'  | Inflorescences /plant | Fruits/ inflorescence | Plants/rai | Spacing | Plant Propagation | Pruning | Plantation | Irrigation | Age of plant | Dry seeds (kg)/rai | Oil (litres/rai) | Flower sex ratio F/M/P |
|--------------------|-----------------------|-----|-----------------------|-----------------------|------------|---------|-------------------|---------|------------|------------|--------------|--------------------|------------------|------------------------|
| North              | CM                    | 4.3 | 96                    | 5                     | 266        | 2.0x3.0 | sc                | no      | single     | non        | 2            | 241                | 60               | 1:10:0                 |
|                    | CR                    | 4.5 | 504                   | 8                     | 85         | 2.5x7.5 | sc                | no      | multi      | non        | 5            | 684                | 162              | 1:6:0                  |
|                    | LP                    | 4.1 | 175                   | 7                     | 266        | 2.0x3.0 | sc                | no      | single     | non        | 3            | 616                | 154              | 1:17:0                 |
|                    | LPH                   | 3.8 | 10                    | 7                     | 600        | -       | sc                | yes     | fencing    | non        | 2            | 79                 | 20               | 1:6:0                  |
|                    | NA                    | 3.8 | 120                   | 7                     | 177        | 3.0x3.0 | sc                | no      | single     | non        | 3            | 281                | 70               | 1:12:0                 |
|                    | PHY                   | 3.8 | 185                   | 5                     | 266        | 2.0x3.0 | sc                | no      | single     | non        | 2            | 465                | 116              | 1:12:0                 |
| Northeast          | KS                    | 3.1 | 30                    | 14                    | 266        | 2.0x3.0 | sc                | yes     | single     | non        | 3            | 211                | 53               | 1:16:0                 |
|                    | KK                    | 2.5 | 6                     | 9                     | 533        | 2.0x1.5 | sc                | no      | single     | non        | 2            | 54                 | 14               | 1:15:0                 |
|                    | MS                    | 2.5 | 25                    | 3                     | 800        | 2.0x1.0 | sc                | no      | single     | non        | 2            | 113                | 28               | 1:12:0                 |
|                    | NR                    | 2.9 | 198                   | 7                     | 533        | 2.0x1.5 | sc                | no      | single     | non        | 2            | 1,396              | 349              | 1:26:0                 |
|                    | SN                    | 2.8 | 60                    | 2                     | 400        | 2.0x2.0 | sc                | no      | single     | non        | 3            | 91                 | 23               | 1:10:0                 |
|                    | UT                    | 2.8 | 48                    | 8                     | 400        | 2.0x2.0 | sc                | no      | single     | non        | 1            | 290                | 73               | 1:11:0                 |

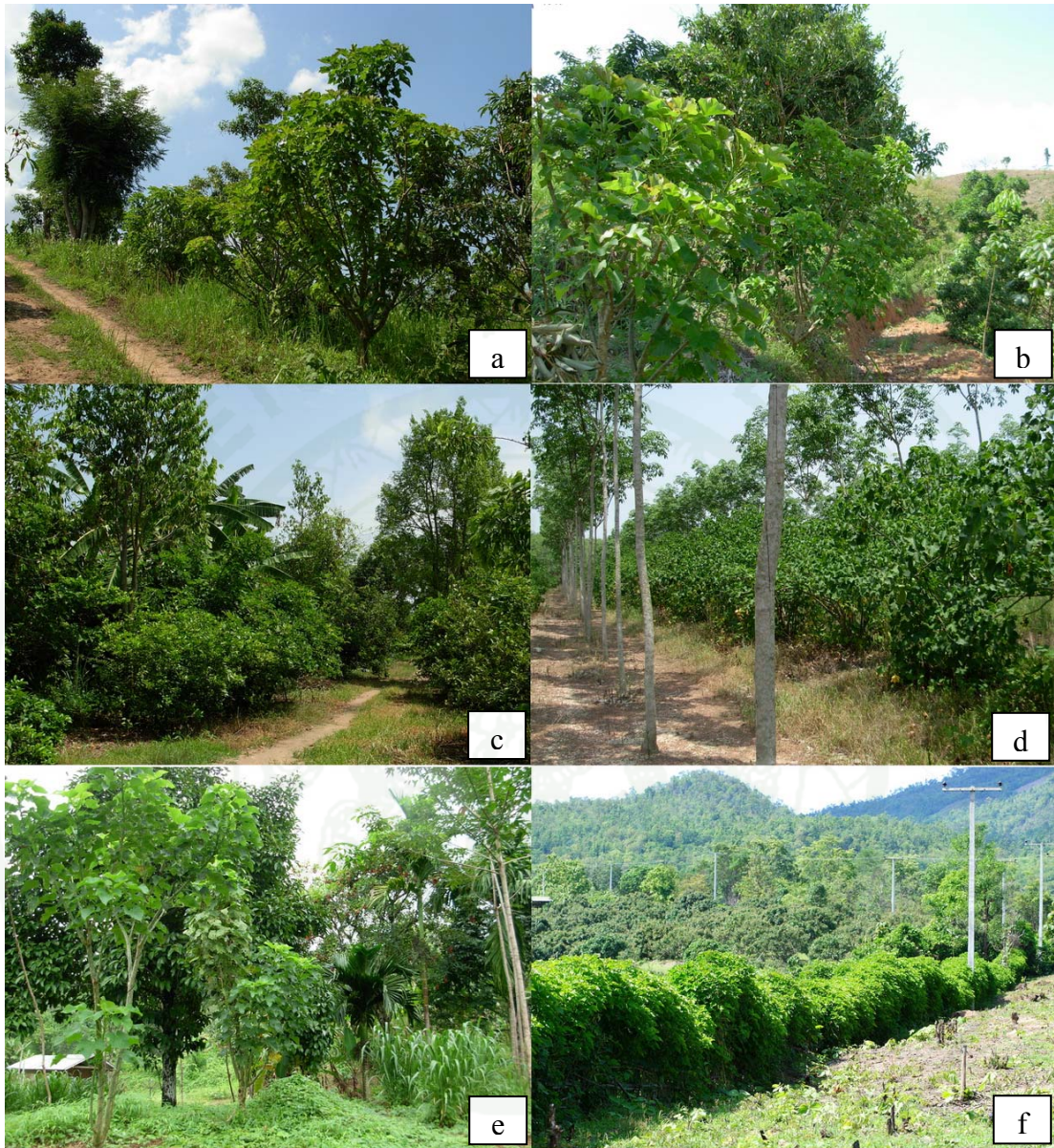
**Table 8** (Continued)

| Region of Thailand | Province <sup>1</sup> | H'  | Inflorescences /plant | Fruits/ inflorescence | Plants/rai | Spacing | Plant Propagation | Pruning | Plantation | Irrigation   | Age of plant | Dry seeds (kg)/rai | Oil (litres/rai) | Flower sex ratio F/M/P |
|--------------------|-----------------------|-----|-----------------------|-----------------------|------------|---------|-------------------|---------|------------|--------------|--------------|--------------------|------------------|------------------------|
| Central            | CN                    | 3.4 | 270                   | 6                     | 289        | 2.5x3.0 | sc                | no      | single     | non          | 3            | 885                | 221              | 1:23:0                 |
|                    | KP                    | 3.4 | 108                   | 8                     | 289        | 2.5x3.0 | sc                | no      | multi      | non          | 3            | 472                | 118              | 1:9:0                  |
|                    | NP                    | 3.2 | 170                   | 10                    | 266        | 2.0x3.0 | sc                | yes     | single     | level furrow | 3            | 855                | 214              | 1:7:0                  |
|                    | SB                    | 2.9 | 45                    | 8                     | 400        | 2.0x2.0 | s                 | no      | single     | non          | 2            | 272                | 68               | 1:23:6                 |
| East               | CB                    | 3.0 | 30                    | 6                     | 266        | 2.0x3.0 | sc                | yes     | single     | non          | 3            | 90                 | 23               | 1:10:0                 |
|                    | RY                    | 3.3 | 100                   | 8                     | 85         | 2.5x7.5 | sc                | no      | multi      | non          | 4            | 129                | 32               | 1:11:0                 |
| South              | CP                    | 2.5 | 12                    | 5                     | 85         | 2.5x7.5 | sc                | no      | multi      | non          | 1            | 10                 | 2                | 1:10:0                 |
|                    | PNG                   | 2.9 | 36                    | 5                     | 400        | 2.0x2.0 | sc                | no      | single     | non          | 2            | 136                | 34               | 1:10:0                 |

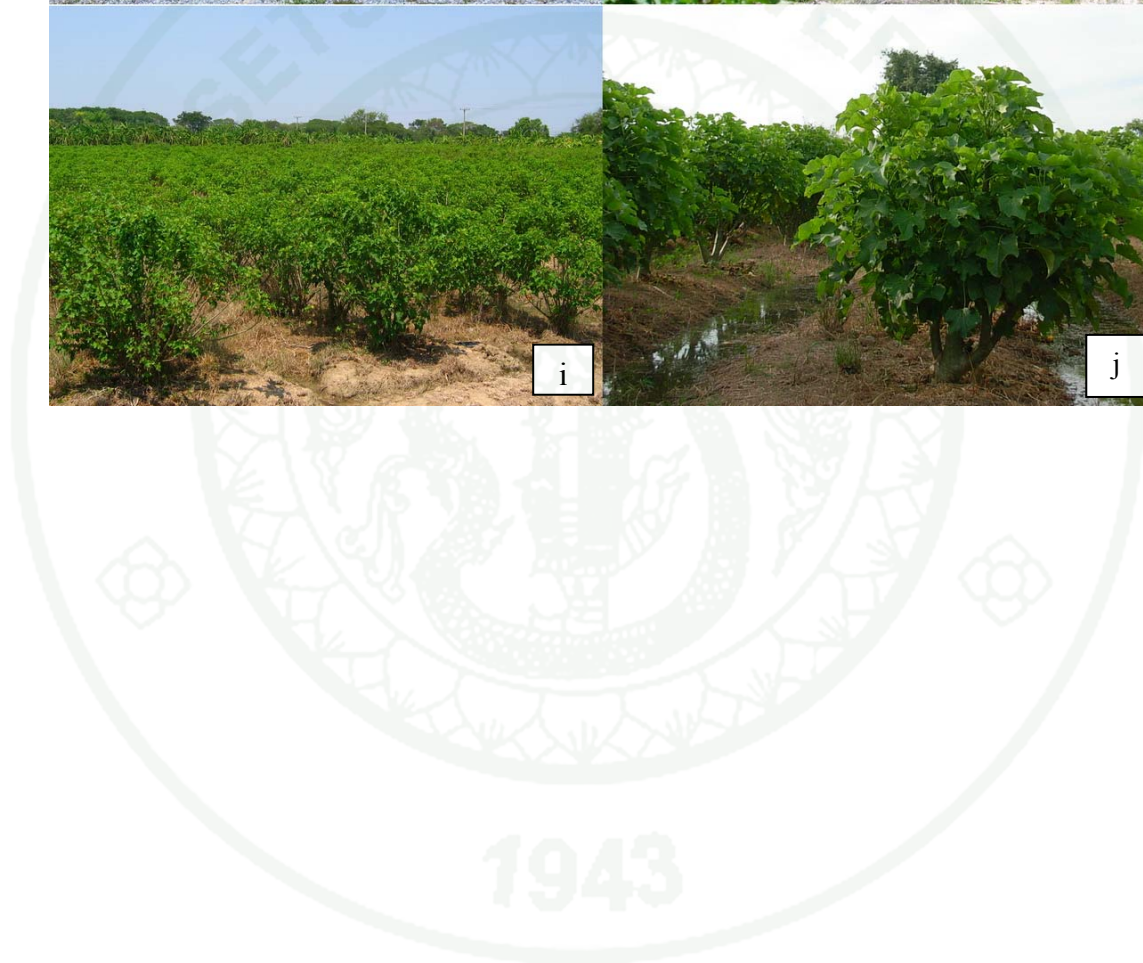
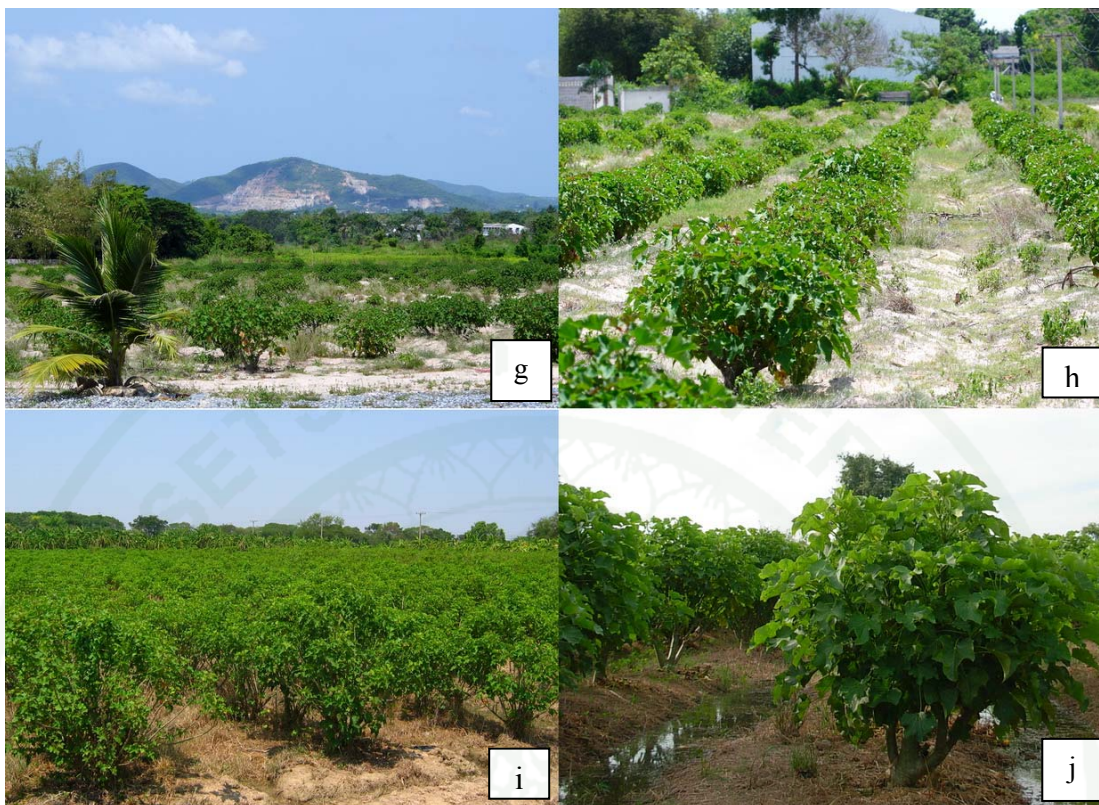
<sup>1</sup> CM = Chiang Mai, CR = Chiang Rai, LP = Lampang, LPH = Lamphun, NA = Nan, PHY = Phayao, KS = Kalasin, KK = Khon Kaen, MS = Maha Sarakham, NR = Nakhon Ratchasima, SN = Sakon Nakhon, UT = Udon Thani, CN = Chai Nat, KP = Kamphaeng Phet, NP = Nakhon Pathom, SB = Suphan Buri, CB = Chon Buri, RY = Rayong, CP = Chumphon, and PNG = Phangnga

**Figure 22** *Jatropha curcas* L. plantation in Thailand

- a, b = Multi-plantation at Chiang Rai province
- c = Multi-plantation at Kamphaeng Phet province
- d = Multi-plantation at Rayong province
- e = Multi-plantation at Chumphon province
- f = Physic nut fencing at Lamphun province
- g, h = Single-plantation at Chon Buri province
- i, j = Single-plantation and level furrow irrigation at Nakhon Pathom province



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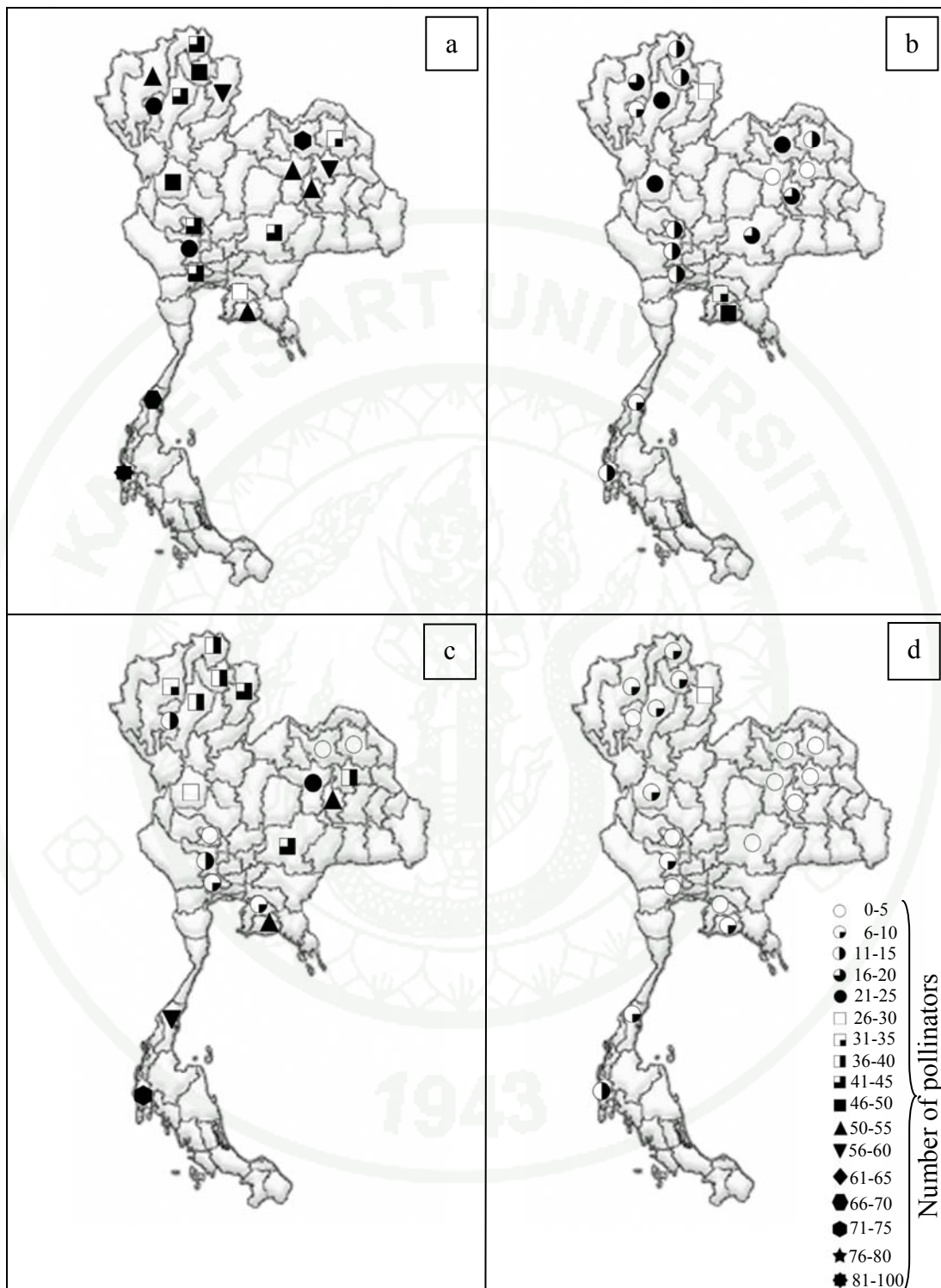
## 2.4 Number of insect pollinators on physic nut flowers

Number of insect pollinators visited on female and male flowers of physic nut is shown in Table 9 and Figure 23. Numbers of insect pollinators per 100 flowers (female or male) were variation ranges among 20 provinces, that range of insect pollinator numbers was 22-100 individuals/100 female flowers (mean 50 individuals); 3-46 individuals/100 male flowers (mean 17 individuals). In the same way, the range of insect pollinator numbers in Apoidea was 0-75 individuals/100 female flowers (mean 30 individuals); 0-28 individuals/100 male flowers (mean 6 individuals). The highest insect pollinator number was found in Phangnga (100 individuals/100 female flowers) and Rayong (46 individuals/100 male flowers), while the lowest was found in Lamphun (22 individuals/100 female flowers) and Khon Kaen (3 individuals/100 male flowers). In Apoidea, the highest number of individual was found in Phangnga (75 individuals/100 female flowers), and Nan (28 individuals/100 male flowers) while the lowest was found in three provinces i.e. Sakon Nakhon, Udon Thani and Chai Nat (non visitors on female flowers), and Udon Thani (non visitors on male flowers). Summarize, the result showed number of insect pollinators and Apoidea that average number of insect pollinators on female flowers higher than male flowers, and average number of Apoidea on female flowers higher than male flowers too.

In the observation field insect pollinators always found visited male flowers more frequency of occurrence than female flower, because of male flowers were larger blooming. The study of Chang-wei *et al.* (2007a) was supported that the anther of the male flowers is bright yellow and looks conspicuous. In this population, an inflorescence had 184 (88-238) male flowers, blooming about 10 flowers daily in average to display them more conspicuously, for big blooming it could attract more insects visiting. It seems that insect pollinators preferred visit on male flowers than female flowers, because of large population of male flowers, and bright yellow of anthers was more attractive pollinators than female flowers.

**Table 9** Number of insect pollinators per 100 flowers of *Jatropha curcas* L. during 08.00-12.00 h in 20 provinces from April to August 2009

| Thailand<br>Regions | Provinces         | Number of individuals/100 flowers |                 |                   |                 |
|---------------------|-------------------|-----------------------------------|-----------------|-------------------|-----------------|
|                     |                   | Insect pollinators                |                 | Apoidea           |                 |
|                     |                   | female<br>flowers                 | male<br>flowers | female<br>flowers | male<br>flowers |
| North               | Chiang Mai        | 53                                | 20              | 31                | 7               |
|                     | Chiang Rai        | 42                                | 15              | 36                | 10              |
|                     | Lampang           | 42                                | 22              | 36                | 9               |
|                     | Lamphun           | 22                                | 8               | 14                | 3               |
|                     | Nan               | 56                                | 29              | 45                | 28              |
|                     | Phayao            | 49                                | 13              | 36                | 7               |
| Northeast           | Kalasin           | 60                                | 5               | 40                | 3               |
|                     | Khon Kaen         | 50                                | 3               | 25                | 2               |
|                     | Maha Sarakham     | 50                                | 17              | 50                | 2               |
|                     | Nakhon Ratchasima | 43                                | 20              | 43                | 2               |
|                     | Sakon Nakhon      | 34                                | 12              | 0                 | 2               |
|                     | Udon Thani        | 75                                | 21              | 0                 | 0               |
| Central             | Chai Nat          | 45                                | 13              | 0                 | 1               |
|                     | Kamphaeng Phet    | 64                                | 23              | 28                | 7               |
|                     | Nakhon Pathom     | 44                                | 11              | 7                 | 5               |
|                     | Suphan Buri       | 24                                | 11              | 12                | 7               |
| East                | Chon Buri         | 30                                | 35              | 9                 | 5               |
|                     | Rayong            | 50                                | 46              | 50                | 7               |
| South               | Chumphon          | 67                                | 8               | 56                | 6               |
|                     | Phangnga          | 100                               | 12              | 75                | 11              |
|                     | Average           | 50                                | 17.2            | 29.65             | 6.2             |



**Figure 23** The density of insect pollinators on *Jatropha curcas* L. flowers during 08.00-12.00 h in 20 provinces from April to August 2009  
 a, b = Number of insect pollinators visited on 100 female, male flowers  
 c, d = Number of Apoidea visited on 100 female, male flowers

## 2.5 Amount of pollen grains on stigmas of physic nut flowers

Amount of pollen grains on stigmas were estimated into the pollen grains grade level in the evening during 17.00-18.00 h. All of the study sites showed non F-grade, that the female flowers were always pollinated by insect pollinator in 20 provinces in the rainy season. The highest average numbers of female flowers were observed in A-grade of pollen grains on stigmas (44.00%) followed by C-grade (37.09%), while the lowest value was found on B-grade (18.91%) (Table 10).

In addition, this observation found pollen foragers of bees about 10% in the morning. Bee pollen foragers (*Apis cerana indica*, *A. dorsata*, *A. florea*, *A. mellifera ligustica*, and *Trigona pagdeni*) (Figure 24) were found in the north, some provinces in northeast (Maha Sarakham, and Nakhon Ratchasima), central, east, and south of Thailand. This result showed number of female flowers in A-grade, in Chiang Mai, Chiang Rai, Lampang, Lamphun, Nan, Phayao, Maha Sarakham, Nakhon Ratchasima, Chai Nat, Kamphaeng Phet, Nakhon Pathom, Suphan Buri, Chon Buri, Rayong, Chumphon, and Phangnga, while Kalasin, Khon Kaen, Sakon Nakhon, and Udon Thani were not found A-grade of pollen grains grade on stigmas.

Pollen grains grade study seem to the first observation of physic nut in Asia or the world, this study followed by Malaipan *et al.* (2007) in pollen grains grade classification method. Different among pollen grains grade level on stigma is caused by diverse of insect pollinators and behavior collection of visitors. This study was observed in the evening (17.00-18.00 h), that was estimated the effective visitors for pollination, and predicted pollination problem in the near future. This data was important to physic nut pollination management. It can apply to the others pollination studies.

**Table 10** Percentage of female flowers in each amount of pollen grains during 17.00-18.00 h on *Jatropha curcas* L. in 20 provinces from April to August 2009

|           | Provinces <sup>1</sup> | Number of female flowers (%) |       |        |   | Average (%) |
|-----------|------------------------|------------------------------|-------|--------|---|-------------|
|           |                        | A                            | B     | C      | F |             |
| North     | Chiang Mai             | 93.94                        | -     | 6.06   | - | 96.97       |
|           | Chiang Rai             | 88.89                        | 8.89  | 2.22   | - | 96.67       |
|           | Lampang                | 100.00                       | -     | -      | - | 100.00      |
|           | Lamphun                | 15.73                        | 29.09 | 55.18  | - | 65.14       |
|           | Nan                    | 55.56                        | 33.33 | 11.11  | - | 86.11       |
|           | Phayao                 | 94.34                        | 5.66  | -      | - | 98.59       |
| Northeast | Kalasin                | -                            | -     | 100.00 | - | 50.00       |
|           | Khon Kaen              | -                            | -     | 100.00 | - | 50.00       |
|           | Maha Sarakham          | 50.00                        | 50.00 | -      | - | 87.50       |
|           | Nakhon Ratchasima      | 28.57                        | -     | 71.43  | - | 64.29       |
|           | Sakon Nakhon           | -                            | 66.67 | 33.33  | - | 66.67       |
|           | Udon Thani             | -                            | 50.00 | 50.00  | - | 62.50       |
| Central   | Chai Nat               | 88.89                        | -     | 11.11  | - | 94.45       |
|           | Kamphaeng Phet         | 45.45                        | -     | 54.55  | - | 72.73       |
|           | Nakhon Pathom          | 10.00                        | 44.00 | 46.00  | - | 66.00       |
|           | Suphan Buri            | 76.00                        | 16.00 | 8.00   | - | 92.00       |
| East      | Chon Buri              | 2.00                         | 24.58 | 73.42  | - | 57.15       |
|           | Rayong                 | 33.33                        | 16.67 | 50.00  | - | 70.83       |
| South     | Chumphon               | 22.22                        | 33.34 | 44.44  | - | 69.45       |
|           | Phangnga               | 75.00                        | -     | 25.00  | - | 87.50       |
|           | Average                | 44.00                        | 18.91 | 37.09  |   | 76.73       |



**Figure 24** Bee foragers on *Jatropha curcas* L. flowers were gathering collecting nectar and pollen grains in pollen baskets (corbicular).

a, b = *Apis cerana indica*; c = *Apis dorsata*; d = *Apis floreae*;  
 e = *Apis mellifera ligustica*; f = *Trigona pagdeni*  
 Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm

### 3. General botanical characteristic of physic nut (*Jatropha curcas* L.)

#### 3.1 Types and sex ratio of physic nut flowers

Table 11 summarized types and flower sex ratio data from four locations; Bangkhen (BK) location on plot-B in 2011, Kamphaeng Saen (KPS), Aviation School (AS) and Agriculture Research and Development Center (ARDC) locations in 2009 among different original sources (four original sources such as toxic variety at Kamphaeng Saen (KPSt), non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunhiboon (SSnt), between varieties (toxic and non-toxic), seasons (the summer, rainy and winter). The results showed the variation different in flower sex ratio and type of flowers.

##### 3.1.1 Different varieties of physic nut

In the same area at BK location in the rainy season (June-October; at 29.51 °C, 1,611.7 mm of accumulative rainfall, and 79.6% RH); the mixed variety of physic nut flowers on the plot-B (four original sources; KPSt, ARDCnt, ASnt, SSnt, that were planted with stem cutting in pots) were observed. Flowers were divided into two types, female and male flower, and flowers sex ratio in each original source revealed slightly different in sex ratio (female:male). Such flower sex ratio of ASnt was 1:18, flower sex ratio of ARDCnt and KPSt were 1:19, but flower ratio of SSnt was 1:33. Same condition, character of each variety was express distinct difference in SSnt original source.

##### 3.1.2 Different plantation locations of physic nut

In the same variety but different location; comparison flowers sex ratio of ARDCnt original source between BK and ARDC locations. ASnt was compared flowers sex ratio between BK and AS locations, and sex ratio of KPSt at

BK location was compared with KPS location. They were observed in the rainy season.

1) The flowers type and sex ratio of ARDCnt were different between BK and ARDC locations, type of flowers in BK location was divided female and male, and flower sex ratio (female : male) was 1:19, but in ARDC location (at 27.89 °C, 607.9 mm of accumulative rainfall, and 78% RH) had three types of flowers i.e. female, male and perfect flowers. Flower sex ratio of this site was 9:83:7.

2) The flowers type and sex ratio of ASnt were different between BK and AS locations, type of flowers in BK location (at 29.51 °C, 1,611.7 mm of accumulative rainfall, and 79.6% RH) was divided female and male, and flower sex ratio (female:male) was 1:18, but in AS location (at 28.66 °C, 550.9 mm of accumulative rainfall, and 75.6% RH) had three types of flowers i.e. female, male and perfect flowers. Flower sex ratio of this site was 13:29:8.

3) The flowers type of KPSt on plot-B at BK location was similar to KPS location, these flowers were divided into two types i.e. female and male, but flower sex ratio were different between BK and KPS locations, flower sex ratio (female:male) in KPS location (1:7) was higher than in BK location (1:18).

### 3.1.3 Different seasons

In the toxic variety, and in the area at KPS location, but in different seasons the summer, rainy and winter in 2009 were observed flowers type of KPSt in KPS location were similar among three seasons. The flowers were divided into two types female and male flower. But flower sex ratios were different among the summer, rainy and winter season; the highest flower sex ratio female:male was 1:7, observed in the rainy season followed by the summer season (1:23), while the lowest flower sex ratio was 1:41 found in the winter season.

### 3.1.4 Different months

In the toxic variety, same season in the rainy season, but different month at KPS location, Kasetsart University, Nakhon Pathom Province, in 2009. Flower sex ratio were non significantly different among month during the rainy season, flower ratio; female:male in March, August and September were 1:8, 1:6, and 1:7, respectively.

Variation of environment factors such as air temperature, accumulative rainfall, and relative humidity in months, seasons, years, and locations these influenced to different types and sex ratio of physic nut. Figure 25 and Appendix Table 6 showed methodology of air temperature, accumulative rainfall, and relative humidity from four locations in 2009-2011.

Different location had effect on flowers type of non-toxic and sex ratio expression. ASnt and ARDCnt are the same variety from Mexico origin variety. When they were planted in AS and ARDC locations, that perfect flowers were often presence about 16% in AS location, and 7% in ARDC location. But in the same clones of all non-toxic variety was never found perfect flowers in BK location (plot-A: cleft grafting plants and plot-B: stem cutting plants). Also the phenotype of physic nut in BK location differs from mother plants from AS and ARDC locations.

Various seasons in toxic variety were found different sex ratio of physic nut flowers. At KPS location flowers sex ratio in the rainy season was higher than the summer and the winter season, because high accumulative rainfall and humidity occurred in the rainy season. Similarly, Raju and Ezradanam (2002) reported that flowering occurred during the wet season and two flowering peaks were often seen, i.e. during the summer and autumn. The number of female and male flowers per inflorescence varies a lot among the observations (female:1-19 and male:25-238), and the female to male flower ratio of the two studies reporting it was similar [1: 24.5 (Raju and Ezradanam , 2002), and 1:29 (Chang-wei *et al.*, 2007a)].

A perfect flower or hermaphrodite flower of physic nut is formed terminally, individually, with female flowers (tricarpellary, syncarpous with trilocular ovary) usually slightly larger and occurs in the hot seasons (Dehgan and Webster, 1979). Whereas these results in non-toxic variety showed that seasons had no effect on the perfect flower occurring, but in the different locations and cultivations are influence to the hermapodite expression. Similarly, the perfect flowers of toxic variety were very rare to find, two hermaphrodite flowers were found on this observation at KPS location during 2008-2010. Likewise, Kumar and Sharma (2008) said that the rare hermaphrodite flowers could be self-pollinating. It seems to conclude that perfect flowers in non-toxic variety can be found easier than hermaphrodite flowers in the toxic variety.

**Table 11** Type of flowers and flower sex ratio of four original sources of *Jatropha curcas* L. from four locations in 2009-2011

| Locations <sup>1</sup> | Seasons   | Original sources <sup>2</sup> | Flower sex ratios and types of flowers |      |         |
|------------------------|-----------|-------------------------------|--|------|---------|
|                        |           |                               | female                                 | male | perfect |
| KPS (2009)             | Summer    | KPSt                          | 1                                      | 23   | -       |
|                        | Rainy     | KPSt                          | 1                                      | 7    | -       |
|                        | March     | KPSt                          | 1                                      | 8    | -       |
|                        | August    | KPSt                          | 1                                      | 6    | -       |
|                        | September | KPSt                          | 1                                      | 7    | -       |
|                        | Winter    | KPSt                          | 1                                      | 41   | -       |
| ARDC (2009)            | Rainy     | ARDCnt                        | 9                                      | 83   | 7       |
| AS (2009)              | Rainy     | ASnt                          | 13                                     | 29   | 8       |
| BK (2011)              | Rainy     | KPSt                          | 1                                      | 19   | -       |
|                        |           | ARDCnt                        | 1                                      | 19   | -       |
|                        |           | ASnt                          | 1                                      | 18   | -       |
|                        |           | SSnt                          | 1                                      | 33   | -       |

<sup>1</sup> KPS = Kamphaeng Saen location in 2009, plant propagation by stem cutting in the field.

ARDC = Agriculture Research and Development Center location in 2009, plant propagation by seeding in the field.

AS = Aviation School location in 2009, plant propagation by stem cutting in the field.

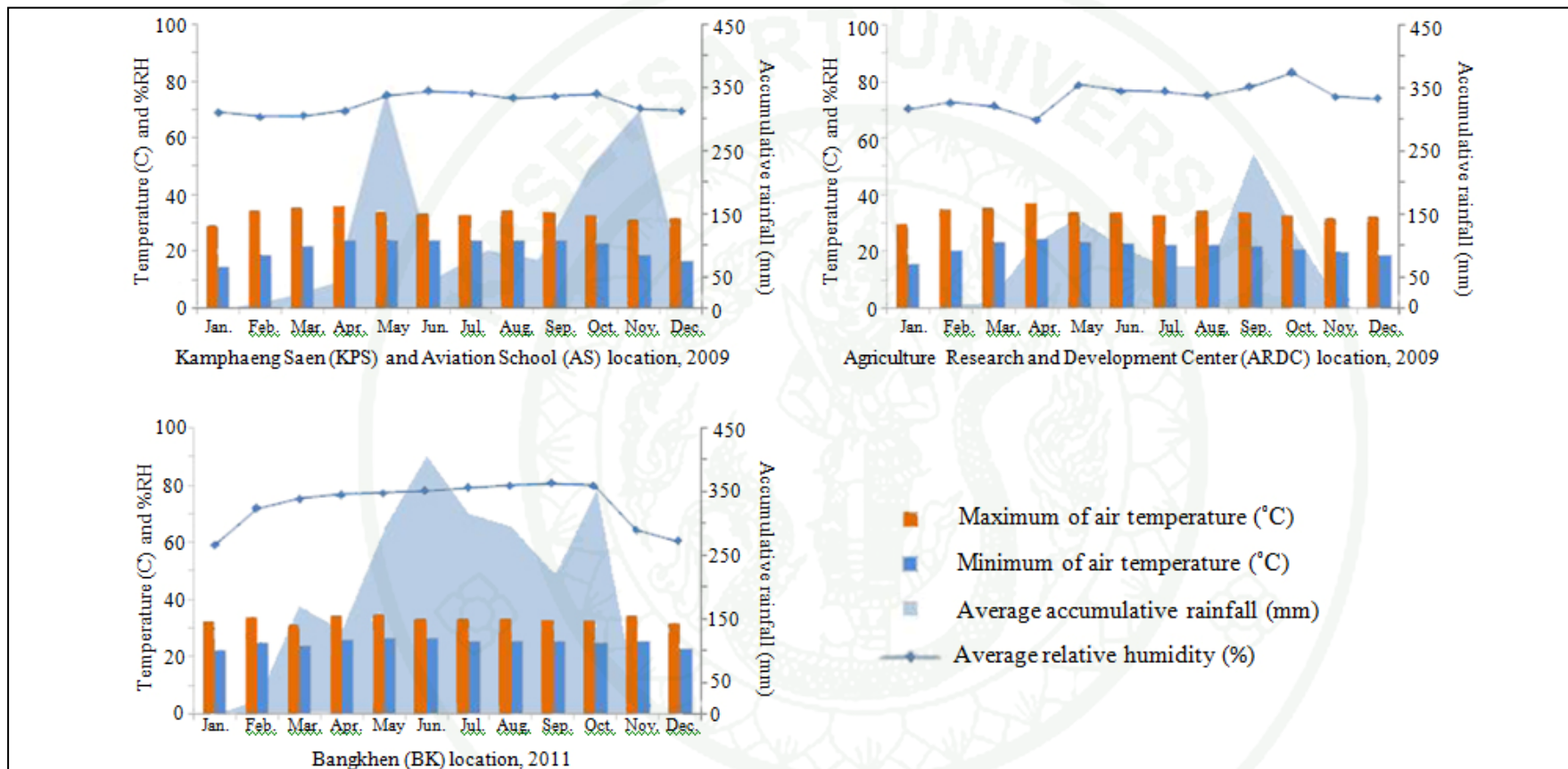
BK = Bangkhen location in 2011, plant propagation by stem cutting in pots.

<sup>2</sup> KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon



**Figure 25** Metrodology of air temperature, accumulative rainfall, and relative humidity from four locations in 2009-2011

**Sources:** Nakhon Pathom Meteorological Station (2009); Thai Meteorological Department (2009, 2011)

### 3.2 Flowering period

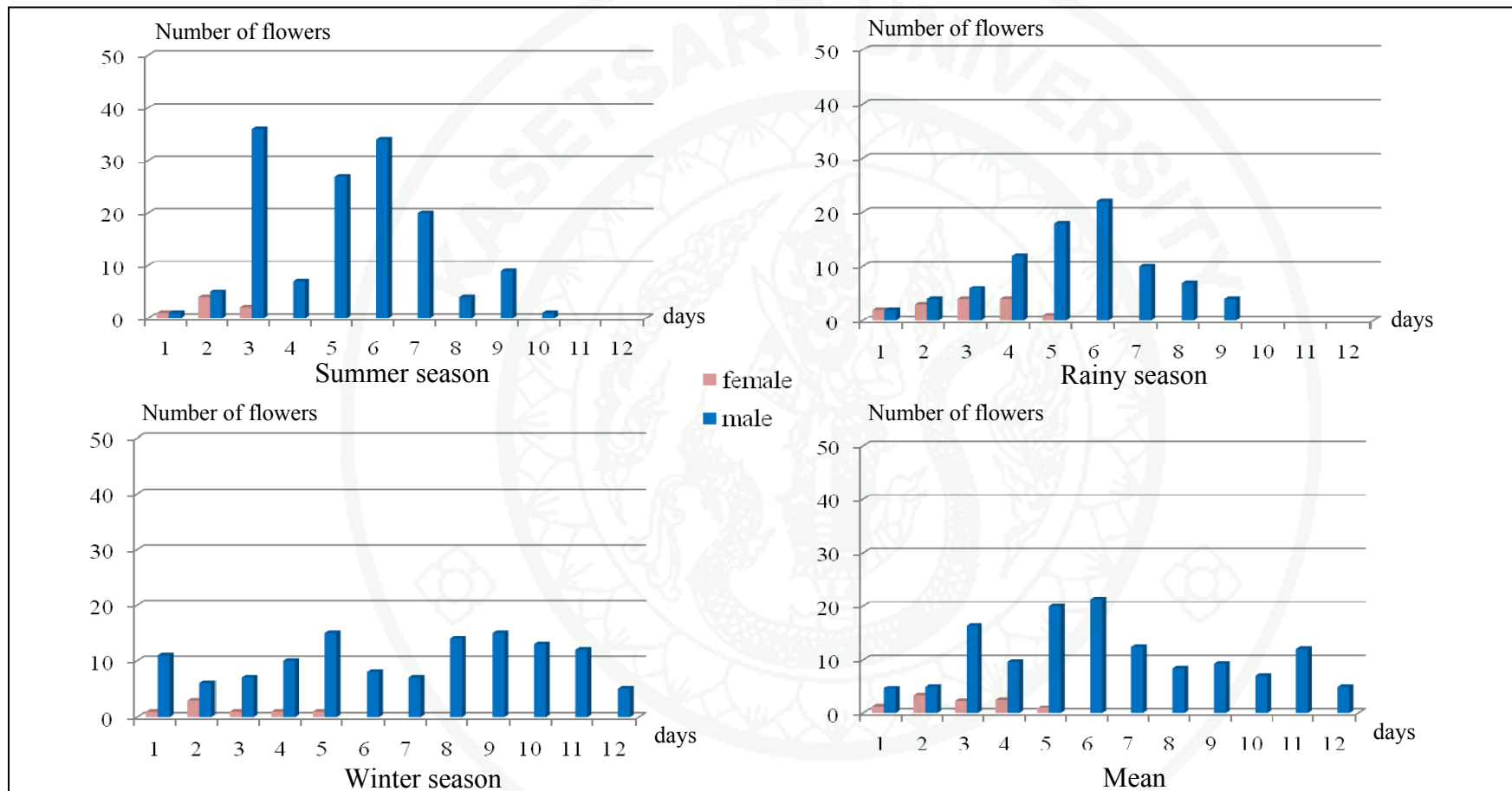
Figure 26 summarized flowers on inflorescences display of *Jatropha curcas* L., toxic variety, in different season (the summer, rainy and winter) at KPS location in 2008. The observation showed slight different in blooming period, that female flowers opened during the 1<sup>st</sup>-3<sup>rd</sup> day in the summer season, the 1<sup>st</sup>-5<sup>th</sup> day in the rainy season, and the 1<sup>st</sup>-6<sup>th</sup> day in the winter season. The male flowers opened for a period of 10 days in the inflorescences in both the summer and rainy season, but in the winter season the flowers opened during 12 days. Usually the male flowers opened for only one day long all the days, in addition the female flowers can opened about 5-7 days, if that female flowers not pollinated yet.

Most flowering of physic nut in the first blooming was self-incompatibility plant to promote outcrossing, that were divided into three types of inflorescence blooming (Figure 27). Only female flowers were opened on physic nut inflorescences, which is called “type I”, whereas only male flowers were blooming, which is called “type II”, but some inflorescences both flowers (female and male) are blooming, which is called “type III”, because of the male parts can pollinate the female parts of the same inflorescences. Three inflorescence blooming types were different frequency of occurrence, the highest value was found in type I (72%) followed by type II (18%), while the lowest value was observed in type III (10%).

Chang-wei *et al.* (2007a) reported that the male flowers normally open first at the branch joint, followed by female ones. The female flowers at the top of inflorescence bloom earlier than flowers at other places. Some male flowers remain un-opened even when all female flowers have opened. Very few inflorescences had their female flowers open before male ones. Each inflorescence's flower lasts from 13-19 days. Normally, a few male flowers open at the first one or two days, followed by the quick opening of the female flowers. About 60% of the female flowers opened from the 3<sup>rd</sup>-5<sup>th</sup> day. The flowering pattern showed that male flowers open gently with a small peak between the 9<sup>th</sup> and 13<sup>th</sup> day. The results contrast with previously observations in that most female flowers opened from the first which

more than 60%, each inflorescence's flower lasts from 10-12 days, the female flower opened during 5-12 days, and male flowers open gently with a small peak between the 3<sup>rd</sup> and 6<sup>th</sup> day in the summer season, that open was faster than the rainy and winter season. In addition, a few racemes, and the female flowers opened firstly shows a tendency to promote xenogamy (cross-pollination) and minimize geitonogamy (self-pollination) (Chang-wei *et al.*, 2007b).

Jongschaap (2007) suggested that flowering was one the most important crop phonological stages for physic nut oil production, as the number of female flowers and their fertilization determines how many fruits and seeds eventually will develop. Flowering normally starts after a dry and dormant period and is induced and continued by prolonged periods of soil water availability, either by precipitation or irrigation. Nutrient limitation seem to provoke the end of flowering (Aker, 1997)



**Figure 26** Flowering period of *Jatropha curcas* L. at Kamphaeng Saen (KPS) location in 2008



**Figure 27** Blooming inflorescence types on toxic variety of *Jatropha curcas* L. flowers at Kamphaeng Saen (KPS) location in 2008

a, b = type I (female flowers open at the first blooming stage)

c, d = type II (male flowers open at the first blooming stage)

e, f = type III (both female and male flower at the first blooming stage)

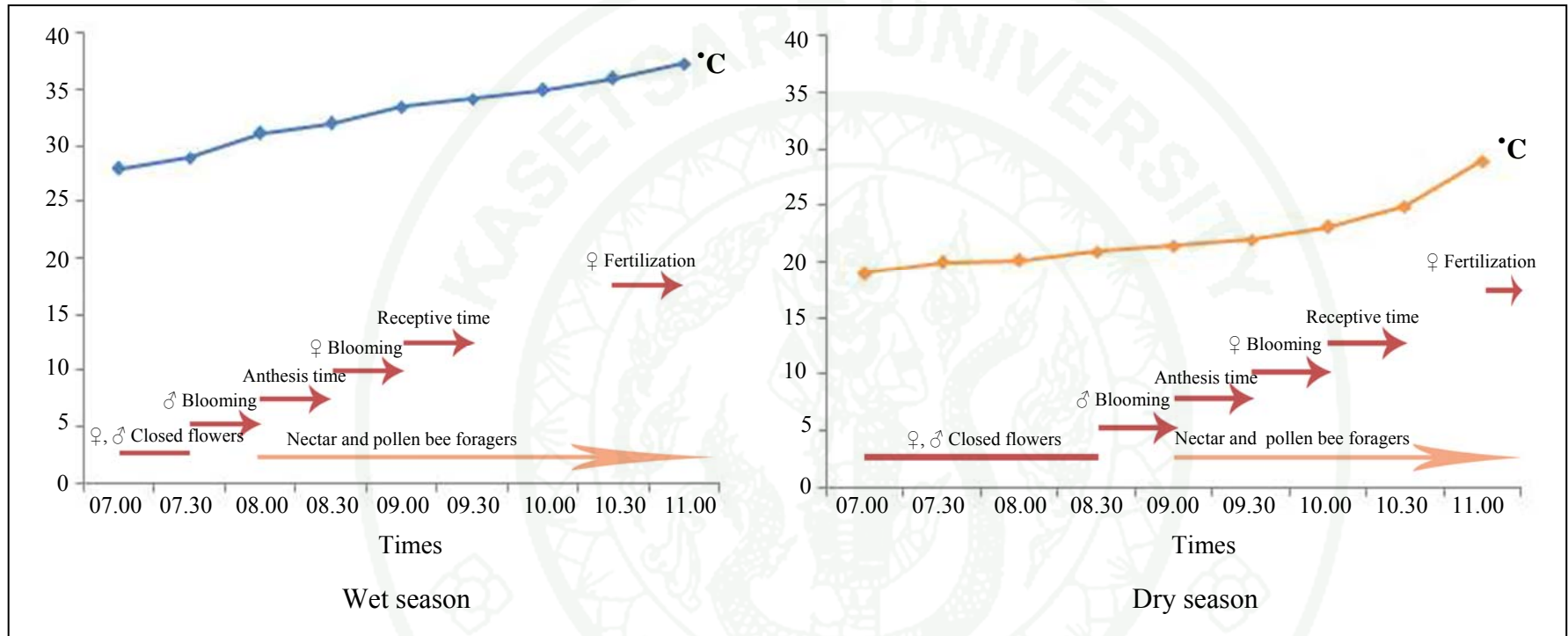
Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm.

Diameter of physic nut female and larger male flowers is  $0.9 \pm 0.1$  cm.

### 3.3 Anthesis and receptive time

At KPS location, Kasetsart University, Nakhon Pathom Province on April and December in 2009, *Jatropha curcas* L. flowers were observed anthesis and receptive time (Figure 28 and Appendix Table 7). The anthesis and receptive time in the wet season (April 16<sup>th</sup>) were earlier than in the dry season (December 27<sup>th</sup>). Anthesis time of the male flowers were observed during 08.00-08.30 h in the wet season (long day), while in the dry season (short day) anthesis time occurred during 09.00-09.30 h. Similarly, receptive time of the female flowers occurred after anthesis time about 30-60 minutes, these wet stigmas were already period for pollination. If these female flowers were not pollinated with insect pollinators, the stigmas receptive will continue active during 5-7 days, but when they were pollinated by visitors, that petals of female flowers to be rapidly declining after pollinated 10 days.

Chang-wei *et al.* (2007a) supported that the anthers dehisce 1 hour after flower opening by longitudinal slits. The pollens from male flower blooming at the first day are adhesive. In additional, Raju and Ezradanam (2002) stated that the stigmas are receptive during 3 days after the opening of the flower. Likewise, the lifespan of the female flower was about 5-12 days. The stigma receptivity was strong during 1<sup>st</sup>-4<sup>th</sup> day and begins to decline on the fifth day onwards and completely loses its receptivity on the ninth day. There were still many unflowering male flowers after all female flowers blooming. This provides time for receptive stigma to get pollens from male flowers, and enhance the opportunity of reproductive success. In addition, this result showed that the air temperature had effect on anthesis and receptive time. Temperature of air was 31.1-35.0°C in the morning (during 08.00-10.00 h) on April 16<sup>th</sup>, while on December 27<sup>th</sup> was 20.2-23.1°C in the morning.



**Figure 28** Anthesis and receptive time on *Jatropha curcas* L. flowers during 07.00-11.00 h on April 16<sup>th</sup> (wet season) and December 27<sup>th</sup> (dry season) in 2009 at Kamphaeng Saen (KPS) location

♀ = female flowers, and ♂ = male flowers

### 3.4 Pollen germination of physic nut (*Jatropha curcas* L.) flowers

Figure 29 and Appendix Table 8 showed the percentage of pollen germination variation were occurred among intra-variety (toxic) in different seasons at KPS location in 2009, between inter-variety (toxic and non-toxic) in the dry season at BK location in 2011, and between male and perfect flowers at AS location in 2009. In additional, Figure 30 and Appendix Table 9 showed the comparison pollen germination percentage from pollen grains on the bodies of the three species of honey bees (*Apis cerana indica*, *A. dorsata*, and *A. florea*) in the nature at KPS location in 2009.

#### 3.4.1 Toxic variety of physic nut

Comparison of pollen germination percentage in intra-variety (toxic) among three seasons at KPS location were significantly different pollen germination percentage. The highest value was pollen germination percentage in the summer season (70.50%, at 35.0°C), followed by value in the rainy season (60.33%, at 32.8°C), and the lowest value was pollen germination percentage in the winter season (55.60%, at 23.0°C).

#### 3.4.2 Toxic and non-toxic varieties of physic nut

Comparisons of pollen germination were done in inter-variety among four original sources i.e. KPSt, ARDCnt, ASnt, and SSnt. The experiments were done in the rainy season at BK location, there were significantly different in pollen germination percentage. The highest value of pollen germination percentage belong to SSnt original source (85.00%, at 33.0°C), followed by value in KPSt original source (62.00%, at 33.0°C), and the lowest value was ARDCnt and ASnt original sources (21.00% and 19.00%, at 33.0°C).

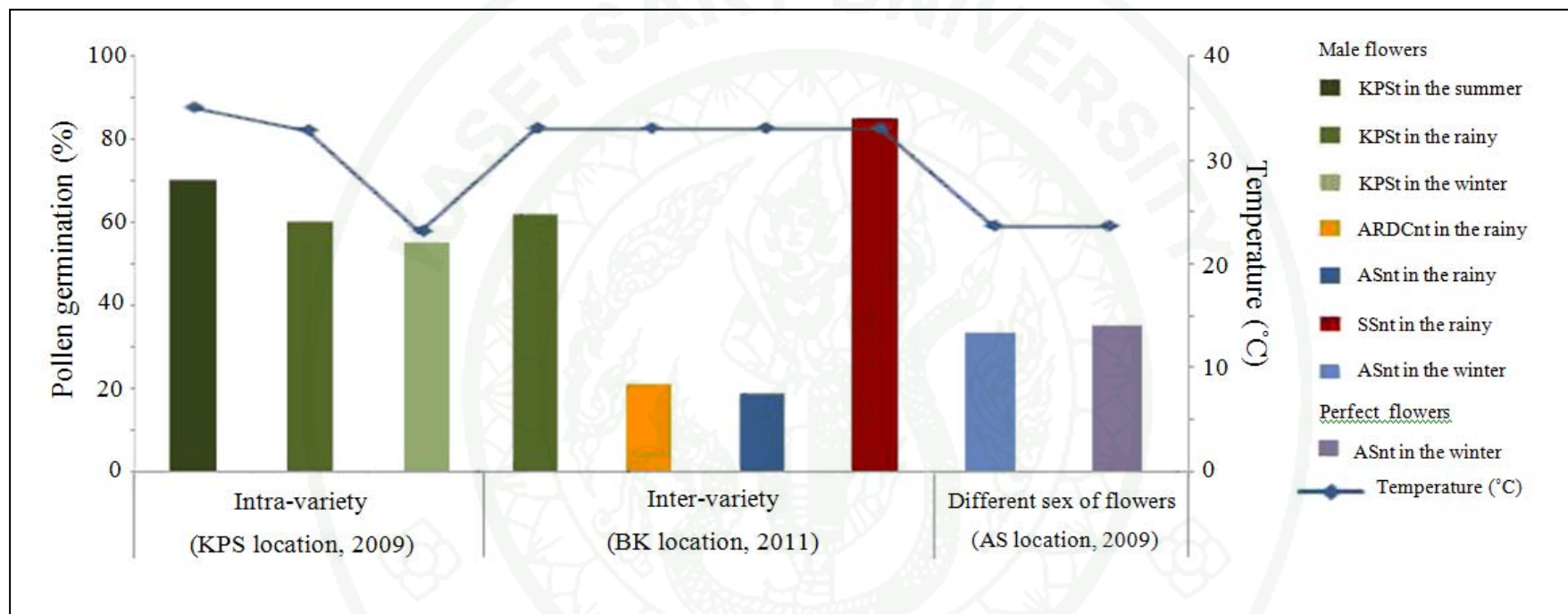
### 3.4.3 Male and perfect flowers

Comparison between pollen germination percentage of male and perfect flowers of non-toxic variety, in the field at AS location were non significantly different pollen germination percentage. In the male flowers were 34.00% at 23.5°C similar the perfect flowers which were 35.50% at 23.5°C.

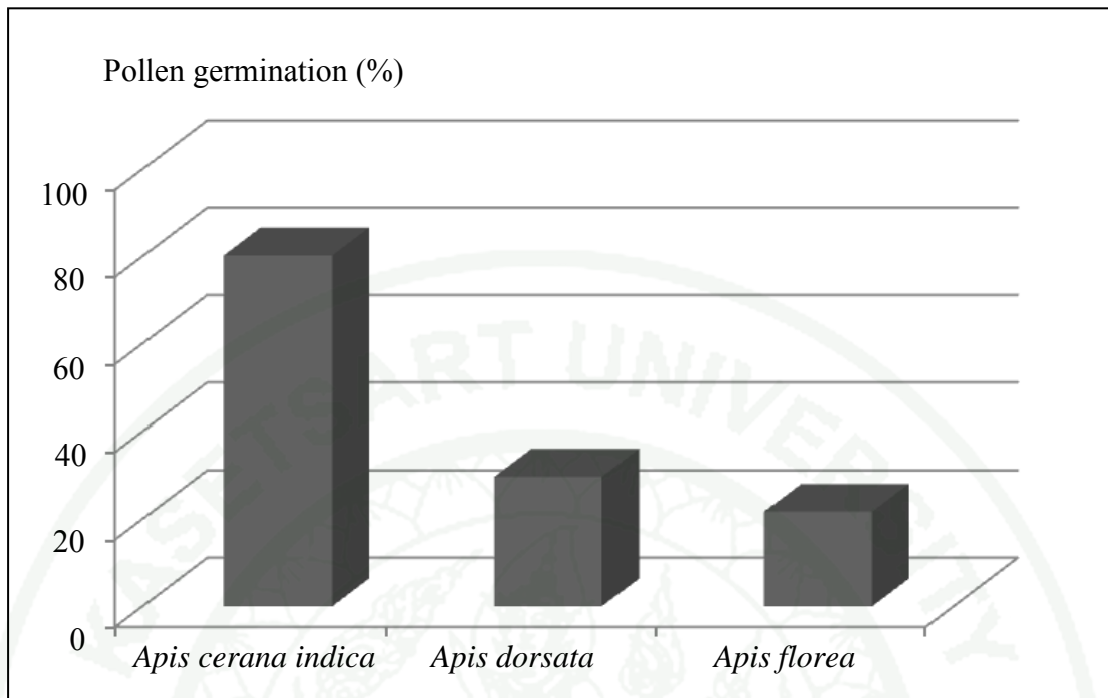
### 3.4.4 On these species of honey bee bodies

Comparison of pollen germination percentage from pollen grains on the body except from corbicular of three species of honey bees (*Apis cerana indica*, *A. dorsata*, and *A. florea*), in the summer (April) season 2009 at KPS location in the experimental plant fields. Pollen germination percentage was to be significantly different among three species of bees. The highest value was found on *A. cerana indica* (80.17%), followed by on *A. dorsata* (29.67%), and the lowest on *A. florea* (21.67%), all trials were done around temperature at 35.5°C.

Temperature had effect on pollen germination in the nature, pollen germination in vitro that various values followed by high or low temperature. Similarly, Malaipan *et al.*, (2007) indicated that various factors had effect on pollen germination; times, directions of sun, positions of flowers on inflorescence and inflorescences on branches, inflorescences stage, and variety. These results were different pollen germination among three season (the rainy, winter, and summer), four original sources of physic nut, whereas pollen germination of male flowers were similar to perfect flowers. In additional, this result supported that *A. cerana indica* was the most effective bees for pollination, because of the high pollen germination was observed pollen grains on their bodies. Pollen grains on bodies of *A. dorsata* and *A. florea* were low pollen germination; because of some factors (enzyme or bee individual behavior) influenced inhibition of pollen germination.



**Figure 29** Pollen germination percentage comparison in intra-variety (toxic) in different seasons at Kamphaeng Saen (KPS) location in 2009, inter-variety (toxic and non-toxic) in the dry season at Bangkhen (BK) location in 2011, and pollen grains from male and perfect flowers of *Jatropha curcas* L. at Aviation School (AS) location in 2009



**Figure 30** Comparison of pollen germination percentage from pollen grains on the bodies of three species of honey bees in the nature, in the summer season (April) 2009 at Kamphaeng Saen (KPS) location, at 35.5°C

### 3.5 Amount of pollen grains

The relationship between pollen grade on stigmas of *Jatropha curcas* L. flowers and frequency of effective bee or dwarf bees (*Apis florea*) visiting were studied, and fruit setting. The experiments were set in the toxic variety of physic nut plantation, in the summer season (April) 2009 at KPS location (Figure 31 and Appendix Table 10).

#### 3.5.1 Frequency of visiting

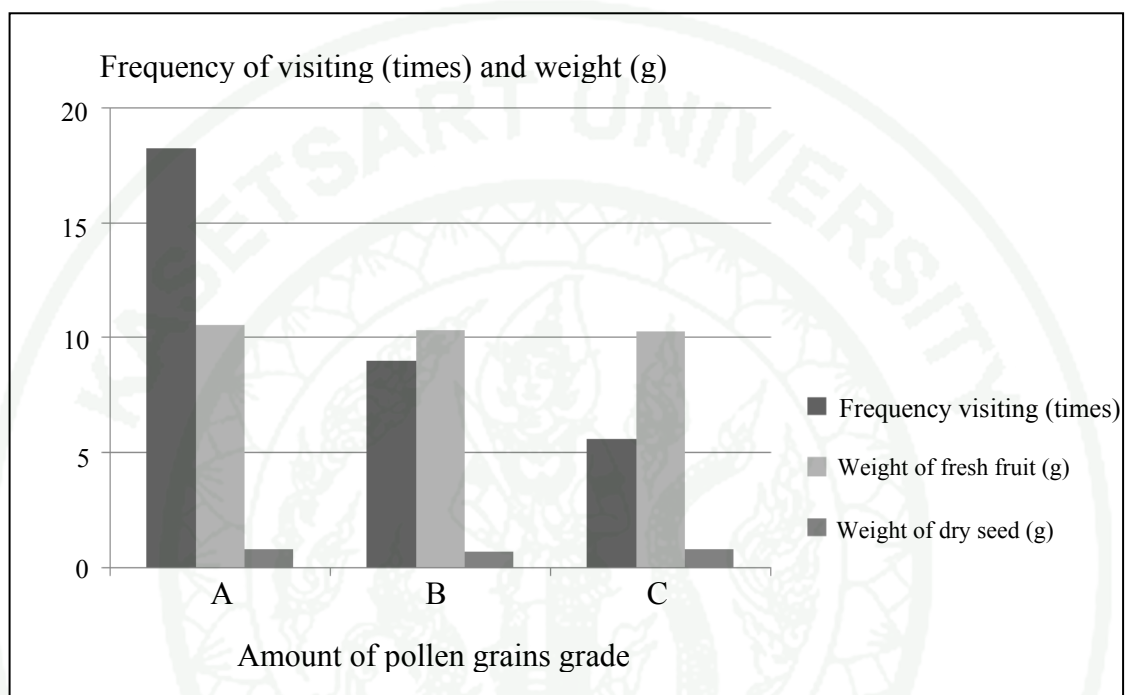
One of the effective bee, dwarf bees (*A. florea*) in the nature were to be significantly different frequency of visiting on physic nut flowers. The highest value was observed on female flowers, which had pollen A-grade (18.2 times), followed by on pollen B-grade (9 times), and the lowest frequency of visiting was pollen C-grade flowers (5.6 times). High frequency of visiting A-grade on stigmas were found in visiting of *A. florea*.

#### 3.5.2 Fruit setting

After flowers were pollinated with each grade of amount of pollen grains, the flowers changed to set fruits. The results showed similarity weight (g) of fresh fruit and dry seed, these parameters were non significant. The fresh fruit from female flowers, which amount of pollen grains in A, B, and C grade had 10.54 g, 10.30 g, and 10.25 g respectively. Likewise, weight of dry seed in each amount of pollen grains grades; A (0.78 g), B (0.71 g), and C (0.79 g) were resemblance.

Amount of pollen grains were increased respectively after stigmas were visited by pollinators. Abdelgadir *et al.* (2008) supported that flowers exposed to single and multi visit by honey bees set significantly more set fruits than those which received no visit, indicating that honey bees were effective pollinators. Correspondingly, the effect of number of pollen grains deposited, and pollen compatibility in seed set and fruit quality should be better studied in sweet pepper

crop. It probably happens because *Melipona subnitida* deposited a great number of viable pollen grains on the stigmas of sweet pepper flower, assuring many seeds set per fruit. More seeds developing inside each fruit would lead to bigger and heavier fruits, and reduce the percentage of malformed ones (Darci *et al.*, 2005).



**Figure 31** Average frequency of visiting of *Apis florea* on stigmas, average of fresh fruit, and dry seed weight (g) of *Jatropha curcas* L. flowers, in each amount of pollen grains grade, in the summer season (April) 2009 at Kamphaeng Saen (KPS) location

Amount of pollen grains grade

A = very fulfillment of pollen grains (>1,500 pollen grains/stigma)

B = moderate pollen grains (300-1,500 pollen grains/stigma)

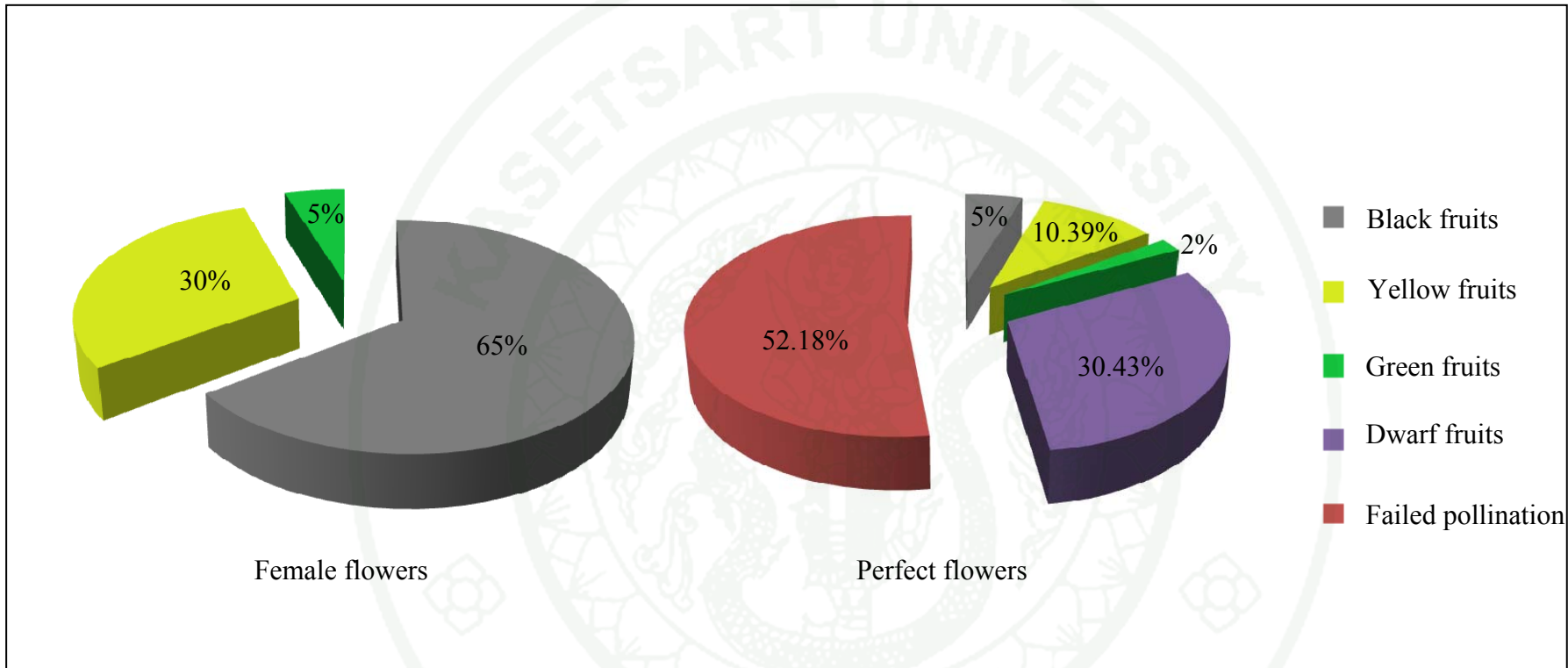
C = slight pollen grains (< 300 pollen grains/stigma)

### 3.6 Fruits and fruit set

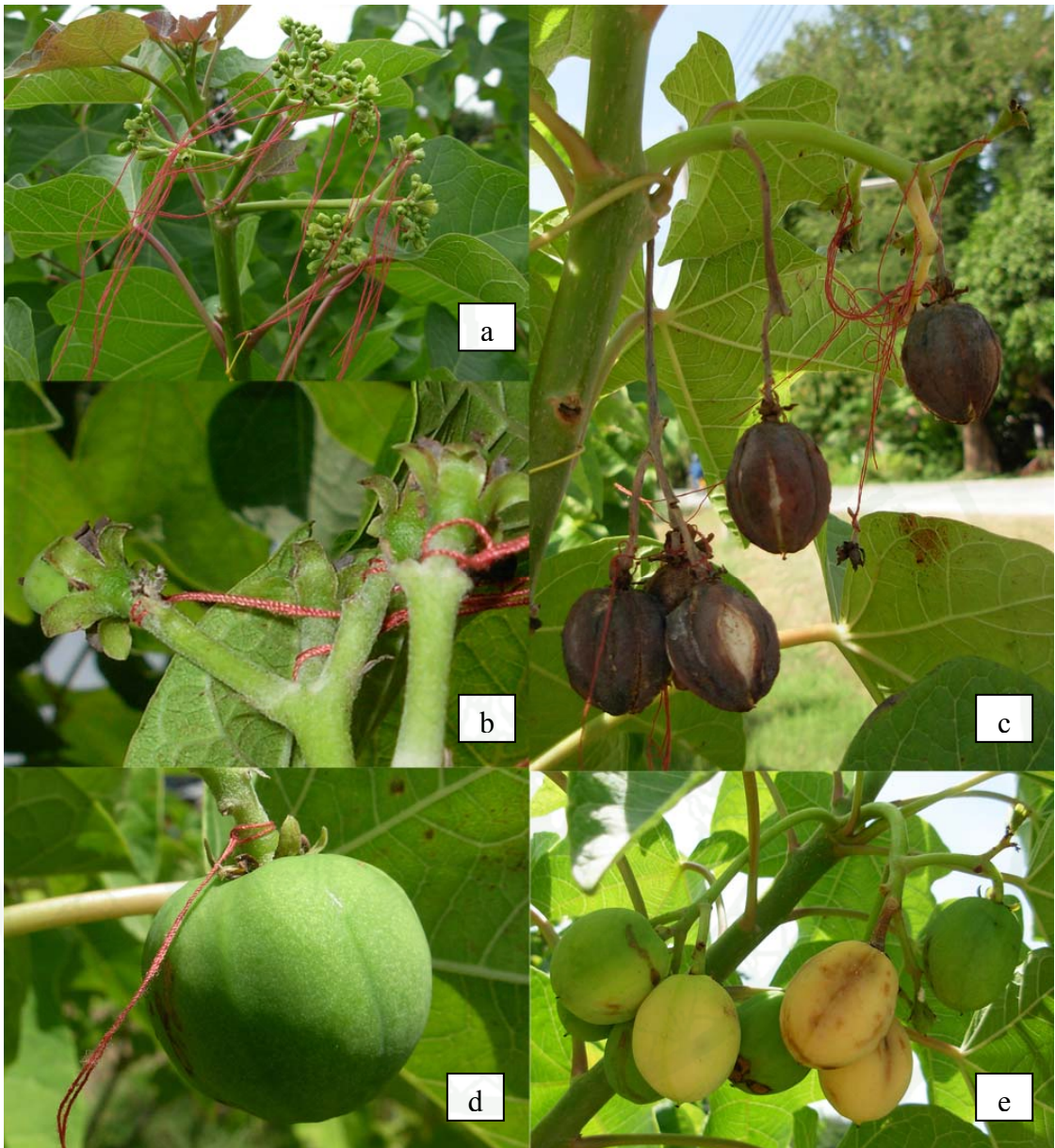
#### 3.6.1 Fruits from female and perfect flowers

At ARDC location in non-toxic variety, comparison of fruit development between female and perfect flowers was absolute difference. Female flowers can developed 100% complete fruits, while perfect flowers developed only 17.39% complete fruits, dwarf fruits (30.43%), and high percentage of fail set fruits (52.18%). In additional, the fruits from female and perfect flowers in the same age (64 days after open-pollinated; June 23<sup>rd</sup> to August 25<sup>th</sup>) had different fruits color. Black fruits (65%), yellow fruits (30%), and green fruits (5%) (Figure 32, 33 and Appendix Table 11).

Normally the fruits mature after 64 days of pollination and the seeds turn from green to yellow, but some fruits different color. The cause seems to be the long period of receptive time (5-7 days) that had effect on fruit development. Likewise, Chang-wei *et al.* (2007a) said that the stigma receptivity is strong during the 1<sup>st</sup> - 4<sup>th</sup> day and begins to decline on the fifth day onwards and completely loses its receptivity on the ninth day. Different fruits color occurred in equally period after pollination.



**Figure 32** Characteristics of fruit setting from female and perfect flowers in *Jatropha curcas* L. of the non-toxic variety on August 25<sup>th</sup> 2009 at Agriculture Research and Development Center (ARDC) location



**Figure 33** Fruit set from female and perfect flowers in *Jatropha curcas* L. non-toxic variety observation on June 23<sup>rd</sup> to August 25<sup>th</sup> 2009 at Agriculture Research and Development Center (ARDC) location

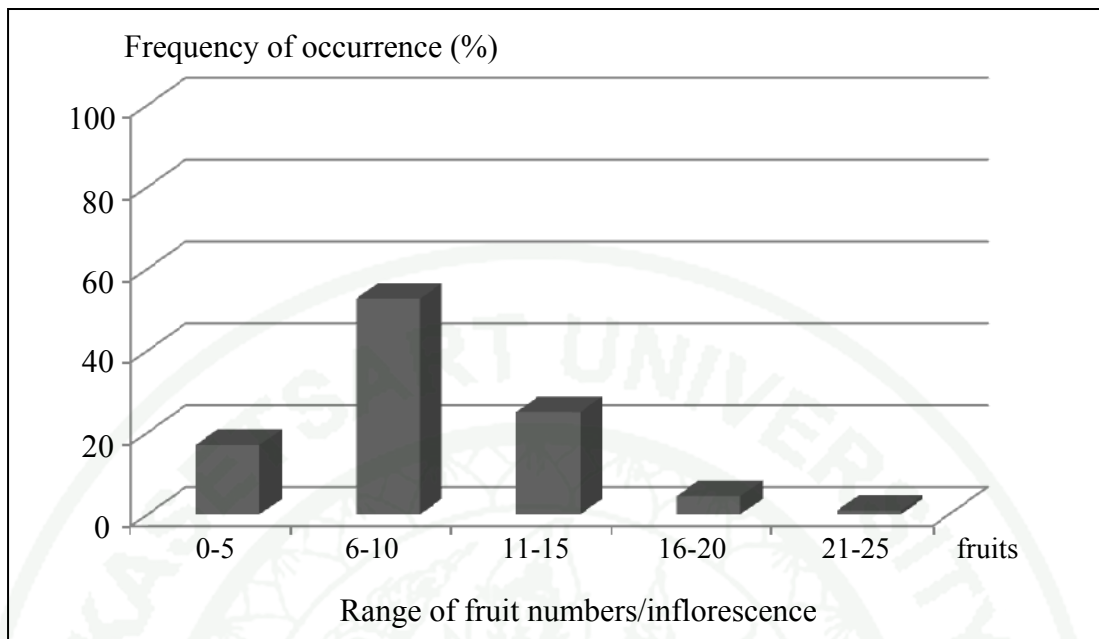
- a = Female and perfect flowers on the same inflorescence were tagged on June 23<sup>rd</sup> 2009, b = Dwarf fruits from perfect flowers  
 c = Fruit set from inflorescence in the a-figure, on August 25<sup>th</sup> 2009  
 d = Green fruit of four seeds developing, and e = Yellow fruits

### 3.6.2 Frequency of occurrence of fruit setting per inflorescence

Randomized large numbers of fruits were observed in the various number of fruits per inflorescence of toxic variety, at Kamphaeng Saen (KPS) location, on October 2008. The results showed various numbers of fruits per inflorescence, that were divided into five ranges i.e. 0-5, 6-10, 11-15, 16-20, and 21-25 fruits/inflorescence. Frequency of occurrence of fruits were to be significantly different in among vary ranges of fruit numbers per inflorescence, the highest frequency of occurrence of fruits were found on 6-10 fruits/inflorescence (52.68%), followed by 11-15 fruits/inflorescence (25.00%), 0-5 fruits/inflorescence (16.96%), and 16-20 fruits/inflorescence (4.46%), while the lowest frequency of occurrence of fruits were found on 21-25 fruits/inflorescence (0.89%) (Figure 34 and Appendix Table 12).

Saimaneerat *et al.* (2006) reported that fruit numbers per inflorescence in 52 accessions of physic nut were observed from various locations of the Northeastern, the Central Plain and the Southern part of Thailand. Range of fruit number per inflorescence showed 2-8 fruits/inflorescence in the Northeastern, and 4-13 fruits/inflorescence in the Central Plain and the Southern part of Thailand. While this observation revealed range of fruit setting per inflorescence about 0-25 fruits/inflorescence, that high variation fruit setting of physic nut.

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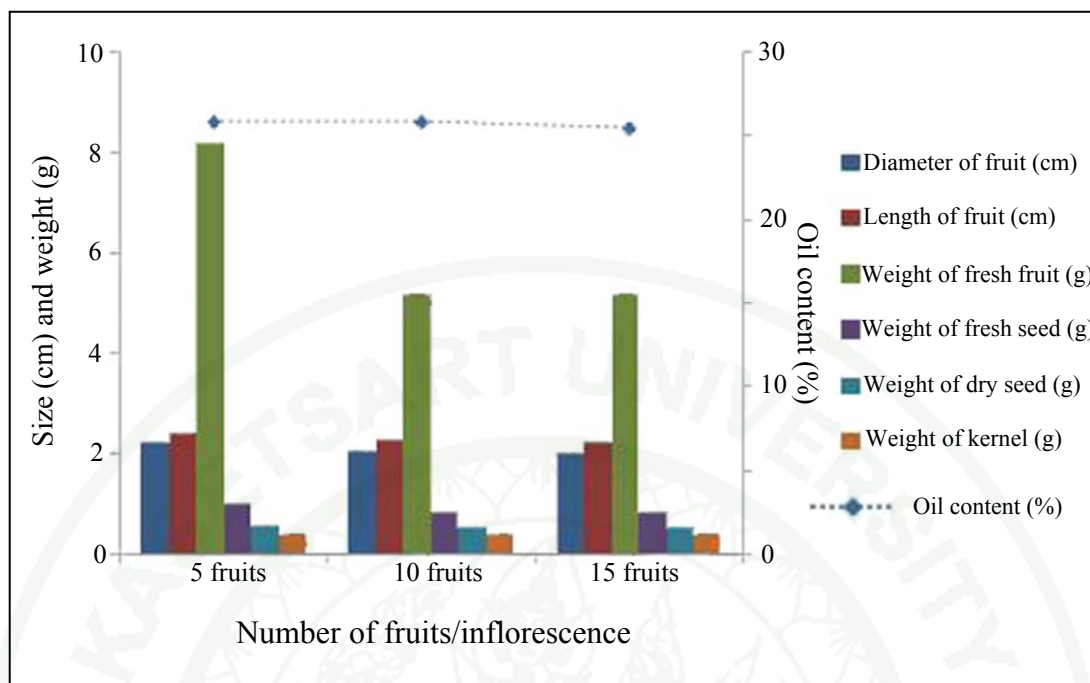


**Figure 34** Frequency of occurrence of fruit numbers per inflorescence of *Jatropha curcas* L. in toxic variety, on October, 2008 at Kamphaeng Saen (KPS) location

### 3.6.3 Effect of fruit numbers per inflorescence

At KPS location in 2009 the fruit set experiments were observed, after female flowers were pollinated by various insect pollinators. Size of fruit, weight of seed, kernel and oil content were non significantly different among various fruit numbers per inflorescence (5, 10, and 15, respectively). Fresh fruits diameter of 5, 10, and 15 fruits/inflorescence were 2.24 cm, 2.07 cm, and 2.01 cm, respectively. Fresh fruits length of 5, 10, and 15 fruits/inflorescence were 2.41 cm, 2.29 cm, and 2.23 cm, respectively. Fresh seed weight in 5 fruits/inflorescence was 1.03 g, similar to fresh seed weight of 10, and 15 fruits/inflorescence were 0.87 g. Dry seed weight of 5, 10, and 15 fruits/inflorescence were 0.61 g, 0.56 g, and 0.55 g, respectively. Kernel weight in 5 fruits/inflorescence was 0.42 g, similar to kernel weight of 10, and 15 fruits/inflorescence were 0.41 g. Oil content in 5, and 10 fruits/inflorescence were 25.87%, similar to oil content of 15 fruits/inflorescence was 25.48%. Weight of fresh fruit was significantly different among various numbers of fruit settings. Weight of fresh fruit; the highest value were found in 5 fruits/inflorescence (8.20 g), while the lowest weight was observed in 10, and 15 fruits/inflorescence; 5.22 g, and 5.21 g respectively (Figure 35 and Appendix Table 13).

Fruit number (5, 10, and 15) per inflorescence had effect on weight of fresh fruit only, while that were had no effect on the oil content. Also, the full potential of fruitfulness seems to exceed 15 fruits/inflorescence of physic nut. Even though fruitful of many horticulture have a large negatively impact on fruits. For example, plant in family Curcubitaceae (watermelon, melon, cucumber, and pumkin) the number of fruits per plant absolutely had effect on their size and seed production. Only one fruit is retained per vine and the maximum of 2 vines are retained per plant for seed production of watermelon (*Citrulus lanatus* Thumb) (Tomar, 2011).



**Figure 35** Effect of fruit numbers per inflorescence to size of fruits, weight of fruit and seed, and oil content of *Jatropha curcas* L. in toxic variety at Kamphaeng Saen (KPS) location in 2009

## 4. Pollination factors

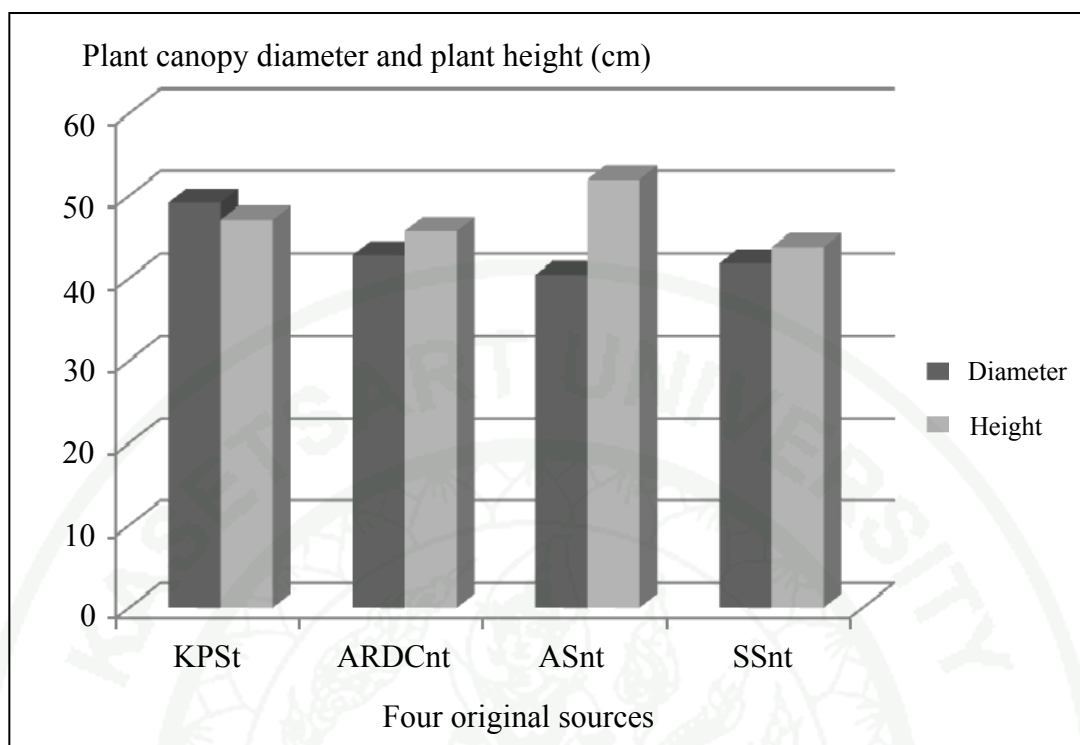
### 4.1 Varieties of physic nut (*Jatropha curcas* L.)

At Bangkhen (BK) location, the mixed variety of physic nut from the plot-A; toxic variety at Nong Yai (NYt), were planted mixed variety of non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunphiboon (SSnt) on the same plants and the different plants with cleft grafting method, and plot-B; four original sources such as toxic variety at Kamphaeng Saen (KPSt), non-toxic at Agriculture Research and Development Center (ARDCnt), non-toxic at Aviation School (ASnt), and non-toxic from Mr. Suksan Suttipunphiboon (SSnt) were planted in the pots with stem cutting method.

#### 4.1.1 Plant canopy diameter and plant height

Ninety days later, plant canopy diameter and plant height were measured on plot-B. These data were non significantly different among four original sources, ASnt (diameter, height: 40.40 cm, 51.98 cm), ARDCnt (42.78 cm, 45.72 cm), SSnt (41.78 cm, 43.78 cm), and KPSt (49.15 cm, 47.10 cm) (Figure 36 and Appendix Table 14).

Different original sources not influenced to plant canopy diameter and plant height. Likewise, Kamphaengkaew and Chinawong (2007) indicated that study on patterns of pruning and nutrition management for increase yield in physic nut on February 2006 to February 2007 at Agronomy Department field laboratory faculty of Agriculture at Kamphaeng Saen Kasetsart University Nakhon Pathom Thailand. Four different patterns of pruning have no different with plant canopy diameter which pattern unpruned (control), and nutrition management with five different patterns have no effect with plant height, plant canopy diameter, stem diameter and yield.



**Figure 36** Plant canopy diameter and plant height of four original sources *Jatropha curcas* L., on plot-B in the rainy season 2011, 90 days after planting at Bangkhen (BK) location

KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

#### 4.1.2 Leaf area of plants

Table 12 summarized leaves area of physic nuts. The 10<sup>th</sup> of leaf on branches on plot-A and B were measured leaf area (cm<sup>2</sup>). The results showed variation of leaf area in four original sources and difference propagation methods. Leaf area of plants on the plot-A was significantly different among four original sources. The highest leaf area was ASnt (270.58 cm<sup>2</sup>), followed by ARDCnt (260.17 cm<sup>2</sup>), and SSnt (164.17 cm<sup>2</sup>), the lowest leaf area was NYt (154.58 cm<sup>2</sup>), whereas leaf area of plants on the plot-B were significantly different among four original sources. The highest leaf area was ARDCnt (109.50 cm<sup>2</sup>), followed by SSnt (78.12 cm<sup>2</sup>), ASnt (63.19 cm<sup>2</sup>), and KPSt (62.56 cm<sup>2</sup>). Thus, these leaf area comparison between Plot-A and Plot-B of three original sources (ARDCnt, ASnt, and SSnt) were significantly different between plant propagation methods; Plot-A-cleft grafting method, and Plot-B-stem cutting methods. The leaves of physic nut from cleft grafting method were bigger than the leaves from stem cutting method about 4.28 times in ASnt, followed by ARDCnt (2.10 times), and SSnt (2.10 times).

These results showed that the variation of plants morphology, such as leaves area of plants is dominant character. Different cultivar technique of plant propagation had effect on vegetative development that showed stem cutting plants in pots were smaller than the leaves growing of cleft grafting plants which were similar to the same original sources from the field. Correspondingly, Kobilke (1989) in Cape Verde, conducted comparative research on the influence of different propagation methods on survival and vegetative development. In better rainfall or good moisture condition the plantation could also be established by direct seeding. The survival rate depended not only on sowing time and depth of sowing, but also on the trial year. When establishing a physic nut crop, the survival rate can be influenced by the choice of cultivation method. Two factors are generally responsible for sprouting: the age of the plant from which cuttings are taken and the position of the cutting within the plant, it might have been due to declines of rooting ability of many woody plants with age, when the source is a seedling derived mother plant. Distal portion of the stock plants are first to exhibit this reduced rooting

potential, while cuttings from the lower or juvenile regions of the plants generally maintain a higher rooting capacity than those from the upper regions (Hartmann and Kester, 1983).

**Table 12** Leaf area of four *Jatropha curcas* L. original sources, on plot-A and plot-B, in the rainy season 2011 at Bangkhen (BK) location

| Original sources <sup>1</sup> | Average of leaf area (cm <sup>2</sup> ) <sup>2</sup> |                       | Differential ratio |
|-------------------------------|--|-----------------------|--------------------|
|                               | Plot-1(Cleft grafting)                               | Plot-2 (Stem cutting) |                    |
| KPSt                          | -  | 62.56 <sup>b</sup>    | -                  |
| NYt                           | 154.58 <sup>c</sup>                                  | -                     | -                  |
| ARDCnt                        | 260.17 <sup>b</sup>                                  | 109.50 <sup>a</sup>   | 2.38:1             |
| ASnt                          | 270.58 <sup>a</sup>                                  | 63.19 <sup>b</sup>    | 4.28:1             |
| SSnt                          | 164.17 <sup>c</sup>                                  | 78.12 <sup>b</sup>    | 2.10:1             |

<sup>1</sup> KPSt = toxic variety from Kamphaeng Saen location

NYt = toxic variety from Nong Yai, Chon Buri province

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

<sup>2</sup> Average of leaf areas were significantly difference (df = 39, p = 0.05).

#### 4.1.3 Growth development of physic nut

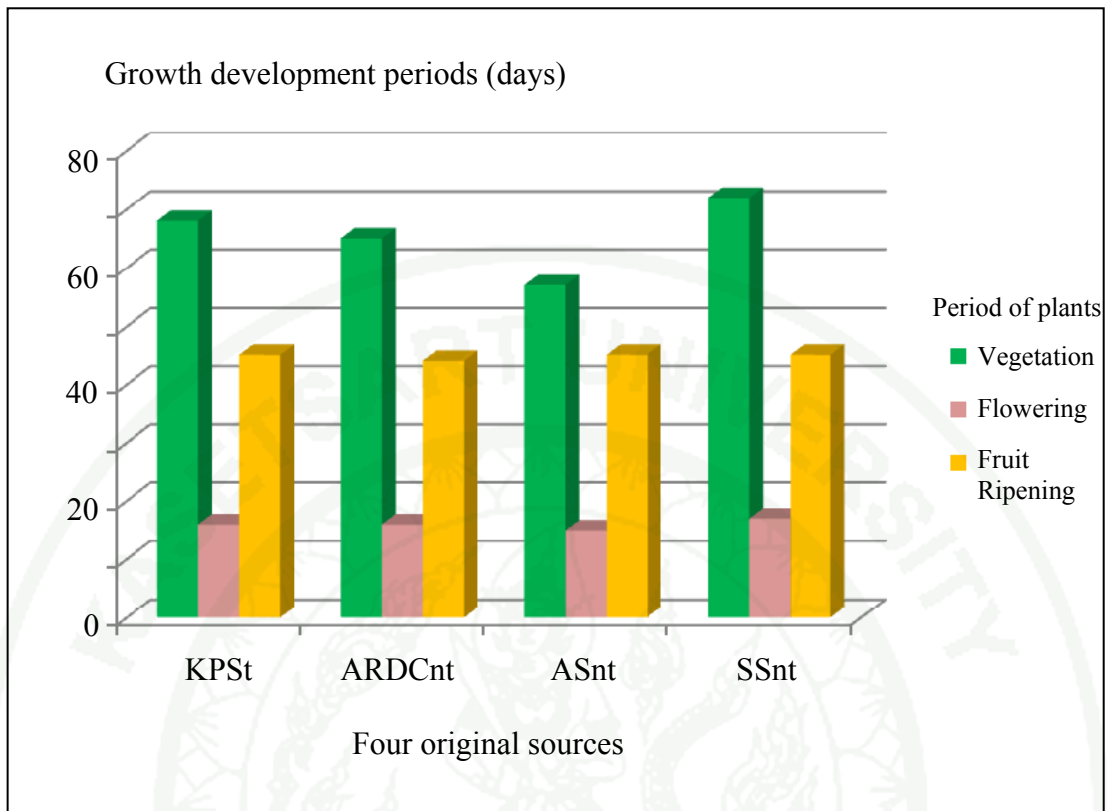
Figure 37 and Appendix Table 14 summarized different growth development periods among the mixed variety of physic nut from the plot-B. Vegetative period, flowering, and fruit ripening were observed.

1) Vegetative period was non significantly different among four original sources, i.e. ASnt (57 days), ARDCnt (65 days), SSnt (72 days), and KPSt (68 days).

2) Flowering was the first flower blooming to the last of flowers blooming, these period was non significantly different among four original sources; ASnt (15 days), ARDCnt (16 days), SSnt (17 days), and KPSt (16 days).

3) Fruit ripening period was the first day after female flowers were pollinated, and developing to yellow fruits, they were collected for oil extraction process. These period were non significantly different among four original sources; ASnt (45 days), ARDCnt (44 days), SSnt (45 days), and KPSt (45 days).

Different original sources have no effect on vegetative growth, whereas variable of location, plant propagation, and cultivation of physic nut had effect on vegetative growth. This vegetative period observation (57-72 days) was shorter than vegetative period of physic nut (77-82 days) of observation from Chutkaew *et al.* (2007). Similarly, this flowering period (15-17 days) slightly different each inflorescence's flower last from 13-19 days (Chang-wei *et al.*, 2007a). Even though, this fruit ripening period was 44-45 days, but Dhillon *et al.* (2006) reported that period of fruit development and maturity ranged from 55-61 days from date of first fruit initiation.



**Figure 37** Growth development of *Jatropha curcas* L. among four original sources, on plot-B in the rainy season 2011, 90 days after planting at Bangkhen (BK) location

KPSst = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

#### 4.1.4 Size and shape of flowers and fruits

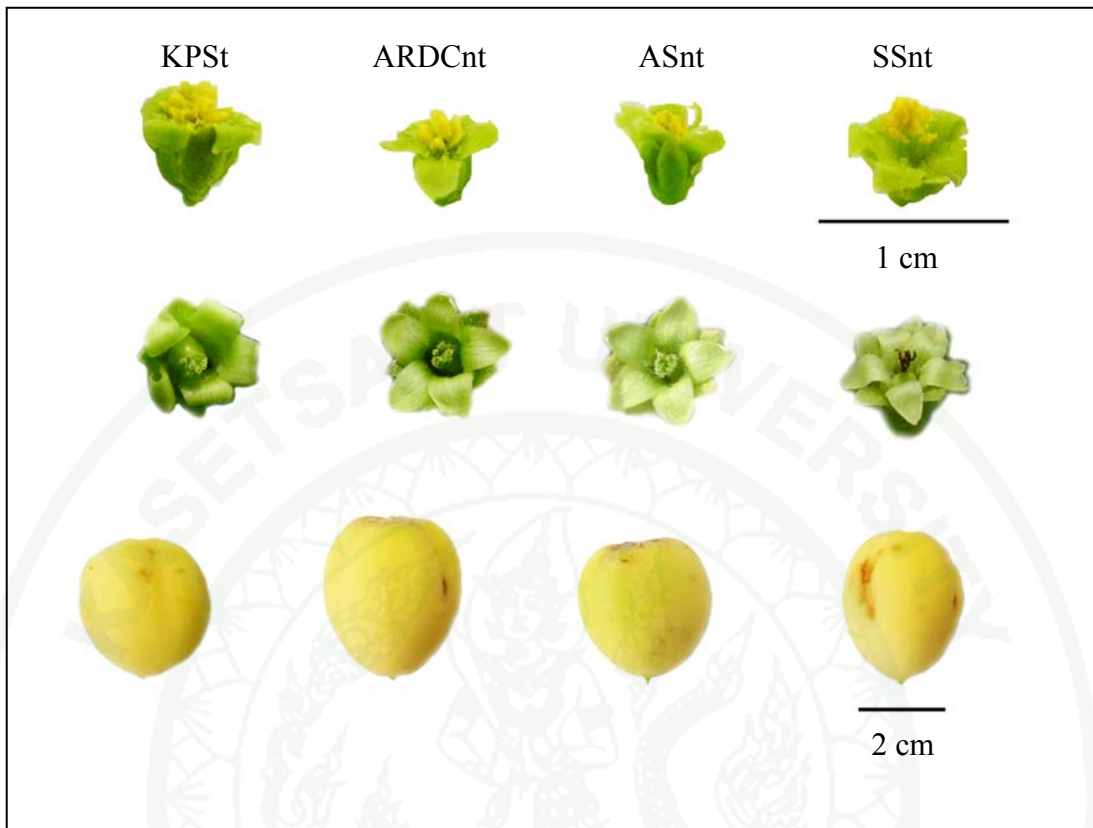
Figure 38 summarized size of flowers and fruits on the plot-B (open-pollination) in the rainy season 2011 at BK location were measured.

1) Male flowers revealed slightly different size and shape, diameter of the male flowers in ARDCnt and ASnt was  $0.6 \pm 0.1$  cm, heights of flowers were  $0.6 \pm 0.1$  cm, and the shapes of flowers was liked-tulip flower. In addition, flower size of SSnt was similar to KPSt, the diameter of flowers was  $0.7 \pm 0.1$  cm, and shape of flowers was different between SSnt and KPSt, petals and anthers of flowers in SSnt were slimmer than flowers in KPSt.

2) Female flowers revealed non significantly size and shape, diameter of the female flowers in four original sources was  $0.9 \pm 0.1$  cm, heights of flowers were  $0.9 \pm 0.1$  cm, and the shape of flowers was similarity.

3) Fruits revealed slightly different size and shape, the biggest fruit was found in ARDCnt ( $2.7 \pm 0.1$  cm), followed by ASnt ( $2.6 \pm 0.1$  cm), and KPSt ( $2.3 \pm 0.1$  cm), while the smallest fruit was observed in SSnt ( $1.9 \pm 0.1$  cm) (average of diameter). The longest fruit was found in ARDCnt ( $2.9 \pm 0.1$  cm), followed by ASnt ( $2.7 \pm 0.1$  cm), and SSnt ( $2.6 \pm 0.1$  cm), while the shortest fruit was observed in KPSt ( $2.4 \pm 0.1$  cm) (average of length). Shape of fruits in ASnt and KPSt was spherical shape, while fruits in ARDCnt and SSnt were oval shape, but fruit in SSnt was narrow oval shape more than fruit from ARDCnt.

Raju and Ezradanam (2002), and Chang-wei *et al.* (2007a) indicated that male flowers are small and plate shaped, the five sepals (0.5 cm long) and five petals (0.7 cm) are free. Female flowers show a similar shape as the male flowers. These flowers contain three styles and bifid stigmas. It might be concluding that size and shape of male was similar to female flowers in the same variety, while size and shape of both flowers were different among various varieties.



**Figure 38** Size and shape of flowers and fruits in *Jatropha curcas* L. on plot-B (open-pollination) in the rainy season 2011 at Bangkokhen (BK) location

KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

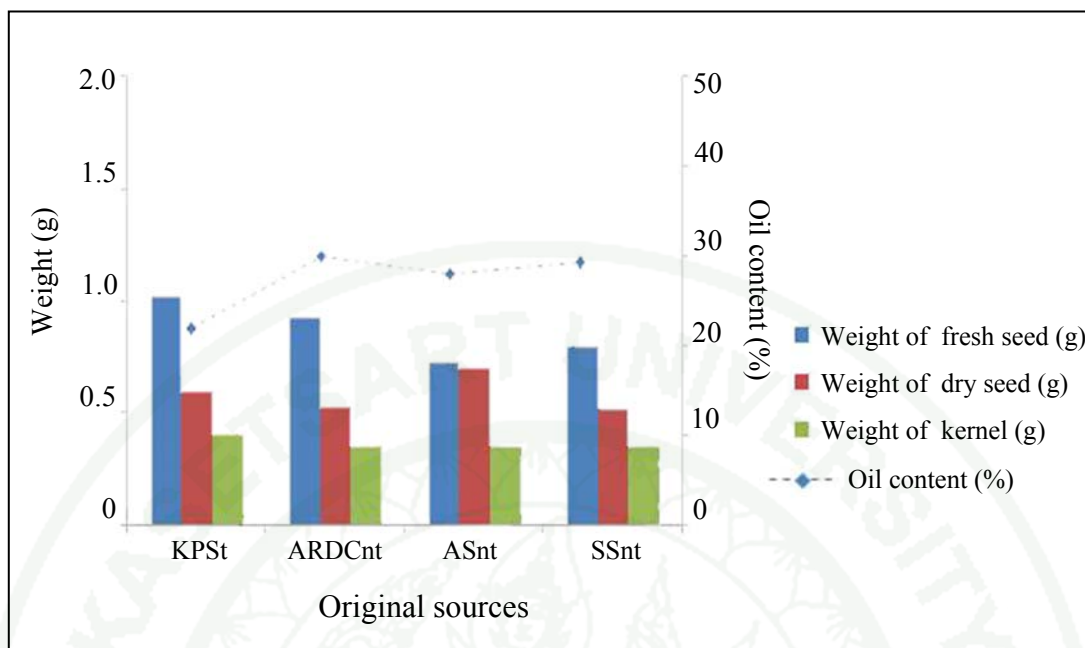
ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

#### 4.1.5 Weight and oil content of physic nut

These experiments were observed on plot-B in the rainy season 2011 at BK location. Figure 39 and Appendix Table 15 summarized different variety of physic nut had effect on size of fruit, weight of fruit and seed, kernel, and oil content. Weight of fresh fruits, fresh seed, dry seed, and oil content were significantly different among four original sources. Weights of fresh fruit in ARDCnt (10.09 g), ASnt (9.50 g), and KPSt (8.13 g) were higher than SSnt (5.74 g). Likewise, weight of fresh seed; the highest value was found in KPSt (1.02 g), followed by ARDCnt (0.93 g), SSnt (0.80 g), and ASnt (0.73 g). Similarly, weights of dry seed in ASnt (0.70 g), and KPSt (0.60 g) were higher than ARDCnt (0.53 g), and SSnt (0.52 g). Correspondingly, oil contents in ASnt (28.20%), ARDCnt (30.10%), and SSnt (29.31%) were higher than KPSt (22.01%). While weight of kernel were non significantly different in four original sources.

Weight and oil content of physic nut were variation among four original sources. The dry seed weight of physic nut around 0.75 g and contain more than 30% by weight of oil that was suitable for conversion into biodiesel. The kernel forms around 60% of the seed (Becker and Francis, 2010). Makkar *et al.* (1997) reported that large variations in contents of crude protein, crude fat, neutral detergent fiber and ash on 18 different provenances of physic nut from countries in the West and East Africa, Americas and Asia. Wani *et al.* (2006) recorded variation in Indian accessions for oil content (27.8-38.4%) and 0.44-0.77 g/seed. Kaushik *et al.* (2007) explored the variability in Haryana-India accessions to find wide variation in 100 seed weight (49-69g) and oil content (28-39%). Similarly, Rao *et al.* (2008) reported that variation in 0.57-0.79 g/seed and oil content (30-37%) for Andhra Pradesh, India, accessions. In additional, Pramanik (2003) indicated that the oil content of physic nut seed ranges from 30-50% by weight and the kernel itself ranges from 45-60%, while these results were lower oil content than previously researches, because oil extraction seem to vary in kind of solvent and condition.



**Figure 39** Weight of fruit, seed and kernel, and oil content of *Jatropha curcas* L. on plot-B in the rainy season 2011 at Bangkhen (BK) location

#### 4.1.6 Diversity of insect pollinators

At BK location, in the rainy season 2011, the studies were done on the diversity of insect pollinators on *Jatropha curcas* L. flowers on plot-B. Figure 40, Table 13 and Appendix Table 16 showed different species list, species diversity index ( $H'$ ) and density of insect pollinators.

##### 1) Species list of insect pollinators

The result represented 11 species, 10 genera and seven families, three orders, namely Diptera, Hemiptera, and Hymenoptera. Species list of insect pollinators on physic nut flowers was slight different among four original sources, the highest species list was observed on flowers of KPSt (9 species), followed by ASnt and ARDCnt (8 species), while the lowest species list was observed on flowers of SSnt (4 species).

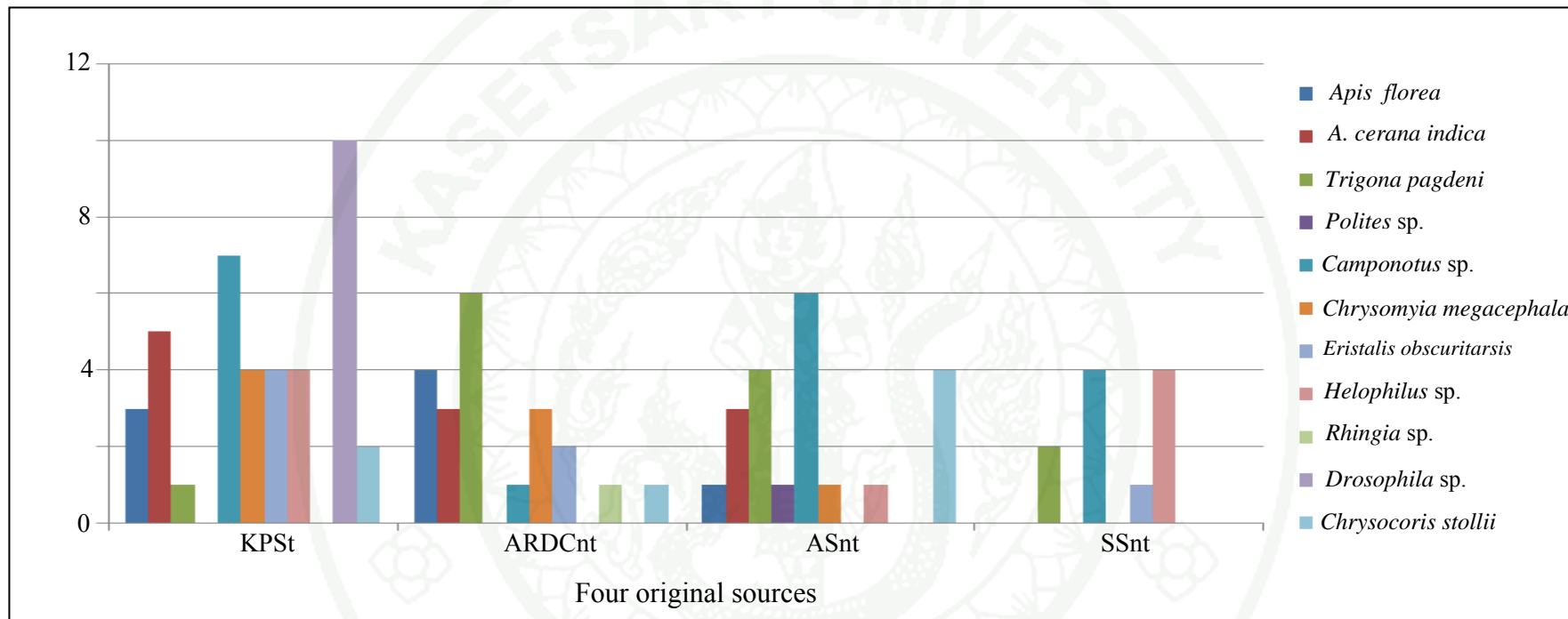
##### 2) Species diversity index

The species diversity indices were different among four original sources. The highest value was found in KPSt (2.04), followed by ASnt (1.85) and ARDCnt (1.89), the lowest value was found in SSnt (1.26).

##### 3) Density of insect pollinators

The density of insect pollinator visited on physic nut flowers among different four original sources showed in Table 13. Density of visitor (individual) per flowers revealed a few difference ratio, the ratio of insect pollinator (individual) per various flowers in KPSt, ARDCnt, and ASnt (1:9) were higher than SSnt (1:17).

These results represented that bee prefer visited on physic nut flowers both toxic and non-toxic variety. Julia (1964) reported that physic nut (*Jatropha curcas*) was other poisonous plants have nectar apparently harmless to bees and provide excellent honey same as poison ivy (*Rhus toxicodendron*), poison wood (*Metopium toxiferum*), manchineel (*Hippomane mancinella*), sea myrtle (*Baccharis halimifolia*) and others which were of value in our honey flora. The castor bean (*Ridnus communis*) was an important source of pollen. In additional, some plants were unsafe sources of nectar or pollen. Poisonous nectar or pollen which kills the bees before they could make honey. Examples were non-Floridian locoweed (*Astragalus lentiginosus*), buckeye (*Aesculus californica*), death camass (*Zygadenus venenosus*), *Veratrum califomicum*. These plants had nectar harmless to bees but which yield honey more or less toxic to humans. Notorious plants of this nature were oleander (*Nerium oleander*); jimson weed, angel's trumpet and other species of *Datura*, which occasion frequent deaths in Mexico; mountain laurel (*Kalmia* spp.); Carolina yellow jessamine (*Gelsemium sempervirens*), reportedly fatal to young bees also; snow-on-the-mountain (*Euphorbia marginata*); and the potent *Serjania lethalis* of Brazil.



**Figure 40** Species list and number (individuals) of insect pollinators on *Jatropha curcas* L. flowers in four original sources on plot-B in the rainy season 2011 at Bangkhen (BK) location

KPSt = toxic variety from Kamphaeng Saen location, ARDCnt = non-toxic variety from Agriculture Research and Development Center location, ASnt = non-toxic variety from Aviation School location, SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

**Table 13** Diversity of insect pollinators on *Jatropha curcas* L. flowers in four original sources on plot-B in the rainy season 2011 at Bangkhen (BK) location

| Diversities                               | Four original sources <sup>1</sup> |        |      |      |
|---|------------------------------------|--------|------|------|
|   | KPSt                               | ARDCnt | ASnt | SSnt |
| Total of species                          | 9                                  | 8      | 8    | 4    |
| Species diversity index (H')              | 2.04                               | 1.89   | 1.85 | 1.26 |
| Total of insect pollinators (individuals) | 40                                 | 21     | 21   | 11   |
| Total of male flowers                     | 329                                | 189    | 176  | 171  |
| Total of female flowers                   | 0                                  | 3      | 2    | 10   |
| Total number of all flowers               | 329                                | 192    | 178  | 181  |
| Ratio of visitor: flowers                 | 1:9                                | 1:9    | 1:9  | 1:17 |

<sup>1</sup> KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

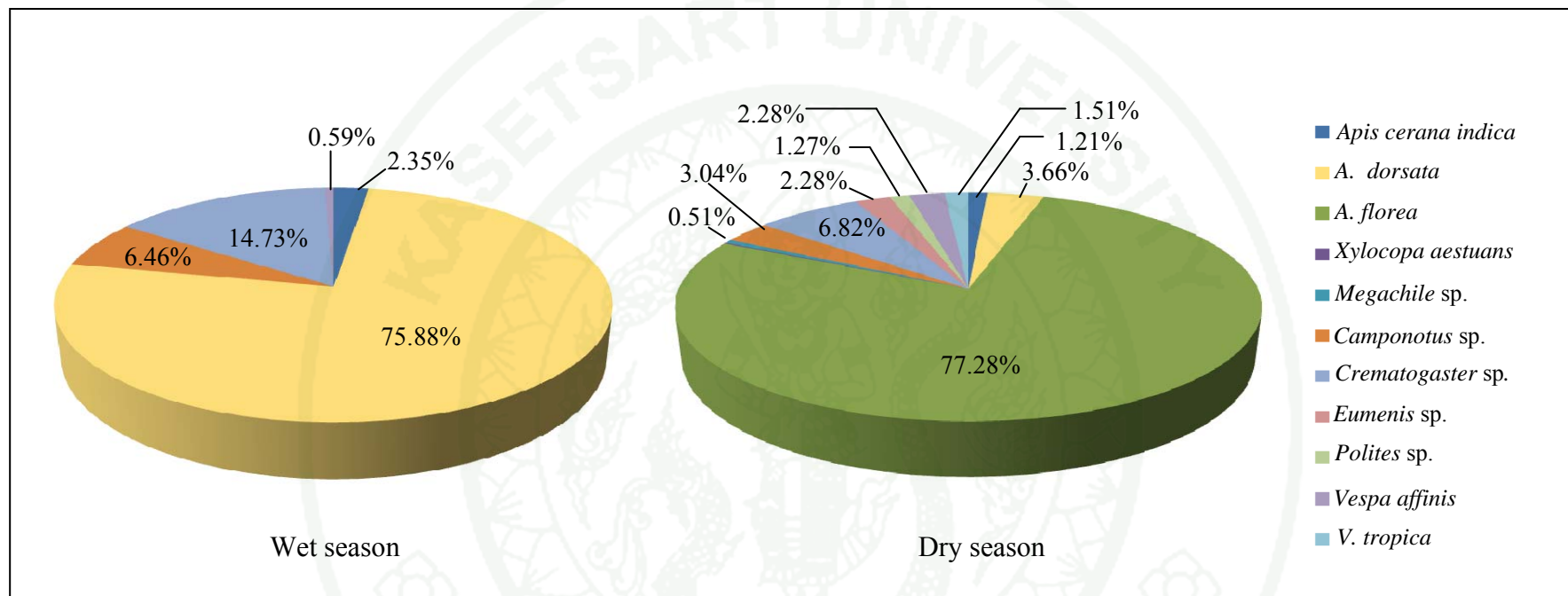
SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

## 4.2 Seasons

At KPS location in 2008-2009, species list, species diversity index ( $H'$ ), and number insect pollinators (Hymenoptera) were difference between the wet and dry seasons. Data were showed in Figure 41 and Appendix Table 17, Species list in the dry season (11 species) was more than the wet season (5 species). Similarly, species diversity index in the dry season (0.98) was slight higher than the wet season (0.79), number of insect pollinators in the dry season (661 individuals) was more than the wet season (142 individuals).

This location was irrigated all year, thus physic nut flowers were always flowering. Insect pollinators in the dry season were found higher species diversity than in the wet season, because of competitive plants (corn, sensitive plants, and caltrops) in the wet season more occurred than in the dry season. Whereas, Malaipan *et al.* (2007) reported few species diversity (>50 species) in the dry season, while species diversity of this research in the wet season was recorded 311 species, these locations were non irrigation.

Vespidae, wasps were not found in the wet season (April-September 2009) because of dominant behavior of wasps usually prepared food for their brood. Leaflets of host plant in the wet season suit for growth development of caterpillars in Lepidoptera, which was a favorite food for female of wasps. The females of a few solitary wasps, may oviposit after they had stored the larval food, while female of subsocial wasps practiced progressive provisioning. They prepared a cell and oviposit, but may not started hunting for and providing food until the egg hatches. Food was then brought in slowly, and the cell may not be sealed until the larva is fully grown (Kenneth and Matthews, 1991). In additional, adult wasps were insectivorous, melliferous and frugivorous; they will also enter houses to hunt for sugary substances. The larvae on the other hand were strictly carnivorous and the workers caught a wide range of insects and other small animals, and also scavenge dead meat, to provide the larvae with the diet they require (Gauld and Bolton, 1988).



**Figure 41** Species list, species diversity index ( $H'$ ), and number of insect pollinators (Hymenoptera) on *Jatropha curcas* L. flowers in the wet and dry season 2008-2009 at Kamphaeng Saen (KPS) location

Insect pollinators visited on both female and male flowers of physic nut

Individual of insect pollinators/10 rais; 1 rai = 1,600 m<sup>2</sup>

Wet season during April-September 2009

Dry season during October 2008-Mar 2009

### 4.3 Times of day and directions of plant canopy

At KPS location on October 2008, flowers from 75 inflorescences were targeted for determinations during 30 minutes of number and species of pollinator every hour during 07.00-18.00 h, in five directions of plant canopy. Numbers of insect pollinator on physic nut flowers were significantly different among times of day, and directions of plant canopy (Figure 42 and Appendix Table 18).

1) In every hour during 07.00-18.00 h, the highest number of insect pollinators was observed on 13.00-14.00 h (42 individuals), followed by 15.00-16.00h (32 individuals), 11.00-12.00 h (30 individuals), 14.00-15.00 h, and 16.00-17.00 h (29 individuals), 10.00-11.00 h (27 individuals), 12.00-13.00 h (25 individuals), 08.00-09.00 h (18 individuals), and 09.00-10.00 h (17 individuals), while the lowest number of insect pollinators was observed on 07.00-08.00 h (8 individuals), and 17.00-18.00 h (6 individuals).

2) In five directions of plant canopy, the highest number of insect pollinator was observed in the east (114 individuals), followed by the north (106 individuals), the west (99 individuals), the south (95 individuals), while the lowest number of insect pollinators was observed in the central of plant canopy (62 individuals).

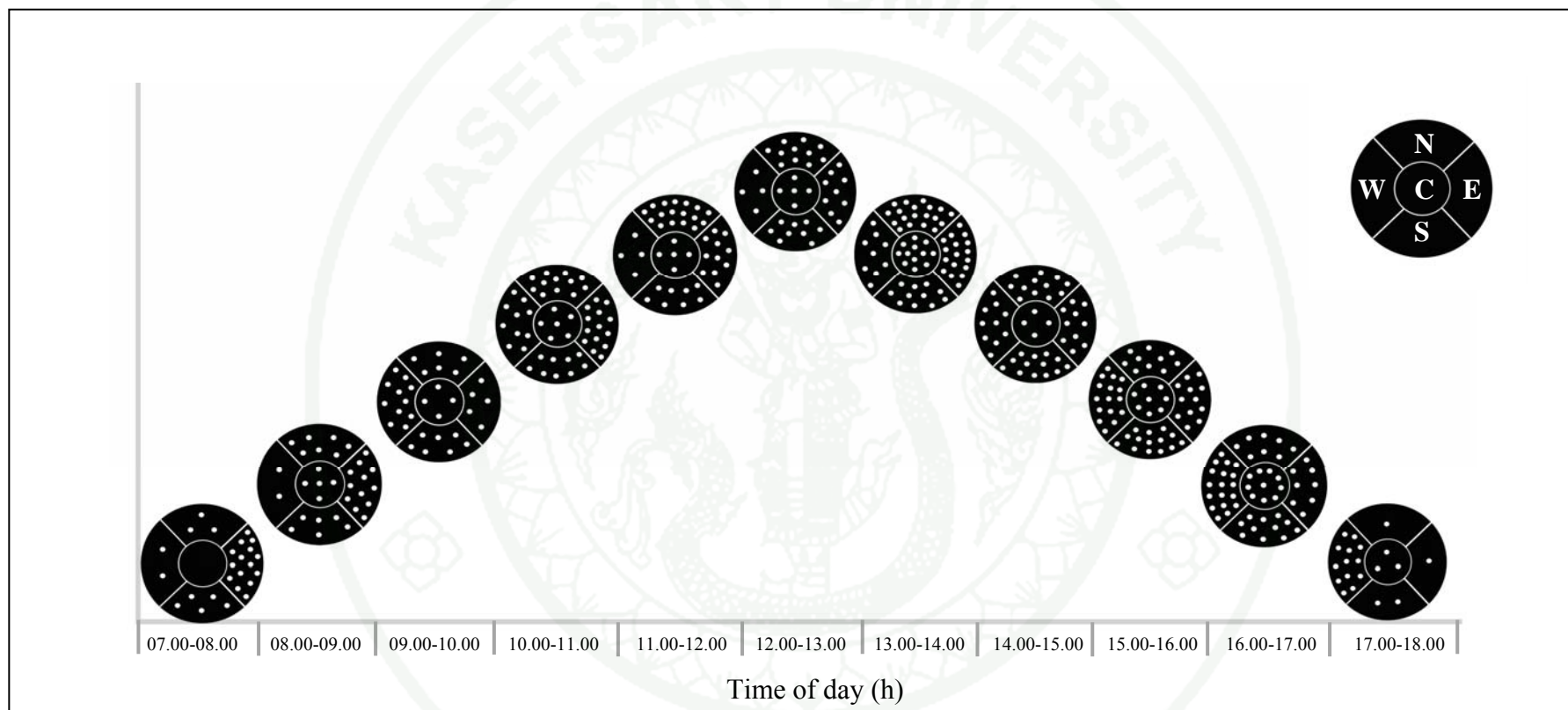
Insect pollinators preferred to visiting physic nut flowers on the east of plant canopy, and visiting peaks at 13.00-14.00 h of the day in the dry season. Similarly, Jinarite (2006) reported that the stingless honey bees to prefer visiting lychee inflorescences on the east side from morning till noon, followed by the south side and the west side, the lowest foraging was found on the north side. From the afternoon to evening, the bees of nearly equal numbers dispersed to visit the inflorescences in all directions. This might be due to the inflorescence of the sun pathway which tilted to the south in this season, thus affecting the bee behavior. In contrast, Qing *et al.* (2007) reported 17 species of floral visitors among which 11 species were pollinators (*Apis dorsata*, *A.florea*, *A.mellifera*, *Eumenes conica*,

*Vespa* sp.) with two floral visiting peaks at 10.00-12.00 h and 16.00-17.00 h every day. Among the different insect visitors *Apis* spp. were the most frequent.

#### 4.4 Position of area in the field plot

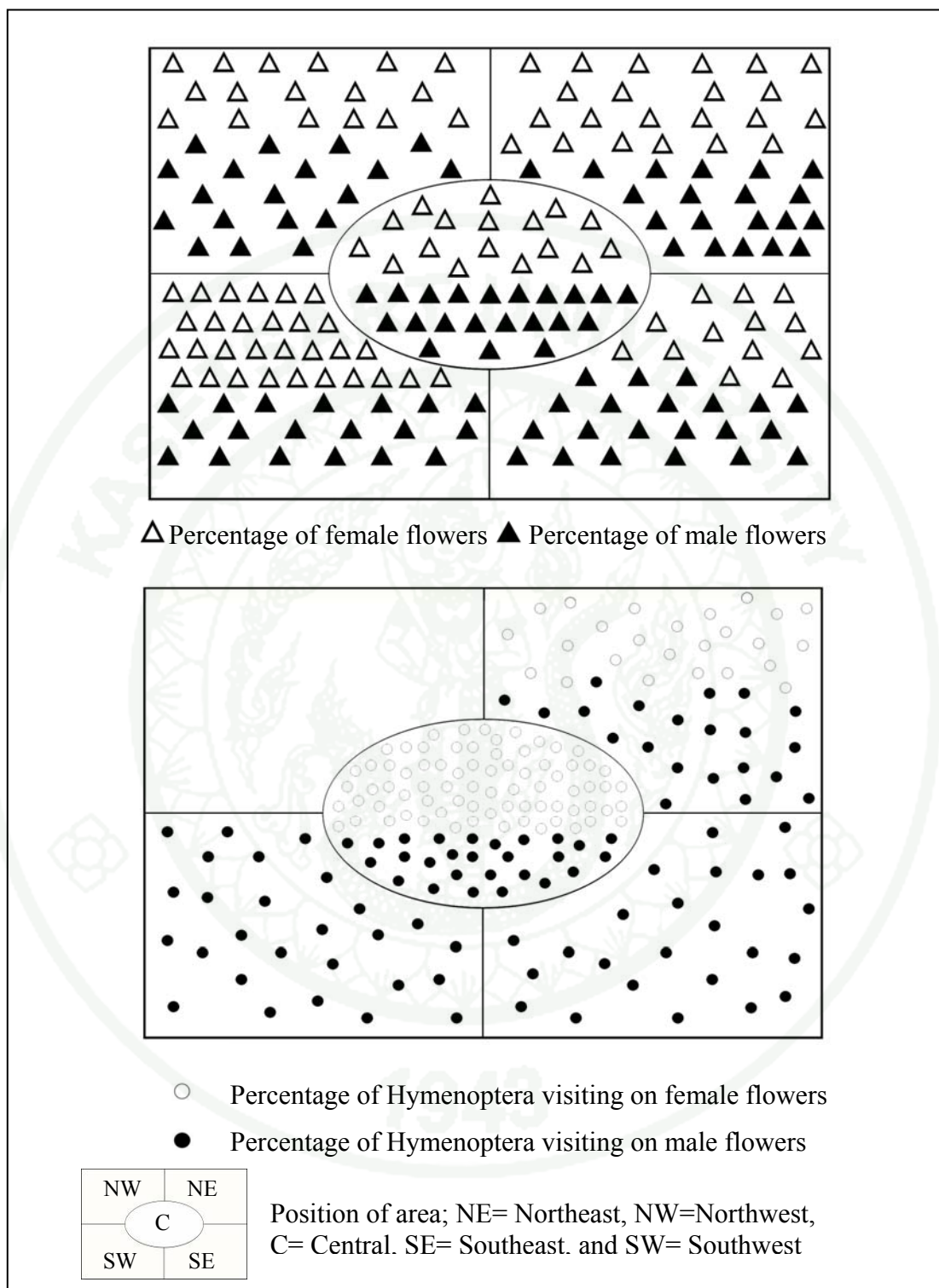
At KPS location on October 2008, the same flowers from 75 inflorescences same in 4.3, were observed in five positions of plot such as the corner of plot in four directions; the northeast, southeast, northwest, southwest, and the central of plot. Visiting percentage of Hymenoptera were slight different among five position of plot. On the female flowers were found Hymenopteran visited in the northeast (25.00%), and central (75.00%) area, but another position of area were not found any insect pollinators. On the male flowers were found Hymenopteran visited in the northeast (21.21%), southeast (24.24%), central (27.27%), southwest (27.28%), while the northwest of plot were not found insect pollinators in the order Hymenoptera (Figure 43 and Appendix Table 19).

Difference position of area had effect on the bee behavior. The important causes is environment such as the northeast, and the northwest be parallel with the main road inside Kamphaeng Saen Campus, Kasetsart University. Because of vehicles to be crowded on this road, direction of wind (the north to the south), thus most insect pollinators move to the central of area. And the end of forrow irrigation of plot, so this corner low humidity in the soil, that had effect on nectar secretion and non attractive pollinators. The position of area was important to application for pollination management. Correspondingly, Jinarite (2006) indicated that placing one stingless honey bee's nest in each corner of 400x500 squarmeter plot was found to give the most efficiency in pollination of the stingless honey bees as well as increasing yields of lychee.



**Figure 42** Number of insect pollinator in five directions of a plant canopy of *Jatropha curcas* L. in toxic variety, every hour during 07.00-18.00 h, on October 2008 at Kamphaeng Saen (KPS) location

Direction of plant canopy; N=North, S=South, C=Central, E=East, and W=West; 1 point = 1 individual of pollinators



**Figure 43** Percentage of Hymenopteran in five positions of *Jatropha curcas* L. plot in toxic variety on October 2008 at Kamphaeng Saen (KPS) location

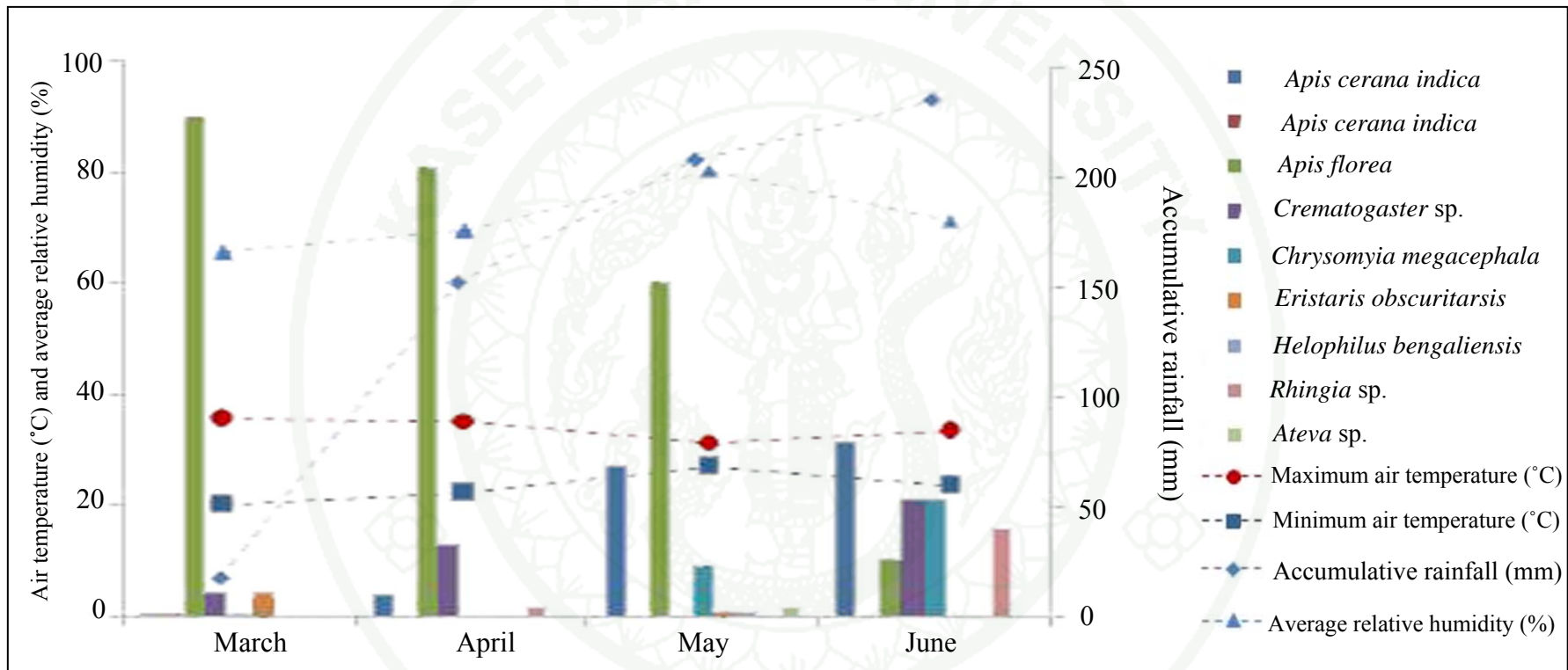
## 5. Effect of insect pollinators to physic nut (*Jatropha curcas* L.)

### 5.1 Diversity of insect pollinators

#### 5.1.1 Insect pollinators in difference months

Figure 44 and Appendix Table 20 summarized observation during March to June 2007 at Kamphaeng Saen (KPS) location. Diversity of insect pollinators (species list and abundance (%)) were different among four month. Insect pollinators were found six species on March; *Apis cerana indica* (0.41%), *A. dorsata* (0.43%), *A. florea* (89.84%), *Crematogaster* sp. (4.54%), *Chrysomyia megacephala* (0.42%), *Eristaris obscuritarsis* (4.36%), and four species on April; *A. cerana indica* (4.00%), *A. florea* (81.00%), *Crematogaster* sp. (13.00%), and *Rhingia* spp. (2.00%), six species on May; *A. cerana indica* (27.00%), *A. florea* (60.00%), *C. megacephala* (9.00%), *E. obscuritarsis* (1.00%), *H. bengaliensis* (1.00%), and *Ateva* sp. (2.00%), and five species on June; *A. cerana indica* (31.58%), *A. florea* (10.53%), *Crematogaster* sp. (21.05%), *C. megacephala* (21.05%), and *Rhingia* spp. (15.79%).

The results showed different abundance among nine species, in each month, *A. dorsata* were found on March only, and *A. cerana indica* and *A. florea* were found every month, they were a resident species, while *A. dorsata* was a passage migrant species. The highest abundance were observed *A. florea* on March (89.84%), April (81.00%), and May (60%), but on June *A. florea* (10.53%) was lower abundance than *A. cerana indica* (31.58%). Population of *A. cerana indica* related to *A. florea* such as abundance of *A. cerana indica* had been increasing from March to June, whereas abundance of *A. florea* had been reducing from March to June. This relation seem to be defend each other, because in each species of bees have footprint pheromone from tarsal glands for attracting other foragers in same species, but defend and protect this physic nut flowers from other bees species. Nasonov and footprint pheromones may prove useful in attracting honey bees to crops needing pollination (Crane, 1990).



**Figure 44** Abundance of insect pollinators on *Jatropha curcas* L. in toxic variety during March to June 2007 at Kamphaeng Saen (KPS) location

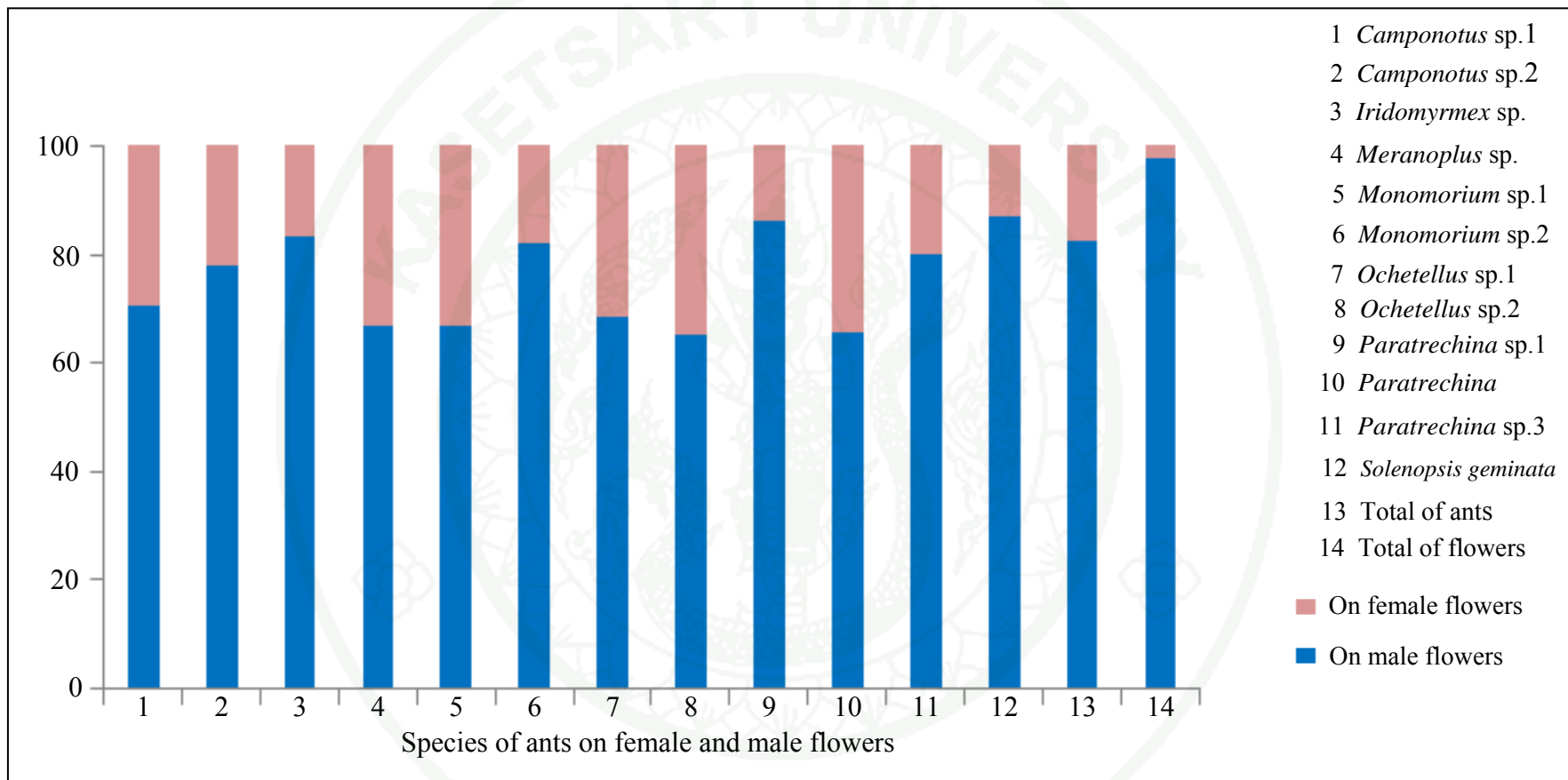
### 5.1.2 Diversity of ants

At KPS location during 10.00-13.00 h on December 2008 on the east of plant canopy in 400 m<sup>2</sup>, the ants were found 12 species seven genera; *Camponotus* (2 spp.), *Iridomyrmex* sp., *Meranoplus* sp., *Monomorium* (2 spp.), *Ochetellus* (2 spp.), *Paratrechina* (3 spp.), and *Solenopsis geminata*. The major of ants visited on female and male flowers were *S. geminata* (35.29%, 50.79%) and *Paratrechina* sp.1 (18.38%, 24.45%). Other ants were slightly abundance on physic nut flowers (Figure 45 and Appendix Table 21).

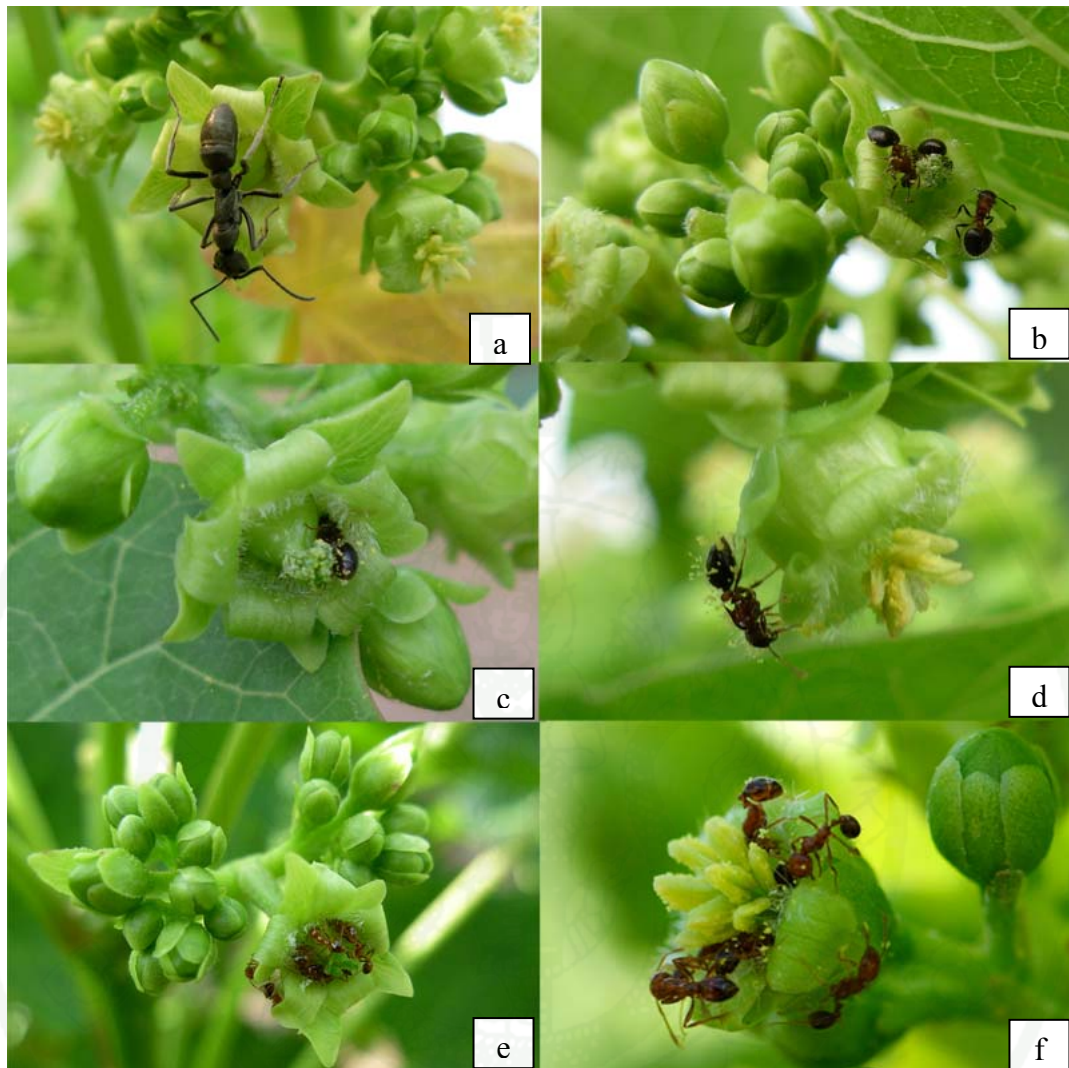
### 5.1.3 Behavior of ants

Twelve species of ants prefer visiting on male flowers 65.22-87.03%, while these ants visited on female flowers about 12.97-34.78% (Figure 45 and Appendix Table 21). In addition, Figure 46 showed three dominant species of ants in pollination behaviors on physic nut flowers during 10.00-13.00 h on December 2008 such as *Camponotus* sp.1 is enormous species on physic nut flowers, they gathered nectar from various plants. Whereas, *Meranoplus* sp. and *S. geminata*. They were usually only species aggregation on female and male flowers of physic nut within the plant, pollen grains on that flowers were non transmitted to outside plants. They were a self-pollination agents, when occurred blooming both female and male flowers. The long hair on the body of *Meranoplus* sp. is a dominant feature, which is more effective pollinator than *S. geminata*.

This results showed the highest species list records of ants, they visited on physic nut flowers, whereas previously reported five species of ants such as *C. compressus*, *Camponotus* sp., *Crematogaster* sp., *S. geminata*, and *Pheidole spathifer* (Raju and Ezradanam, 2002). In addition, *Meranoplus* sp. was more effective pollinator. Likewise, Paul (2011) recorded that *M. bicolor* Guen. was more commonly the ants on chilli (genus *Capsicum*) flowers with pollen grains on their legs.



**Figure 45** Number, preference and species ratio of ants on *Jatropha curcas* L. flowers during 10.00-13.00 h on December 2008 at Kamphaeng Saen (KPS) location, at 18.1 °C, 0.1 mm of accumulative rainfall, and 72% RH



**Figure 46** Dominant species of ants in pollination behavior on *Jatropha curcas* L. (KPS) location, at 18.1°C, 0.1 mm of accumulative rainfall, and 72% RH

Fomicidae; a = *Camponotus* sp.1 on the female flower

b, c = *Meranoplus* sp. on the female flowers

d = *Meranoplus* sp. on the male flowers

e = *Solenopsis geminata* on the female flowers

f = *S. geminata* on the male flowers

Diameter of physic nut male flowers is  $0.7 \pm 0.1$  cm,

female flowers is  $0.9 \pm 0.1$  cm.

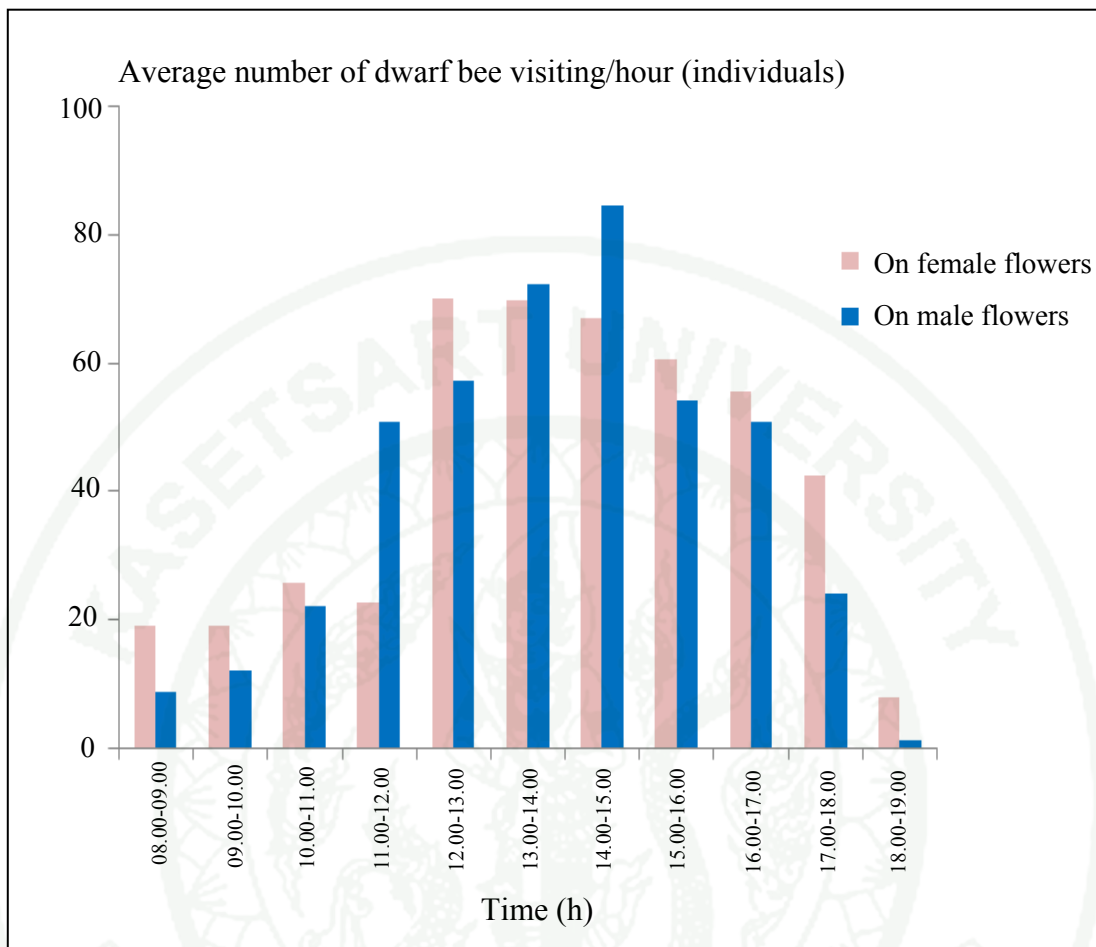
## 5.2 Bee behaviors

### 5.2.1 Bee preferences

#### 1) Preference of resident bee or dwarf honey bee (*Apis florea*)

Figure 47 and Appendix Table 22 showed the preference of *A. florea* visiting on female or male physic nut flowers during 08.00-19.00 h on April 18<sup>th</sup> 2007 at KPS location. Average floral numbers visiting per hour were significantly different among variable times of day. The highest flower number visiting per hour of *A. florea* was observed at 12.00-13.00 h (70.10 individuals), 13.00-14.00 h (69.79 individuals), 14.00-15.00 h (66.95 individuals), and 15.00-16.00 h (60.63 individuals) on female flowers. Similarly, male flowers were found the highest flower number visiting per hour of *A. florea* during 14.00-15.00 h (84.40 individuals). In the other hand, the lowest number flower visiting per hour of *A. florea* was observed at 18.00-19.00 h on female (7.89 individuals), and male flowers (1.29 individuals).

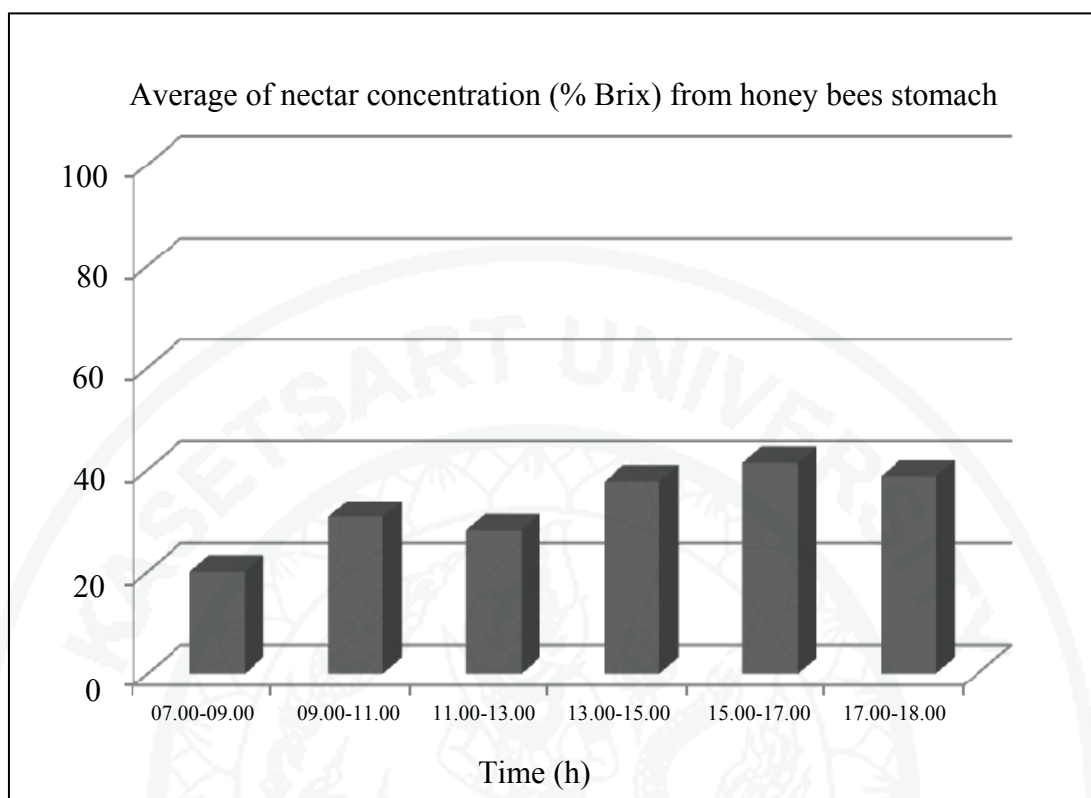
Bhattacharya *et al.* (2005) examined the fore noon pattern of anthesis with subsequent pollen release with each male flower producing  $1,617 \pm 100$  pollen with pollen:ovule ratio as 539:1. The stigma has become receptive two hours after anthesis, coinciding with nectar secretion and pollen presentation schedule. Each female flower ( $4.54 \pm 0.82 \mu\text{l}$ ) produces higher amount of nectar than male flower ( $1.92 \pm 0.44 \mu\text{l}$ ). Perhaps this might have lead to bees prefer female flowers than male flowers in the afternoon (15.00-17.00 h). Similarly, this result showed long peak during 12.00-16.00 h on female flowers, while male flowers were observed the short peak during 14.00-15.00 h.



**Figure 47** Dwarf bees (*Apis florea*) preference on visiting of *Jatropha curcas* L., female or male flowers in toxic variety of during 08.00-19.00 h on April 18<sup>th</sup> 2007 at Kamphaeng Saen (KPS) location, at 30.85 °C, 6.0 mm of accumulative rainfall, and 64% RH

2) Effect of nectar concentration to the bee preference, Figure 48 and Appendix Table 23 showed nectar concentration in the honey stomach of *A. florea* visited on physic nut flowers during 07.00-18.00 h every two hours, on April 19<sup>th</sup> 2007 at KPS location. Average of nectar concentration (% Brix) from honey bee stomachs was significantly different among times of the day. The highest nectar concentration was found during 15.00-17.00 (41.33% Brix), 17.00-18.00 h (38.67 % Brix), and 13.00-18.00 h (37.67% Brix) followed by 09.00-11.00 h (30.67% Brix), and 11.00-13.00 h (28.00 % Brix), while the lowest nectar concentration was observed at 07.00-09.00 h (20.00% Brix).

Indirect method was used to detect nectar concentration, even through nectar in honey sacs were higher concentration than nectar from flowers, but these data were estimated to the trend of concentration. Nectar concentration increased in the afternoon. The nectar concentration related to bee preference, bee prefer visiting flowers in the afternoon. Likewise, Bhattacharya *et al.* (2005) observed that the different grades of sucrose had an influence on insect behavior, flower visits duration, pollen removal and deposition on stigmas by honey bees (*Apis* spp.). Highest number of pollen grains was transported and deposited on stigmas by *A. dorsata* when flowers were treated with 0.9 M sucrose, whereas, it was lowest by *A. florea* in 1.5 M sucrose treated flowers. Nectar factor was possible to lead to increase efficiency of pollination with honey bees. In addition, Julia (1964) recorded that nectar of physic nut abundant flow during April to May that was sought by bees but difficult to collect. Honey was dark-amber, strong, but agreeable in flavor. Nectar concentration of physic nut is the moderate around 20.00-41.33% Brix, while the low nectar concentration was found in durian cultivar Chanee (17.5 % Brix) (Thawiphon, 2003), and the high nectar concentration was observed in rambutan (51-75% Brix) (Boongird and Sauwannasee, 2000).

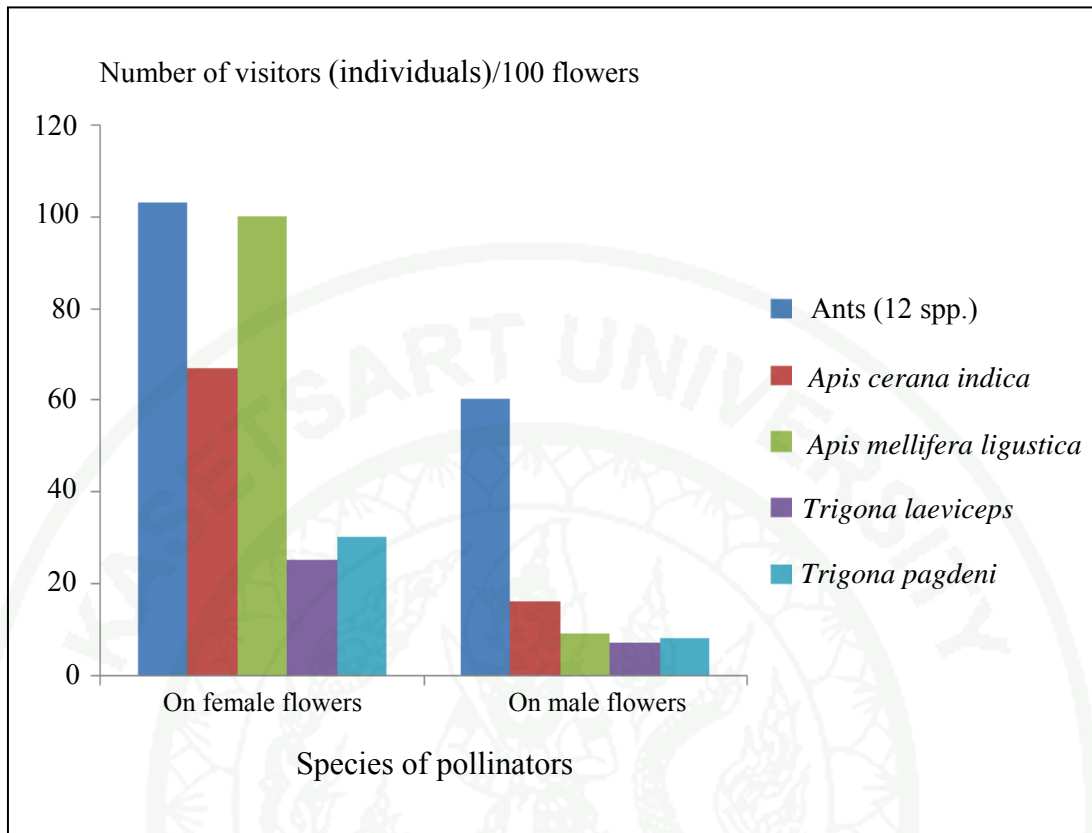


**Figure 48** Nectar concentration in the honey stomach of dwarf bees (*Apis florea*) visited on *Jatropha curcas* L. flowers during 07.00-18.00 h, on April 19<sup>th</sup> 2007 at Kamphaeng Saen (KPS) location, at 29.7 °C, 6.4 mm of accumulative rainfall, and 75.5% RH

3) Different of Hymenoptera preference between female and male flowers at KPS location during 10.00-13.00 h on December 2008, visiting of ants and bees were found to be significantly different in between female and male flowers. In 100 of female flowers, they were found to be significantly with ants (103 individuals) followed by *Apis mellifera ligustica* (100 individuals), *A. cerana indica* (67 individuals), *Trigona pagdeni* (30 individuals) and *T. laeviceps* (25 individuals). Likewise, in 100 of male flowers, they were found to be significantly different with ants (60 individuals) followed by *A. cerana indica* (16 individuals), *A. mellifera ligustica*, *T. pagdeni* (8 individuals), and *T. laeviceps* (7 individuals). All ants have no response so much about sexes of flowers; they visited female flowers quite often 103 individuals, and 60 individuals on male flowers (Figure 49 and Appendix Table 24).

In the field, insect pollinators visited male flowers were more frequency of occurrence than female flower, because of male flowers were larger blooming. The result was supported that the anther of the male flowers was bright yellow and looked conspicuous. In this population, an inflorescence had 88–238 male flowers blooming 0.21–21.57 flowers daily in average to display them more conspicuously, for big blooming it could attract more insects for visiting (Chang-wei *et al.*, 2007a). Perhaps this might have to lead to prefer visited on male flowers than female flowers. These data were not a raw record, but these were estimated data.

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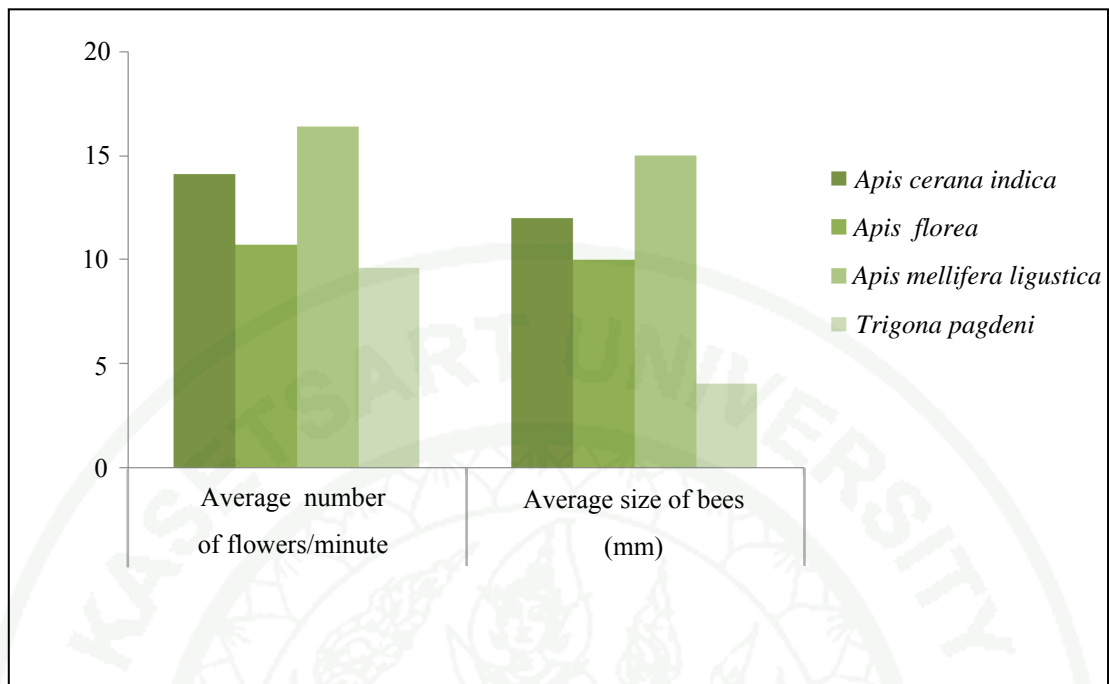
**Figure 49** The preference of the Hymenopteran visiting on female and male *Jatropha curcas* L. flowers during 10.00-13.00 h on December 2008 at Kamphaeng Saen (KPS) location, at 18.1 °C, 0.1 mm of accumulative rainfall, and 72% RH

### 5.2.2 Bees speed

At KPS location during 10.00-13.00 h, on February 2009, Figure 50 and Appendix Table 25 showed the number of flowers, which were visited by four species of 10 bees in one minute. These observations were found to be significantly different in number of visits among these four bees. The most activity were found in *A. mellifera ligustica* (16.40 flowers/minute), and *A. cerana indica* (14.10 flowers/minute), while *A. florea* and *T. pagdeni* were more slow activity, 10.7 and 9.6 flowers/minute respectively. Likewise, this study was found to be significantly different in average of size (mm) among four bees. The biggest bee was observed in *A. mellifera ligustica* (15 mm of body length), following by *A. cerana indica* (12 mm of body length), and *A. florea* (10 mm of body length), while the smallest was found in *T. pagdeni* (4 mm of body length).

Body size of bees had effect on the bees speed. Correspondingly, Boongird (1985) stated that the visiting insect speeds on the mango flower of the *A. cerana* was the highest speed from flower to another within one minute covered  $17.16 \pm 5.01$  flowers, followed by *A. mellifera* ( $8.88 \pm 2.52$  flowers), *A. florea* ( $7.60 \pm 2.47$  flowers), and *Chrysomyia* sp. ( $1.80 \pm 1.16$  flowers) for the speed.

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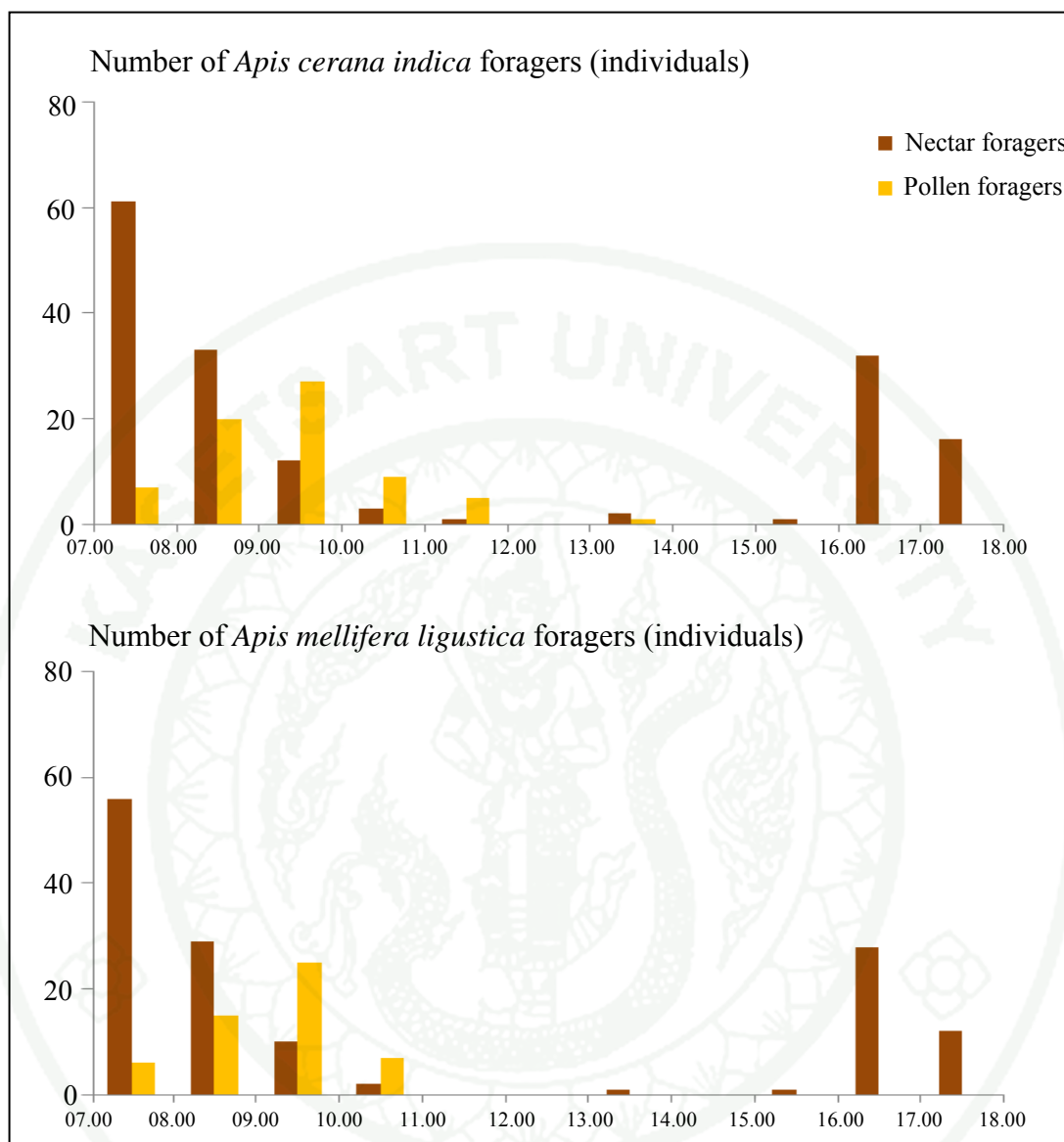


**Figure 50** Bees speed visiting on flowers/minute of *Jatropha curcas* L. during 10.00-13.00 h on February 2009 at Kamphaeng Saen (KPS) location, at 21.8 °C, no rainfall, and 67.5% RH

### 5.2.3 Bee forager

At KPS location, *A. cerana indica*, and *A. mellifera ligustica* were observed number of nectar and pollen forager at the hive entrances every 1 hour during 07.00-18.00 h on November 21<sup>st</sup> 2008. Number of foragers of *A. cerana indica* was similar to *A. mellifera ligustica*, bees foragers prefer collected pollen only peak in the morning (08.00-10.00 h), whereas bee foragers collected nectar two peaks in the morning (07.00-09.00 h), and in the evening (16.00-18.00 h). In the morning bee foragers collected nectar larger than in the evening, but foraging activity stopped or low activity at 11.00-14.00 h. Two species of bees seem to like collected nectar (70-72%) more than pollen (28-30%) in the day (Figure 51 and Appendix Table 26).

Bee foragers prefer collected pollen and nectar of competitive plants in the morning. Similarly, pollen source in dragon fruit (*Hylocereus undatus*), and durian (*Durio zibethinus* L.) studies, Namvong (2003) reported that dragon fruit flowers the highest population observed occurred during 07.30-08.30 h, and Thawiphon (2003) used cross-hand-pollination of durian cultivar Chanee The highest major population; *T. laeviceps*, *A. dorsata*, *T. terminata*, *A. cerana indica*, and *A. florea* that was observed occurred during 08.00-09.00 h. Whereas, nectar source in lychee (*Lichi chinensis* Sonn.), Jinarite (2006) reported that the stingless honey bees to prefer visiting lychee inflorescences from morning till noon. In like manner, *Melipona* observation from one nest specialized as pollen or nectar or resin collectors for their entire adult life; other individuals switched daily, gathering pollen and/or resin in the morning, and nectar in the afternoons. This also had effect on longevity, with pollen foragers active only 1-3 hours in the morning and surviving for 12 days on average, but nectar foragers sometimes active all day and lasting for only 3 days (Peter *et al.*, 2004). Likewise, Boontop (2002) reported that number of stingless honey bee foragers in the morning tended to be higher collecting pollen than in the numbers in the afternoon.



**Figure 51** Number of foragers of *Apis cerana indica* and *Apis mellifera ligustica*, at the hive entrances every hour during 07.00-18.00 h on November 21<sup>st</sup> 2008 at Kamphaeng Saen (KPS) location, at 21.6°C, 14.7 mm of accumulative rainfall, and 74.5% RH

## 5.2.4 Competitive plants of physic nut

### 1) Diversity of competitive plants

At KPS location on November 19<sup>th</sup> 2008, surveyed competitive plants of *Apis cerana indica*, and *A. mellifera ligustica*, these competitive plants in one kilometer radius around the bee hives were observed species of plants. Major competitive plants were found 11 species in six families; three species of Asteraceae; Mexican sunflower weed (*Tithonia diversifolia*), coatbuttons Mexican daisy (*Tridax procumbens*), creeping daisy (*Wedelia trilobata*), one species of Caesalpinoideae; frame tree (*Delonix regia*), one species of Commelinaceae; day flower (*Commelina diffusa*), one species of Convolvulaceae; swamp morning glory (*Ipomoea aquatic*), three species of Mimosaceae; sensitive plant (*Mimosa pudica*), white popinal (*Leuceana leucocephala*, and *Leuceana* sp.), two species of Poaceae; sugar cane (*Saccharum sinense*), corn (*Zea mays*), and one species of Zygophyllaceae; caltrops (*Tribulus cistoides*). Effective bees such as *A. cerana indica*, and *A. mellifera ligustica* from hives not control them, because of distant of food sources located on far from hive, and they prefer competitive plants. In additional, other bee pollinators in field, for example *Pithitis smaragdula* more frequently visited on Mexican fire plant (*Ephorbia geniculata*: Euphorbiaceae) flowers than physic nut (Euphorbiaceae), and so visiting of *Xylocopa aestuans* on *Solanum* sp. (Solanaceae) flowers. In additional, Mexican sunflower weed (*T. diversifolia*: Asteraceae) was visited by *Rhingia* sp. (Figure 52).

### 2) Various pollen grains from bee pollen foragers

At KPS location, number of pollen (9 species of plants) foragers of *A. cerana indica* and *A. mellifera ligustica*, at the hive entrances every 2 hours during 08.3-16.30 h on November 22<sup>nd</sup> 2008. The pollen foragers collected various pollen grains of plants, but there were not found pollen forager from physic nut. In *A. cerana indica*, collected pollen grains from eight species of competitive plants, the highest number of pollen foragers were collected pollen grains of corn

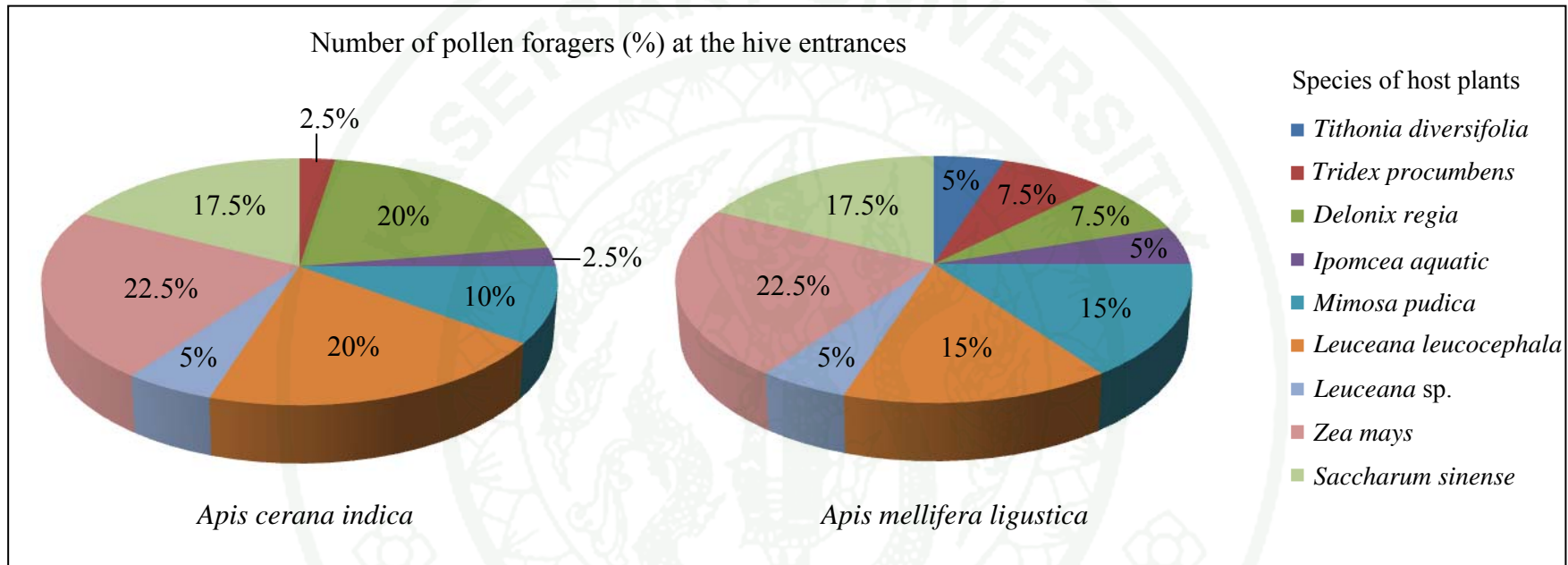
(*Z. mays*) (22.5%) followed by frame tree (*D. regia*), and white popinal (*L. leucocephala*) (20.0%), sugar crane (*S. sinense*) (17.5%), sensitive plant (*M. pudica*) (10.0%), and white popinal (*Leuceana* sp.) (5%), while the lowest number of pollen foragers were collected pollen grains of caltrops (*T. procumbens*), and swamp morning glory (*I. aquatic*) (2.5%). Similarly, *A. mellifera ligustica* collected pollen grains from nine species of competitive plants, the highest number of pollen foragers were collected pollen grains of *Z. mays* (22.5%) followed by *S. sinense* (17.5%), *L. leucocephala*, and *M. pudica* (15.0%), *D. regia*, and *Tridax procumbens* (7.5%), while the lowest number of pollen foragers were collected pollen grains *I. aquatic*, *Leuceana* sp., and *T. diversifolia* (5%) (Figure 53, Appendix Table 27).

Bee pollinators were preferred competitive plants such as corn and sensitive plants, caltrops flowers were more attractive than physic nut flowers. Thus, physic nut flowers were alternative plants of honey bees on November 19<sup>th</sup> 2008 at KPS location. Corn blooming in the winter and produced much pollen, gathered by bees, while nectar was none but under some conditions, bees may fill frames with corn pollen. Reports of corn honey have arisen from rare occurrence of much honeydew from aphids which bees gather. Bees also collect sap from cut stalks. In addition, caltrops blooming in the morning, these flowers were attracted with primary attractants (pollen and nectar) and secondary attractants (orange-yellow color, odour, small size and fully open architecture and high floral density) that influenced visitors and brought out reproductive success (Showkat, 2011).



**Figure 52** Competitive plants on November 19<sup>th</sup> 2008 at Kamphaeng Saen (KPS) location

Asteraceae; a = Mexican sunflower weed (*Tithonia diversifolia*) flower was visited by *Rhingia* sp., and b = coatbuttons Mexican daisy (*Tridax procumbens*) flower., Mimosaceae; c = sensitive plant (*Mimosa pudica*) flower was visited by *Apis cerana indica*., Poaceae; d = corn (*Zea mays*) flower was visited by *A. cerana indica*., Solanaceae; e = *Solonum* sp. flower was visited by *Xylocopa aestuans*., and Zygophyllaceae; f = caltrops (*Tribulus cistoides*) flower was visited by *A. mellifera ligustica*.



**Figure 53** Number of pollen (9 species of plants) foragers of *Apis cerana indica* and *A. mellifera ligustica*, at the hive entrances every 2 hours during 08.30-16.30 h on November 22<sup>nd</sup> 2008 at Kamphaeng Saen (KPS) location, at 21.6°C, 14.7 mm of accumulative rainfall, and 74.5% RH

### 5.3 Pollination experiments

#### 5.3.1 Comparisons among various botanical factors

Figure 54 and Appendix Table 28 comparisons among four pollination experiments in the non-toxic variety during June to August 2009 at ARDC location, weight of fresh and dry seed and kernel were slightly different in four treatments, while in weight of fresh fruit and oil content were significantly different among four pollination experiments.

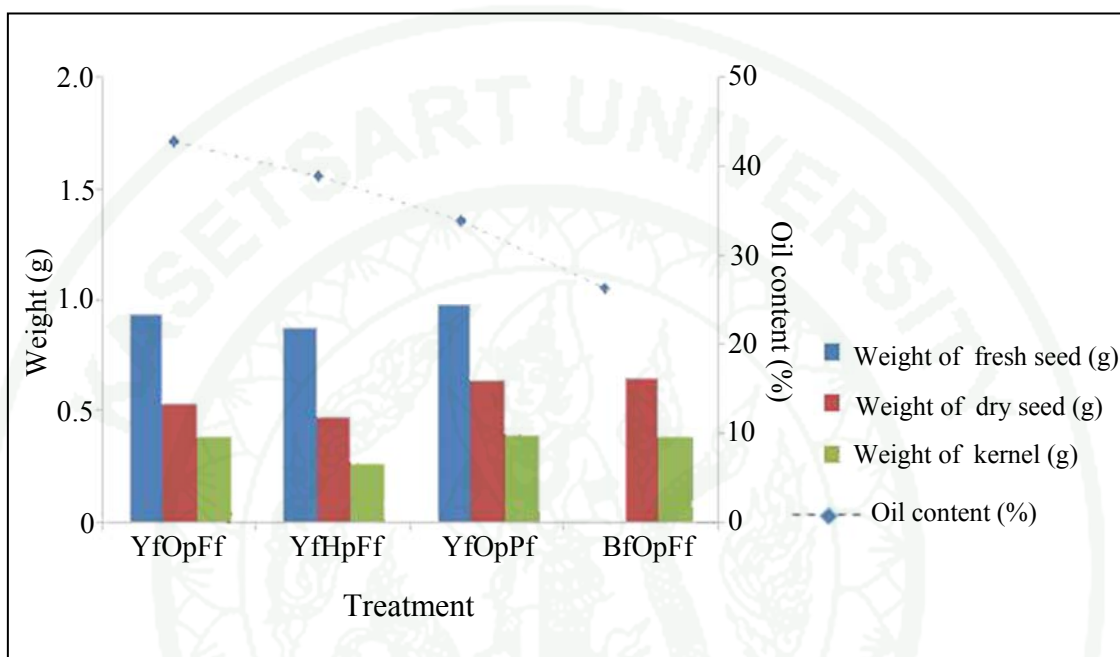
1) Weight of fresh fruit was significantly different among four treatments. The highest value was found in YfOpFf (yellow fruit, open-pollination, on female flower) (10.52 g), followed by YfHpFf (yellow fruit, hand-pollination, on female flower) (8.83 g), and YfOpPf (yellow fruit, open-pollination, on perfect flower) (8.03 g), while the lowest value was observed in BfOpFf (black fruit, open-pollination, on female flower) (3.02 g).

2) Weight of fresh or dry seed, and kernel were slightly different; weight of fresh and dry seed in YfOpPf (0.98 g, 0.64 g) were slightly higher than YfOpFf (0.94 g, 0.54 g), YfHpFf (0.88 g, 0.48 g), and in BfOpFf had weight of dry seed only (0.65 g), because these seed from black fruit was very dry. In additional, weight of kernel in YfHpFf (0.26 g) was slightly lower than YfOpPf (0.40 g), YfOpFf (0.40 g), and BfOpFf (0.39 g).

3) Oil contents were significantly different among various pollinations. The highest oil content was extracted from YfOpFf (42.8%) and YfHpFf (39.0%) followed by YfOpPf (34.0%), while the lowest oil content was found in BfOpFf (26.4%).

Quality of seed from yellow fruit better than black fruit, similar to study of Dhillon *et al.* (2006) stated that the period of fruit development and maturity ranged from 55-61 days from date of first fruit initiation. Seeds of

physic nut were harvested at physiological maturity, which color of fruits turn yellow was proof to be the best seed germination and seedling vigor comparing to the seeds of immature green fruits and dark brown fruits, which passed through timing of physiological maturity (Tongpumnug *et al.*, 2007).



**Figure 54** Effect of various botanical factor to weight of fruit and seed, and oil content during June to August 2009 at Agriculture Research and Development Center (ARDC) location

YfOpFf = yellow fruit, open-pollination, on female flower

YfHpFf = yellow fruit, hand-pollination, stigma pollen A-grade, on female flower

YfOpPf = yellow fruit, open-pollination, on perfect flower

BfOpFf = black fruit, open-pollination, on female flower

### 5.3.2 Comparisons between self - and cross-pollination

Figure 55 and Appendix Table 29 comparisons among four pollination experiments in the non-toxic variety during November, 2009 to January, 2010 at AS location, weight of fresh and dry seed and kernel were slightly different in five treatments, while in weight of fresh fruit and oil contents were significantly different among five pollination experiments.

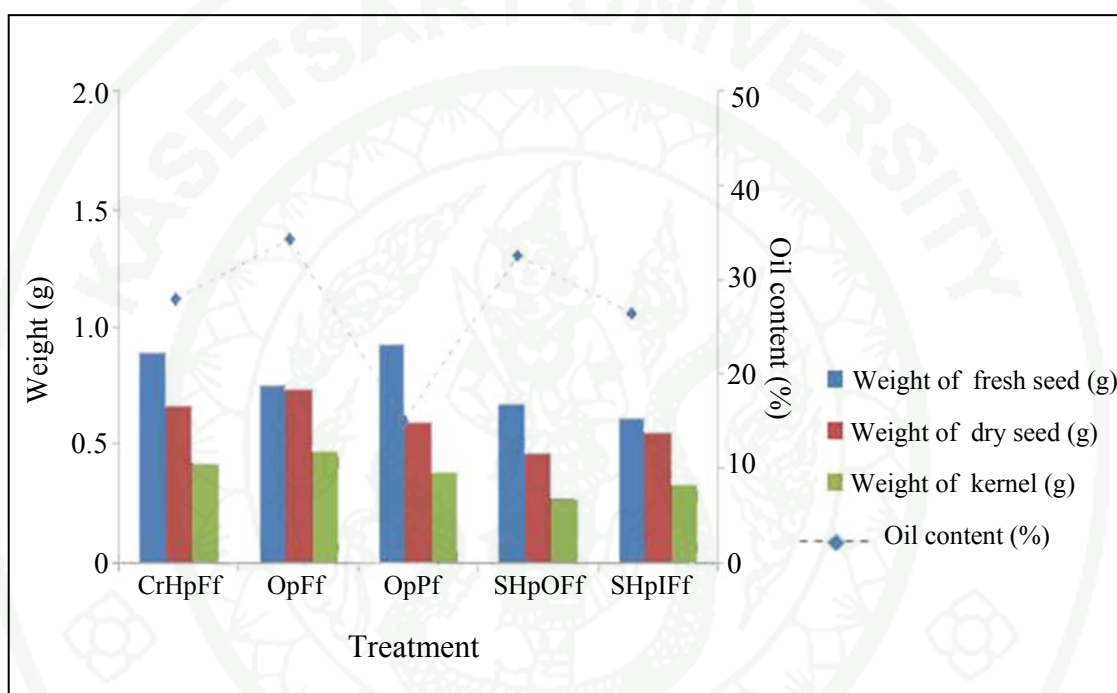
1) Weight of fresh fruit was significantly different among five treatments. The highest value was found in OpPf (open-pollination, on perfect flower) (7.57 g) followed by CrHpFf (cross-hand pollination, on female flower) (4.13 g), while the lowest value was observed in SHpOFF (self-hand-pollination, outside plant, same clone, on female flower) (2.86 g), OpFf (open-pollination, on female flower) (2.73 g), and SHpIFf (self-hand pollination, inside plant, on female flower) (2.52 g).

2) Weight of fresh and dry seed, and kernel were slightly different; weight of fresh and dry seed in five treatments were similarities; OpFf (0.75 g, 0.74 g), SHpOFF (0.67 g, 0.46 g), SHpIFf (0.62 g, 0.55 g), CrHpFf (0.89 g, 0.67 g), and OpPf (0.93 g, 0.60 g). Weight of kernel in SHpOFF (0.28 g) was slightly lower than OpFf (0.48 g), CrHpFf (0.42 g), OpPf (0.39 g), and SHpIFf (0.34 g).

3) Oil contents were significantly different among five pollinations. The highest oil content was extracted from OpFf (34.40%) and SHpOFF (32.72%) followed by CrHpFf (28.00%) and SHpIFf (26.50%), while the lowest oil content was found in OpPf (15.00%).

Self- and cross-pollination had effect on oil content, that oil content of cross-hand-pollination, self-hand-pollination (xenogamy) and open-pollination were higher than self-hand-pollination (geitonogamy) about 1.5-7.9%. Similarly, Abdelgadir *et al.* (2008) indicated that to understand the breeding

system of physic nut, bagged and open, and self- and cross-pollination were studied. Fruits from open-pollinated flowers were significantly more numerous, larger and heavier than those produced from autogamous self-pollinated flowers. Cross-pollinated flowers had significantly higher fruit set than self-pollinated flowers. However, supplemental self- and cross-pollination did not significantly increase fruit set.



**Figure 55** Effect of self- and cross-pollination to weight of fruit and seed, and oil content of *Jatropha curcas* L. during November 2009 to January 2010 at Aviation School (AS) location

OpFf = open-pollination, on female flower

SHpOFF = self-hand-pollination, outside plant, on female flower

SHpIFf = self-hand-pollination, inside plant, on female flower

CrHpFf = cross-hand-pollination, on female flower

OpPf = open-pollination, on perfect flower

### 5.3.3 Comparisons among ants and four introduced bees

Figure 56 and Appendix Table 30 showed comparisons amount of pollen grains grade (17.00-18.00 h), percentage of fruit setting, and effective pollination of pollinators among ants and four kind of bees pollinated in the intra-variety (toxic) at KPS location on November to December 2008, the nests of different kinds of bee pollinators such as two species of the honey bees (*A. cerana indica*, *A. mellifera ligustica*), and two species of the stingless honey bees (*T. laeviceps*, *T. pagdeni*) were introduced three hives/species of bees to the sites of physic nut plantations, in each species per week.

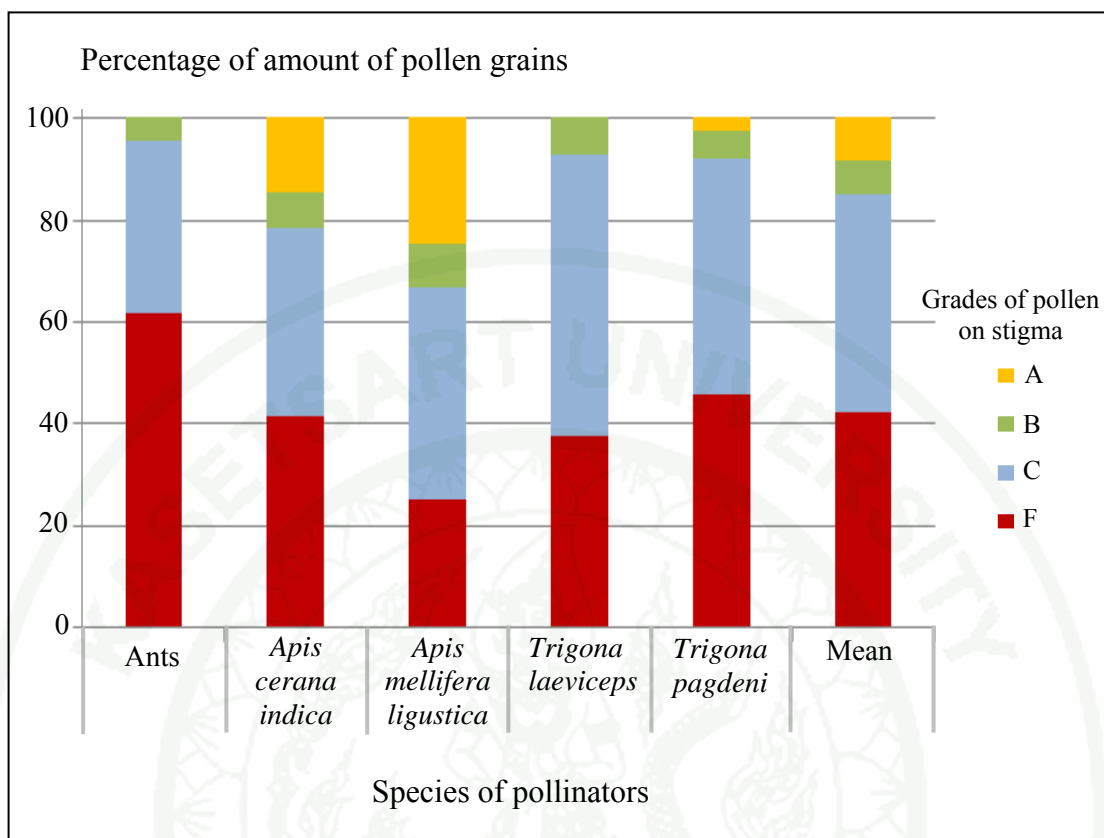
#### 1) Amount of pollen grains grade

The ants and four of bees transferred pollen grains to the stigma, female flower of physic nuts, which were significantly different in amount of pollen grains grade. The largest amount of pollen grains in A-grade flowers (>1,500 pollen grains/stigma) always visited by *A. mellifera ligustica* (25%), followed by *A. cerana indica* (3.99%), and *T. pagdeni* (2.51%), while the A-grade was not occurred on *T. laeviceps* and ants foragers. Whereas, the flowers were not pollinated (F-grade) by ants and four kind of bees; the highest F-grade showed in flowers visiting by ants (60.50%), followed by *T. pagdeni* (45.42%), *A. cerana indica* (41.17%), *T. laeviceps* (37.50%), while the lowest F-grade was observed on flowers, which were visited by *A. mellifera ligustica* (25.00%). In additional, the smallest stingless honey bee, *T. pagdeni* showed about 45.42% on F- grade. Although *T. laeviceps* did not make the flowers leached up to A-grade but by average could be the good one close to *A. mellifera ligustica*. The ants were found in a large number visited on female flowers, but they seem not to good in pollination, may be because of body structure such as less hairs or foraging activities on female flowers.

In commonly, F-grade was not found on stigmas, was caused by most honey bees visited on competitive plants, while ants and stingless honey bees

visited on physic nut flowers, but amount of population were not enough for pollination. Although, Raju and Ezradanam (2002) stated that the stigmas were receptive time during 3 days after the opening of the flowers. The stigma receptivity time was strong during 1<sup>st</sup>-4<sup>th</sup> day and begins to decline the fifth day onwards and completely loses its receptivity on the 9<sup>th</sup> day. Likewise, Chang-wei *et al.* (2007b) said that female flowers are still many un-open male flowers after all female flowers opened. This provides time for receptive stigma to get pollens from male flowers, and enhance the opportunity of reproductive success. Similarly, Darci (2005) reported that the effect of number of pollen grains (sweet pepper) deposited, and pollen compatibility in seed set and fruit quality should be better studied in this crop. The treatments hand cross-pollination and pollination by stingless honey bees (*Melipona subnitida*) produced the higher number of seeds set and lower percentage of malformed fruits, suggesting that a great number of viable, compatible pollen was placed on the flower stigmas. Hand self-pollination also set a high number of seeds, but produced a high percentage of deformed fruits, while self-pollination set the smaller number of seeds and produced a high percentage of malformed fruits. It probably happens because *M. subnitida* deposited a great number of viable pollen grains on the stigmas of sweet pepper flower, assuring many seeds set per fruit. More seeds developing inside each fruit would lead to bigger and heavier fruits, and reduce the percentage of malformed ones.

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**Figure 56** Amount of pollen grains percentage on *Jatropha curcas* L. flowers in different level, were visited by ants and four bees observed during 17.00-18.00 h on November 2008 at Kamphaeng Saen (KPS) location

Amount of pollen grains on stigmas

A-grade > 1,500 pollen grains/stigma

B-grade = 300-1,500 pollen grains/stigma

C-grade < 300 pollen grains/stigma

F-grade is none pollen grain on stigma

## 2) Percentage of fruit setting

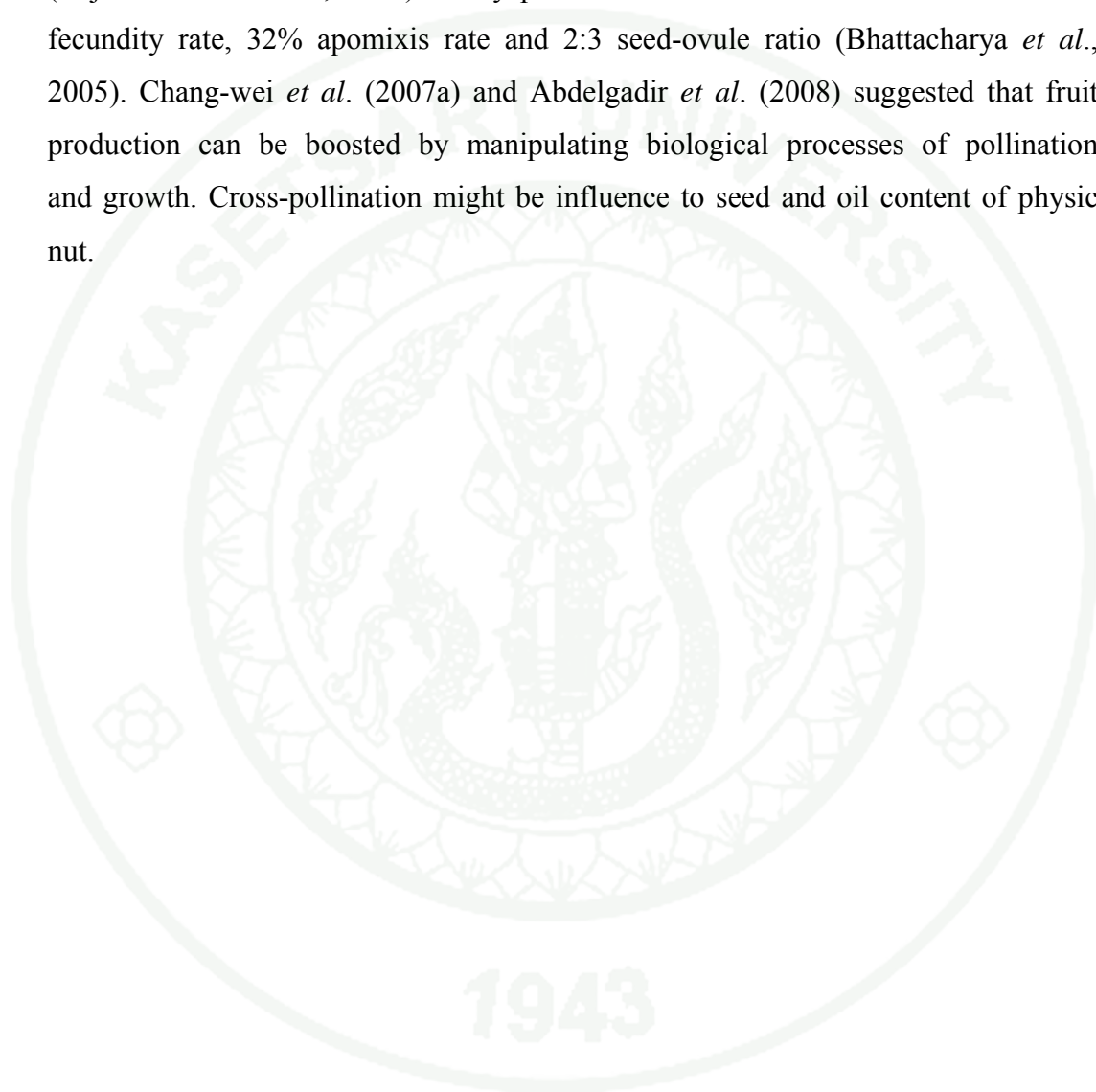
The percentage of fruit set have been recorded after 50 days, it was found that all female flowers that were visited by ants and four kinds of bees always set fruit around 95%. There were no significant different of percentage of fruit set among species of Hymenopteran pollinators. Therefore *Jatropha curcas* L. flowers need pollination but no matter that which kind of insects or amount of pollen grains on the stigma. Full pollination reached to maximize fruit set at 95% (Figure 57 and Appendix Table 31).

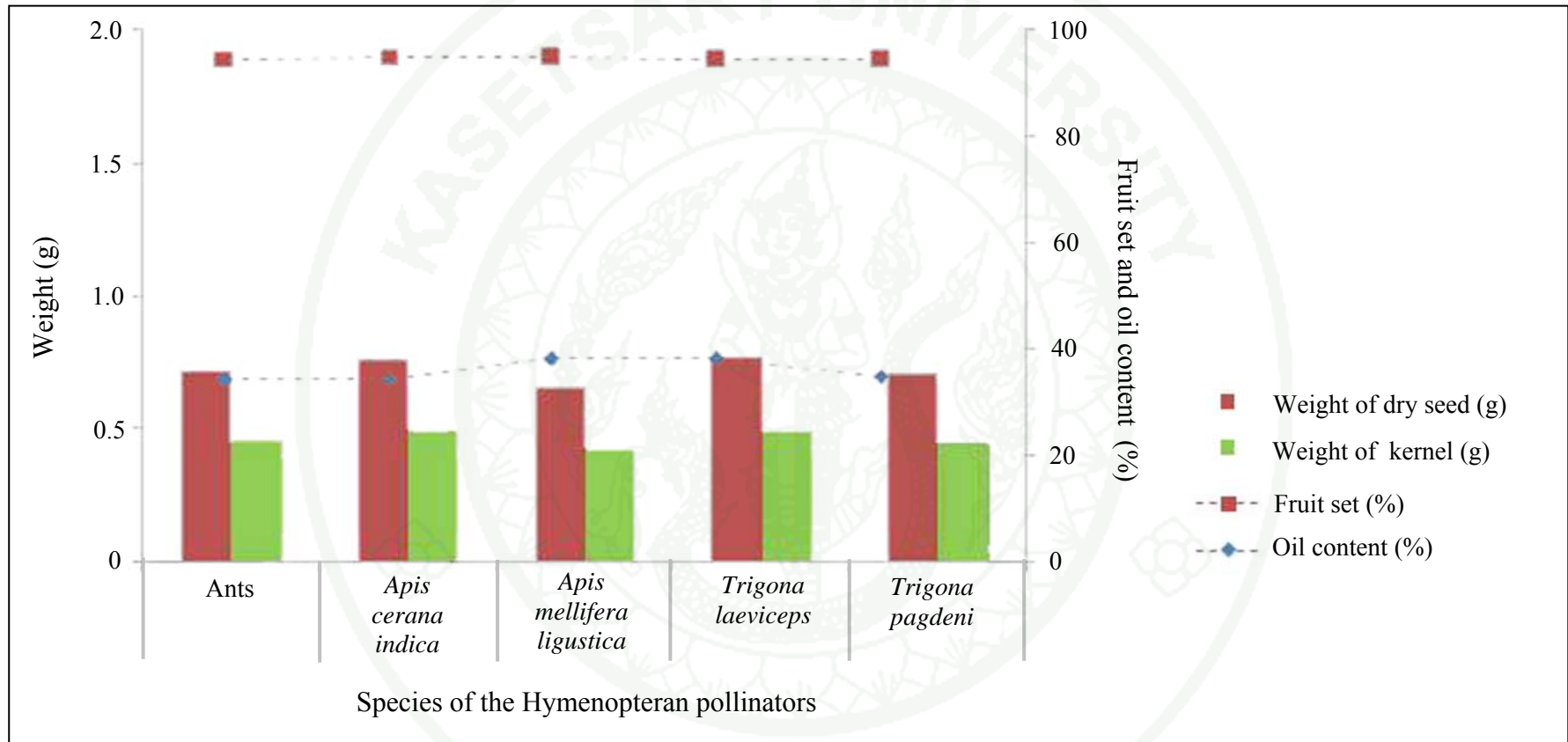
## 3) Effective pollination of Hymenopteran pollinators

Behaviors of pollinators effected to the weight of seed and kernel, and oil content. Data were slightly different among various Hymenoptera pollinators. Weight of dry seed in *T. laeviceps* (0.77 g), ants (0.76 g), *T. pagdeni* (0.72 g), *A. mellifera ligustica* (0.71 g) were slight higher than weight of dry seed, that pollinated by *A. cerana indica* (0.66 g). Percentage of oil content in the full developing seed from *A. mellifera ligustica* (38.23%) and *T. laeviceps* (38.15%) pollination that was slightly higher than the pollination by *T. pagdeni* (34.98%), *A. cerana indica* (34.65%), and ants (34.48%). While, the weight of kernel were non significantly different among variable visitors such as ants and *T. laeviceps* (0.49 g), *T. pagdeni* (0.46 g), *A. mellifera ligustica* (0.45 g), and *A. cerana indica* (0.42 g) (Figure 57 and Appendix Table 31).

High fruit setting (95%) of physic nut occurred on the open-pollination at diversity of insect pollinators location, while vary rare insect pollinators were observed on physic nut flowers at the Lop Buri that had effect on very low of fruit setting. Similarly, Dhillon *et al.* (2006) indicated that 32.9% fruit setting under selfing and 89.7% under natural pollination in physic nut. The high fruit setting under open-pollination revealed that the plant is capable of producing fruits through selfing and cross-pollination. Such a breeding system represents facultative cross-pollination. However, the fruit sets of artificial self-pollination,

artificial cross-pollination and natural cross-pollination were 87.93%, 86.66% and 76.42%, respectively, which indicated that physic nut was self-compatible and tended to cross-pollinate (Qing *et al.*, 2007). The ability to self-pollinate through geitonogamy was considered to be adaptive for physic nut for colonization (Raju and Ezradanam, 2002). Fifty percent of female flowers set fruit with 53% fecundity rate, 32% apomixis rate and 2:3 seed-ovule ratio (Bhattacharya *et al.*, 2005). Chang-wei *et al.* (2007a) and Abdelgadir *et al.* (2008) suggested that fruit production can be boosted by manipulating biological processes of pollination and growth. Cross-pollination might be influence to seed and oil content of physic nut.





**Figure 57** Percentage of fruit sets after female flowers on *Jatropha curcas* L. were visited by various the Hymenopteran pollinators, weight of dry seed and kernel (g), and oil content (%) during November to December 2008 at Kamphaeng Saen (KPS) location

### 5.3.4 Comparisons among experiments with artificial pollination

Figure 58 and Appendix Table 32 comparisons among six pollination experiments in the toxic variety during September to November 2009 at KPS location, the results were variation in weight of fresh fruit and seed. Weight of dry seed and kernel were slightly different in six treatments, while oil contents were significantly different among six pollination methods.

#### 1) Variation in weight of fresh fruit and seed;

- Weight of fresh fruit: the highest weight was found in hand-pollination large amount of pollen grains (11.58 g) followed by open-pollination (8.20 g), full pollinated by *A. florea* (6.95 g), one time visited by *A. florea* (6.23 g), and full pollinated by *T. pagdeni* (5.99 g), while the lowest weight of fresh fruit were found in ants (4.69 g).

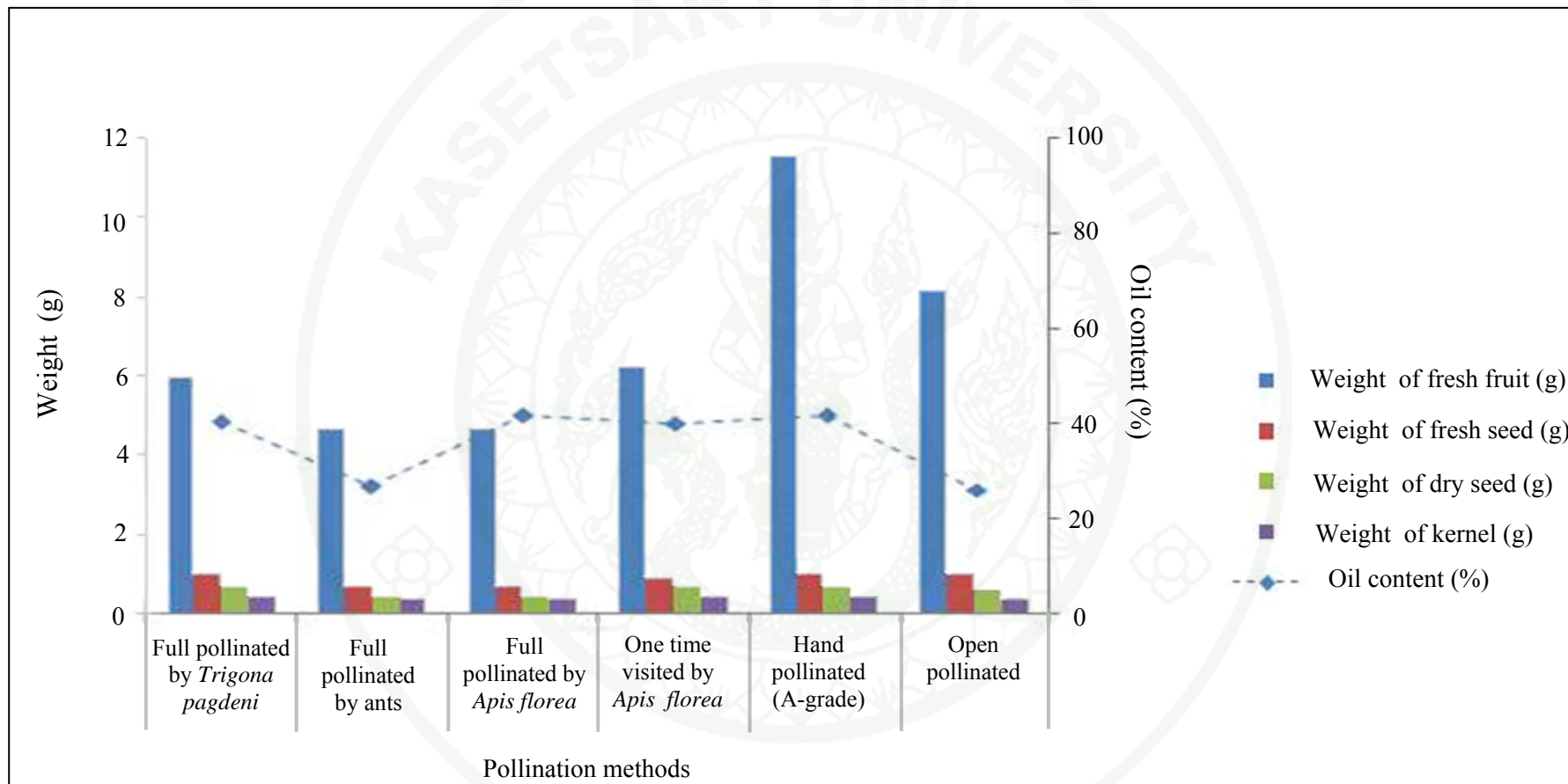
- Weight of fresh seed: in open-pollination (1.04 g), full pollinated by *T. pagdeni* (1.03 g), and hand-pollination (A-grade) (1.01 g) were higher than one time visited by *A. florea* (0.90 g), full pollinated by *A. florea* (0.90 g), full pollinated by ants (0.70 g).

2) Weight of dry seed and kernel were slightly different among six treatments. Weight of dry seed were slightly difference in hand-pollination (A-grade) (0.70 g), full pollinated by *T. pagdeni* (0.69 g), one time visited by *A. florea* (0.69 g), full pollinated by *A. florea* (0.68 g), and open-pollination (0.62 g), except full pollinated by ants (0.47 g). Likewise, weight of the kernel were slightly different in one time visited by *A. florea* (0.45 g), full pollinated by *A. florea* (0.44 g), *T. pagdeni* (0.43 g), hand-pollination (A-grade) (0.43 g), open-pollination (0.42 g), and full pollinate by ants (0.40 g).

3) Percentage of oil content was significantly different among six pollination methods. The highest oil content was found in the full pollinated by

*A. florea* (41.28 %), *T. pagdeni* (40.03 %), hand-pollination (A-grade) (41.02 %) followed by one time visited by *A. florea* (39.70 %), while the lowest values were oil content in full pollinated by ants (26.42 %), and open-pollination (25.87 %).

Distinct results represent effect of cross-pollination method such as full pollinated by *A. florea*, *T. pagdeni*, and hand-pollination (A-grade) to oil content of *Jatropha curcas* L., this pollination methods revealed oil contain higher than open-pollination about 14.16-15.41% oil content. Also *T. pagdeni* was the best efficient bees for physic nut, because of they can colonized, and can control visiting on physic nut flowers. Whereas *A. florea* can not colonized, and control similar to *A. mellifera ligustica* and *A. cerana indica*. In contrast, Abdelgadir *et al.* (2008) indicated that flowers exposed to single and multi visits by honey bees set significantly more fruits than those which received no visits, indicating that honey bees were effective pollinators.



**Figure 58** Effect of various pollination methods to weight of fruit and seed, and oil content of *Jatropha curcas* L. during September to November in 2009 at Kamphaeng Saen (KPS) location

## CONCLUSION AND RECOMMENDATIONS

### Conclusions

From the observation and experimental results and discussion of this study, conclusions can be drawn as follows:

1) 311 species of insect pollinators were found on *Jatropha curcas* L. flowers. Species diversity indices of insect pollinators showed the highest in the north region of Thailand. The bees (*Apis* spp. and *Trigona* spp.) are likely to be the most important pollinators and could be chosen for further studies.

2) Insect pollinators were divided into two groups, major and minor. The major group included honey bees, stingless honey bees, blow fly, and syrphid flies. While minor group consisted of other Apidae, Chrysididae, Evaniidae, Formicidae, Halictidae, Megachilidae, Pompilidae, Scoliidae, Sphecidae, Vespidae, other insects in Blattodea, Coleoptera, Diptera, Hemiptera, Lepidoptera, and Orthoptera.

3) Apoidea were found in the highest number of species, and the highest frequency of occurrence in 20 provinces. Apiformes of Apoidea were observed 60 species. *Apis cerana indica* was found in 11 provinces that was the widest distribution of Apoidea.

4) There are three types of physic nut plants, androgynous gynocious and polygamous plants and three types of flowers, female, male, and perfect flowers. These physic nut plants were divided into toxic and non- toxic variety.

5) Flower sex ratios of toxic variety were found in variable ratios 1:6-26 (female:male) at most locations, except the flower sex ratio of non-toxic variety was found at 9: 23:7 (female:male:perfect) in Suphan Buri.

6) Dry seed yields of physic nut in the rainy season showed high variations of weights (10-1,369 kg/rai; 1 rai=1,600 m<sup>2</sup>), and oil content (2-349 litres/rai). Thus, variable numbers of inflorescences/plant, fruits/inflorescence, plants/rai, spacing (m x m), age, propagation, pruning, plantation, and irrigation had effects on yield and oil content of physic nuts.

7) Density of insect pollinators/100 flowers of physic nuts were found in variable ranges (22-100 individuals/100 female flowers, and 3-46 individuals/100 male flowers), Apoidea were found at 0-75 individuals/100 female flowers, and 0-28 individuals/100 male flowers.

8) Physic nut flowers were pollinated by insect pollinators resulting in all stigmas were covered with pollen grains.

9) Original sources and propagation had effect on leaf area of plants, size and shape of flowers and fruits, weight of fresh fruits, dry seeds, and oil content, flower sex ratio, and diversity of insect pollinators such as species list, species diversity index, and density of insect pollinators.

10) Locations had effect on flowers sex ratio, and type of flowers, while seasons or temperature had effect on flowers sex ratio, flowering, anthesis and receptive time, and pollen germination.

11) Species of insect pollinators carrying pollen grains had effect on pollen germination. Amount of pollen grains on stigmas of physic nut flowers had no effect on weights of fresh fruits and dry seeds.

12) Types of flowers had effect on the fruit development; fruits developed from female flowers were more complete than fruits developed from perfect flowers. The occurrence of 6-10 fruits/inflorescence was found in the highest frequency of 52-68%. In additional, variable numbers of fruits/inflorescence had effect on weights of fresh fruits.

13) Species diversity of insect pollinators on physic nut flowers at Kamphaeng Saen (KPS) location depended on times of day, directions of plant canopy, months or seasons, and positions of area (plot).

14) Behavior of *Apis florea* showed that they preferred to visit on physic nut flowers during 12.00-16.00 h, which was related to the peak of nectar concentration during 13.00-18.00 h (37.67-41.33%).

15) Insect pollinators were more frequency of occurrence on male flowers than female flowers, but the calculation on number of insects/100 flowers showed that they preferred female flowers.

16) Twelve species of ants were found on physic nut flowers in 400 m<sup>2</sup> at KPS location, and *Meranoplus* sp. was the most effective pollinator on physic nut flowers, but they were self-pollination (geitonogamy).

17) *Apis cerana indica* and *A. mellifera ligustica* were highly active on physic nut flowers, they preferred pollen and nectar collecting in the morning and they liked to collect nectar more than pollen. Eleven species of competitive plants were found in six families, they attracted *A. cerana indica* and *A. mellifera ligustica* especially pollen grains from corn (*Zea mays*).

18) The physic nut flowers can be self-pollination, but absolutely need cross-pollination by honey bees and stingless honey bees. *A. cerana indica*, *A. mellifera ligustica*, and *T. pagdeni* were very efficient species to increase oil content.

### **Recommendations**

1) The observation in 20 provinces is a good data base of insect pollinator diversity for application on pollination of physic nuts or other economic plants. For example, the major group (honey bees, stingless honey bees, blow fly, and syrphid flies) can be pollinating other economic plants in each province.

2) The results presented here will provide base line data on Thailand's pollinators of physic nuts, which will be essential for monitoring population, and management of pollination in physic nut plantations.

3) Information acquired in this study forms a basic and supporting knowledge resource for the development of a pollination management program in Thailand on physic nuts or other Euphorbiaceae plants.

4) The information presented here offers new insights into the ecology of honey bee and stingless honey bees in physic nut plantations. The competitive plants are important cause for the different behaviors of insect pollinators.

5) The results of pollen germination of physic nut flowers presented differences among four original sources which could be applied to breeding of physic nuts in the future. For example non-toxic variety of physic nut from Mr. Suksan Suttipunphiboon (SSnt) had high pollen germination that was good character for father stock breeder, whereas low pollen germination in ARDCnt (non-toxic variety from Agriculture Research and Development Center location), and ASnt (non-toxic variety from Aviation School location) original source was suitable character for mother stock breeder.

6) No attempt has been made so far to use molecular methods to study genetic in seeds of physic nuts from self- and cross-pollination.

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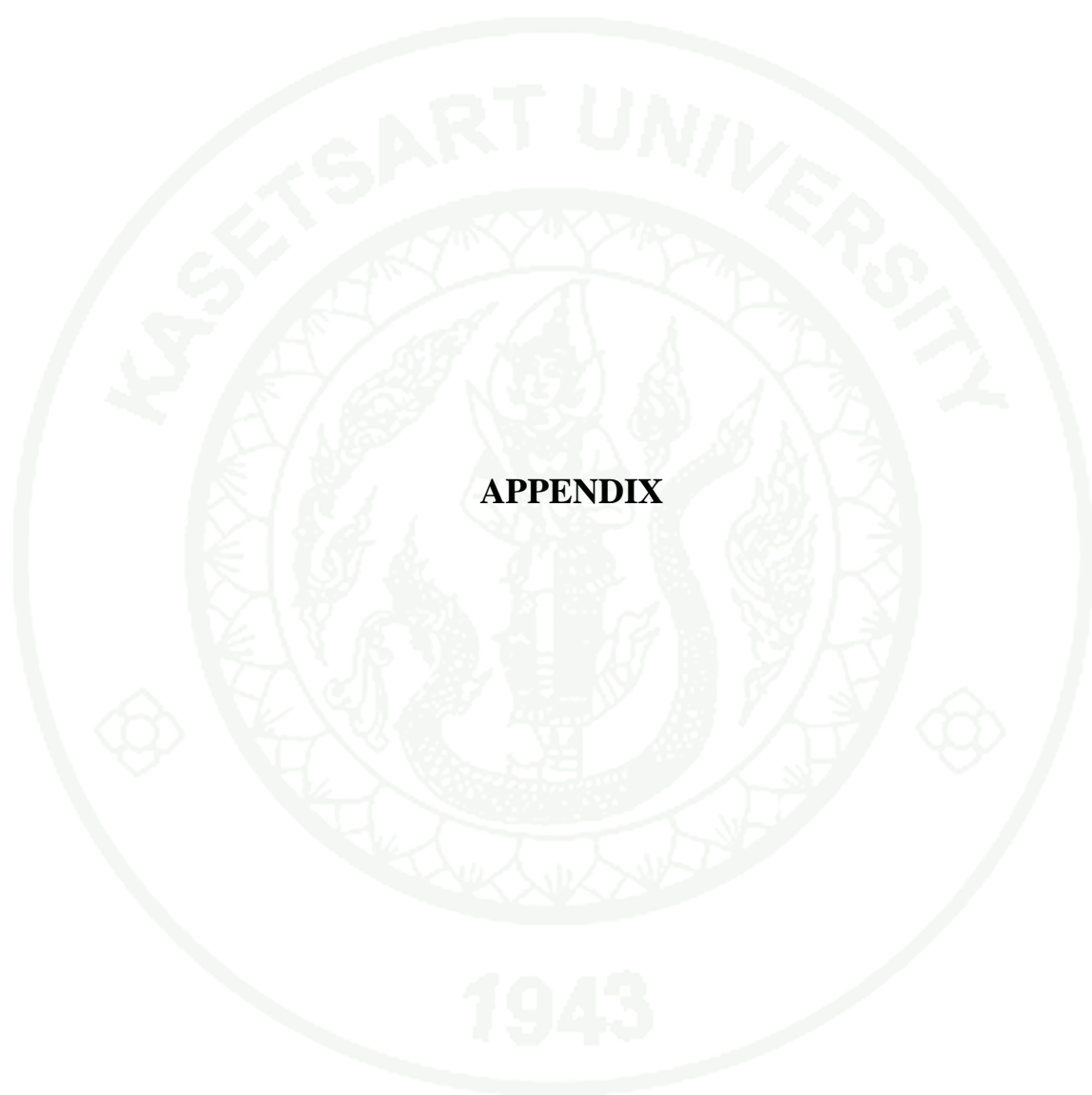
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**Appendix Table 1** Relation of species code number and species of insect pollinators on *Jatropha curcas* L. flowers in 20 provinces, Thailand from April to August

| Code numbers            | Families      | Genera              | Species          | Author name |
|-------------------------|---------------|---------------------|------------------|-------------|
| <b>Order Blattodea</b>  |               |                     |                  |             |
| sp.1                    | Blattellidae  | unidentified        | sp.              |             |
| <b>Order Coleoptera</b> |               |                     |                  |             |
| sp.2                    | Brentidae     | <i>Eubactus</i>     | sp.              |             |
| sp.3                    | Bruchidae     | unidentified        | sp.1             |             |
| sp.4                    |               | unidentified        | sp.2             |             |
| sp.5                    | Cerambycidae  | <i>Chlorophorus</i> | <i>annularis</i> | Fabricius   |
| sp.6                    |               | <i>Polyzonus</i>    | <i>obtusus</i>   | Bates       |
| sp.7                    |               | <i>Polyzonus</i>    | sp.              |             |
| sp.8                    | Chrysomelidae | <i>Aulacophora</i>  | sp.              |             |
| sp.9                    |               | <i>Chrysochus</i>   | sp.              |             |
| sp.10                   |               | <i>Donacia</i>      | <i>aenaria</i>   | Baly        |
| sp.11                   |               | <i>Galerupipla</i>  | sp.              |             |
| sp.12                   |               | <i>Luperomorpha</i> | sp.              |             |
| sp.13                   | Cleridae      | unidentified        | sp.              |             |
| sp.14                   | Curculionidae | <i>Ectatorhinus</i> | sp.              |             |
| sp.15                   |               | <i>Episomus</i>     | sp.              |             |
| sp.16                   | Elateridae    | <i>Alaus</i>        | sp.              |             |
| sp.17                   |               | <i>Diploconus</i>   | sp.1             |             |
| sp.18                   |               | <i>Diploconus</i>   | sp.2             |             |
| sp.19                   | Lycidae       | <i>Lycostomus</i>   | sp.1             |             |
| sp.20                   |               | <i>Lycostomus</i>   | sp.2             |             |
| sp.21                   |               | <i>Lycostomus</i>   | sp.3             |             |
| sp.22                   |               | <i>Lycostomus</i>   | sp.4             |             |

Appendix Table 1 (Continued)

| Code numbers            | Families       | Genera                | Species            | Author name |
|-------------------------|----------------|-----------------------|--------------------|-------------|
| <b>Order Coleoptera</b> |                |                       |                    |             |
| sp.23                   | Cantharidae    | unidentified          | sp.                |             |
| sp.24                   | Nitidulidae    | Unidentified          | sp.1               |             |
| sp.25                   |                | Unidentified          | sp.2               |             |
| sp.26                   | Scarabaeidae   | <i>Gametis</i>        | <i>histrion</i>    | Olivier     |
| sp.27                   |                | <i>Glycyphana</i>     | <i>nicobarica</i>  | Janson      |
| sp.28                   |                | <i>Glycyphana</i>     | <i>horsfieldi</i>  | Hope        |
| sp.29                   |                | <i>Glycyphana</i>     | <i>quadricolor</i> | Wiedemann   |
|                         |                |                       | <i>quadricolor</i> |             |
| sp.30                   |                | <i>Ixorida</i>        | <i>mouhotii</i>    | Wallace     |
| sp.31                   |                | unidentified          | sp.                |             |
| sp.32                   | Staphylinidae  | unidentified          | sp.                |             |
| <b>Order Diptera</b>    |                |                       |                    |             |
| sp.33                   | Asilidae       | <i>Proctacantella</i> | sp.                |             |
| sp.34                   |                | <i>Promachus</i>      | sp.                |             |
| sp.35                   | Bombycidae     | <i>Systropus</i>      | sp.1               |             |
| sp.36                   |                | <i>Systropus</i>      | sp.2               |             |
| sp.37                   |                | <i>Systropus</i>      | sp.3               |             |
| sp.38                   | Calliphoridae  | <i>Chrysomyia</i>     | <i>megacephala</i> | Fabricius   |
| sp.39                   |                | <i>Chrysomyia</i>     | sp.1               |             |
| sp.40                   |                | <i>Chrysomyia</i>     | sp.2               |             |
| sp.41                   |                | <i>Hypopygropsis</i>  | sp.                |             |
| sp.42                   |                | unidentified          | sp.                |             |
| sp.43                   | Dolichopodidae | unidentified          | sp.                |             |
| sp.44                   | Drosophilidae  | <i>Drosophila</i>     | sp.                |             |
| sp.45                   |                | unidentified          | sp.                |             |
| sp.46                   | Empididae      | <i>Hilara</i>         | sp.                |             |

Appendix Table 1 (Continued)

| Code numbers         | Families      | Genera                | Species              | Author name |
|----------------------|---------------|-----------------------|----------------------|-------------|
| <b>Order Diptera</b> |               |                       |                      |             |
| sp.47                | Muscidae      | unidentified          | sp.1                 |             |
| sp.48                |               | unidentified          | sp.2                 |             |
| sp.49                |               | unidentified          | sp.3                 |             |
| sp.50                |               | unidentified          | sp.4                 |             |
| sp.51                |               | unidentified          | sp.5                 |             |
| sp.52                |               | unidentified          | sp.6                 |             |
| sp.53                |               | unidentified          | sp.7                 |             |
| sp.54                | Sarcophagidae | <i>Parasarcophaga</i> | sp.                  |             |
| sp.55                | Stratiomyidae | <i>Hermetia</i>       | sp.                  |             |
| sp.56                |               | <i>Ptecticus</i>      | sp.                  |             |
| sp.57                |               | <i>Stratiomys</i>     | sp.                  |             |
| sp.58                |               | Unidentified          | sp.                  |             |
| sp.59                | Syrphidae     | <i>Eristalis</i>      | <i>arvorum</i>       | (Fabricius) |
| sp.60                |               | <i>Eristalis</i>      | <i>obscuritarsis</i> | Meijere     |
| sp.61                |               | <i>Helophilus</i>     | <i>bengaliensis</i>  | Wiedemann   |
| sp.62                |               | <i>Helophilus</i>     | sp.1                 |             |
| sp.63                |               | <i>Helophilus</i>     | sp.2                 |             |
| sp.64                |               | <i>Megapis</i>        | sp.                  |             |
| sp.65                |               | <i>Physocephala</i>   | sp.                  |             |
| sp.66                |               | <i>Rhingia</i>        | sp.1                 |             |
| sp.67                |               | <i>Rhingia</i>        | sp.2                 |             |
| sp.68                |               | <i>Rhingia</i>        | sp.3                 |             |
| sp.69                |               | <i>Rhingia</i>        | sp.4                 |             |
| sp.70                |               | <i>Syrphus</i>        | sp.1                 |             |
| sp.71                |               | <i>Syrphus</i>        | sp.2                 |             |
| sp.72                |               | unidentified          | sp.                  |             |

Appendix Table 1 (Continued)

| Code numbers           | Families     | Genera               | Species            | Author name |
|------------------------|--------------|----------------------|--------------------|-------------|
| <b>Order Diptera</b>   |              |                      |                    |             |
| sp.73                  | Tabanidae    | <i>Chrysops</i>      | <i>dispar</i>      | (Fabricius) |
| sp.74                  |              | <i>Chrysops</i>      | <i>fasciata</i>    | Wiedemann   |
| sp.75                  | Tachinidae   | <i>Drino</i>         | sp.1               |             |
| sp.76                  |              | <i>Drino</i>         | sp.2               |             |
| sp.77                  |              | <i>Drino</i>         | sp.3               |             |
| sp.78                  | Tephritidae  | unidentified         | sp.                |             |
| sp.79                  | Therevidae   | unidentified         | sp.                |             |
| sp.80                  | Tipulidae    | <i>Tipula</i>        | sp.                |             |
| <b>Order Hemiptera</b> |              |                      |                    |             |
| sp.81                  | Coreidae     | <i>Clavigralla</i>   | sp.                |             |
| sp.82                  |              | <i>Riptortus</i>     | <i>linearis</i>    | Fabricius   |
| sp.83                  |              | <i>Serinetha</i>     | <i>abdominalis</i> | Fabricius   |
| sp.84                  |              | unidentified         | sp.1               |             |
| sp.85                  |              | unidentified         | sp.2               |             |
| sp.86                  |              | unidentified         | sp.3               |             |
| sp.87                  | Lygaeidae    | <i>Geocoris</i>      | sp.                |             |
| sp.88                  |              | <i>Graptostethus</i> | <i>servus</i>      | Fabricius   |
| sp.89                  |              | unidentified         | sp.1               |             |
| sp.90                  |              | unidentified         | sp.2               |             |
| sp.91                  | Miridae      | unidentified         | sp.                |             |
| sp.92                  | Pentatomidae | <i>Eocanthecona</i>  | <i>furcellata</i>  | (Wolff)     |
| sp.93                  |              | <i>Erothesima</i>    | <i>fullo</i>       | Thunberg    |
| sp.94                  |              | <i>Eusarcocoris</i>  | <i>guttiger</i>    | Thunberg    |
| sp.95                  | Reduviidae   | <i>Chitapa</i>       | sp.                |             |
| sp.96                  |              | <i>Ectomocoris</i>   | sp.                |             |

Appendix Table 1 (Continued)

| Code numbers             | Families      | Genera             | Species              | Author name |
|--------------------------|---------------|--------------------|----------------------|-------------|
| <b>Order Hemiptera</b>   |               |                    |                      |             |
| sp.97                    | Reduviidae    | <i>Rhynocoris</i>  | sp.1                 |             |
| sp.98                    |               | <i>Rhynocoris</i>  | sp.2                 |             |
| sp.99                    |               | <i>Sycanus</i>     | <i>collaris</i>      | Fabricius   |
| sp.100                   |               | unidentified       | sp.                  |             |
| sp.101                   | Scutelleridae | <i>Callidea</i>    | sp.                  |             |
| sp.102                   |               | <i>Chrysocoris</i> | <i>grandis</i>       | Thunberg    |
| sp.103                   |               | <i>Chrysocoris</i> | <i>stolii</i>        | Wolff       |
| <b>Order Hymenoptera</b> |               |                    |                      |             |
| sp.104                   | Apidae        | <i>Amegilla</i>    | sp.                  |             |
| sp.105                   |               | <i>Apis</i>        | <i>andreniformis</i> | Smith       |
| sp.106                   |               | <i>Apis</i>        | <i>cerana indica</i> | Fabricius   |
| sp.107                   |               | <i>Apis</i>        | <i>dorsata</i>       | Fabricius   |
| sp.108                   |               | <i>Apis</i>        | <i>florea</i>        | Fabricius   |
| sp.109                   |               | <i>Apis</i>        | <i>mellifera</i>     | Linnaeus    |
|                          |               |                    | <i>ligustica</i>     |             |
| sp.110                   |               | <i>Ceratina</i>    | sp.1                 |             |
| sp.111                   |               | <i>Ceratina</i>    | sp.2                 |             |
| sp.112                   |               | <i>Ceratina</i>    | sp.3                 |             |
| sp.113                   |               | <i>Pithitis</i>    | <i>smaragudla</i>    | Fabricius   |
| sp.114                   |               | <i>Podalirius</i>  | <i>crocea</i>        | Bingham     |
| sp.115                   |               | <i>Thyreus</i>     | sp.                  |             |
| sp.116                   |               | <i>Trigona</i>     | <i>collina</i>       | Smith       |
| sp.117                   |               | <i>Trigona</i>     | <i>laeviceps</i>     | Smith       |
| sp.118                   |               | <i>Trigona</i>     | <i>melanoleuca</i>   | Cockerell   |
| sp.119                   |               | <i>Trigona</i>     | <i>pagdeni</i>       | Schwarz     |
| sp.120                   |               | <i>Trigona</i>     | <i>ventralis</i>     | Smith       |

Appendix Table 1 (Continued)

| Code numbers             | Families    | Genera              | Species           | Author name |
|--------------------------|-------------|---------------------|-------------------|-------------|
| <b>Order Hymenoptera</b> |             |                     |                   |             |
| sp.121                   | Apidae      | <i>Trigona</i>      | sp.1              |             |
| sp.122                   |             | <i>Trigona</i>      | sp.2              |             |
| sp.123                   |             | <i>Trigona</i>      | sp.3              |             |
| sp.124                   |             | <i>Trigona</i>      | sp.4              |             |
| sp.125                   |             | <i>Xylocopa</i>     | <i>aestuans</i>   | (Linnaeus)  |
| sp.126                   |             | <i>Xylocopa</i>     | <i>collaris</i>   | Cockerell   |
| sp.127                   |             | <i>Xylocopa</i>     | <i>latipes</i>    | (Drury)     |
| sp.128                   | Chrysididae | <i>Stilbum</i>      | <i>cyanarum</i>   | (Förster)   |
| sp.129                   |             | <i>Stilbum</i>      | sp.               |             |
| sp.130                   | Evaniidae   | <i>Evania</i>       | sp.               |             |
| sp.131                   | Formicidae  | <i>Anoplolepis</i>  | <i>gracilipes</i> | (Smith)     |
| sp.132                   |             | <i>Camponotus</i>   | sp.1              |             |
| sp.133                   |             | <i>Camponotus</i>   | sp.2              |             |
| sp.134                   |             | <i>Camponotus</i>   | sp.3              |             |
| sp.135                   |             | <i>Camponotus</i>   | sp.4              |             |
| sp.136                   |             | <i>Iridomyrmex</i>  | sp.               |             |
| sp.137                   |             | <i>Meranoplus</i>   | sp.               |             |
| sp.138                   |             | <i>Monomorium</i>   | sp.1              |             |
| sp.139                   |             | <i>Monomorium</i>   | sp.2              |             |
| sp.140                   |             | <i>Ochetellus</i>   | sp.1              |             |
| sp.141                   |             | <i>Ochetellus</i>   | sp.2              |             |
| sp.142                   |             | <i>Oecophylla</i>   | <i>smaracдина</i> | Fabricius   |
| sp.143                   |             | <i>Paratrechina</i> | sp.1              |             |
| sp.144                   |             | <i>Paratrechina</i> | sp.2              |             |
| sp.145                   |             | <i>Paratrechina</i> | sp.3              |             |
| sp.146                   |             | <i>Solenopsis</i>   | <i>geminata</i>   | (Fabricius) |

Appendix Table 1 (Continued)

| Code numbers             | Families     | Genera              | Species             | Author name |
|--------------------------|--------------|---------------------|---------------------|-------------|
| <b>Order Hymenoptera</b> |              |                     |                     |             |
| sp.147                   | Formicidae   | <i>Tetraponura</i>  | <i>rufonigra</i>    | (Jerdon)    |
| sp.148                   |              | unidentified        | sp.                 |             |
| sp.149                   | Halictidae   | <i>Halictus</i>     | sp.1                |             |
| sp.150                   |              | <i>Halictus</i>     | sp.2                |             |
| sp.151                   |              | <i>Halictus</i>     | sp.3                |             |
| sp.152                   |              | <i>Lasioglossum</i> | sp.1                |             |
| sp.153                   |              | <i>Lasioglossum</i> | sp.2                |             |
| sp.154                   |              | <i>Lasioglossum</i> | sp.3                |             |
| sp.155                   |              | <i>Lasioglossum</i> | sp.4                |             |
| sp.156                   |              | <i>Nomia</i>        | <i>albofasciata</i> | Smith       |
| sp.157                   |              | <i>Nomia</i>        | sp.1                |             |
| sp.158                   |              | <i>Nomia</i>        | sp.2                |             |
| sp.159                   |              | <i>Nomia</i>        | sp.3                |             |
| sp.160                   |              | <i>Nomia</i>        | sp.4                |             |
| sp.161                   |              | <i>Nomia</i>        | sp.5                |             |
| sp.162                   |              | unidentified        | sp.                 |             |
| sp.163                   | Megachilidae | <i>Coelioxys</i>    | sp.                 |             |
| sp.164                   |              | <i>Euaspiis</i>     | sp.1                |             |
| sp.165                   |              | <i>Euaspiis</i>     | sp.2                |             |
| sp.166                   |              | <i>Lithurge</i>     | sp.                 |             |
| sp.167                   |              | <i>Megachile</i>    | <i>hera</i>         | Bingham     |
| sp.168                   |              | <i>Megachile</i>    | <i>disjuncta</i>    | (Fabricius) |
| sp.169                   |              | <i>Megachile</i>    | <i>ampulata</i>     | Smith       |
| sp.170                   |              | <i>Megachile</i>    | sp.1                |             |
| sp.171                   |              | <i>Megachile</i>    | sp.2                |             |
| sp.172                   |              | <i>Megachile</i>    | sp.3                |             |

Appendix Table 1 (Continued)

| Code numbers             | Families     | Genera             | Species                                    | Author name |
|--------------------------|--------------|--------------------|--|-------------|
| <b>Order Hymenoptera</b> |              |                    |  |             |
| sp.173                   | Megachilidae | <i>Megachile</i>   | sp.4                                       |             |
| sp.174                   |              | <i>Megachile</i>   | sp.5                                       |             |
| sp.175                   |              | <i>Megachile</i>   | sp.6                                       |             |
| sp.176                   |              | <i>Megachile</i>   | sp.7                                       |             |
| sp.177                   |              | <i>Megachile</i>   | sp.8                                       |             |
| sp.178                   |              | <i>Megachile</i>   | sp.9                                       |             |
| sp.179                   |              | <i>Megachile</i>   | sp.10                                      |             |
| sp.180                   |              | <i>Megachile</i>   | sp.11                                      |             |
| sp.181                   |              | <i>Megachile</i>   | sp.12                                      |             |
| sp.182                   |              | <i>Megachile</i>   | sp.13                                      |             |
| sp.183                   |              | <i>Megachile</i>   | sp.14                                      |             |
| sp.184                   |              | <i>Megachile</i>   | sp.15                                      |             |
| sp.185                   |              | unidentified       | sp.  |             |
| sp.186                   | Mutillidae   | <i>Trogaspidia</i> | sp.  |             |
| sp.187                   | Pompilidae   | <i>Pompilus</i>    | sp.1                                       |             |
| sp.188                   |              | <i>Pompilus</i>    | sp.2                                       |             |
| sp.189                   | Scoliidae    | <i>Camsomeris</i>  | <i>collaris</i> 4-<br><i>fasciata</i>      | Fabricius   |
| sp.190                   |              | <i>Camsomeris</i>  | <i>phalerata</i>                           | Saussure    |
| sp.191                   |              | <i>Liacos</i>      | sp.  |             |
| sp.192                   |              | <i>Megascolia</i>  | <i>azurea</i><br><i>rubiginosa</i>         | Fabricius   |
| sp.193                   |              | <i>Scolia</i>      | <i>quadripustulata</i><br><i>humeralis</i> | Saussure    |
| sp.194                   |              | <i>Scolia</i>      | sp.1                                       |             |
| sp.195                   |              | <i>Scolia</i>      | sp.2                                       |             |

Appendix Table 1 (Continued)

| Code numbers             | Families  | Genera               | Species           | Author name  |
|--------------------------|-----------|----------------------|-------------------|--------------|
| <b>Order Hymenoptera</b> |           |                      |                   |              |
| sp.196                   | Scoliidae | <i>Scolia</i>        | sp.3              |              |
| sp.197                   |           | <i>Scolia</i>        | sp.4              |              |
| sp.198                   |           | unidentified         | sp.1              |              |
| sp.199                   |           | unidentified         | sp.2              |              |
| sp.200                   |           | unidentified         | sp.3              |              |
| sp.201                   |           | unidentified         | sp.4              |              |
| sp.202                   |           | unidentified         | sp.5              |              |
| sp.203                   | Sphecidae | <i>Chalybion</i>     | <i>benjalense</i> | (Dahlbom)    |
| sp.204                   |           | <i>Chlorion</i>      | <i>lobatum</i>    | (Fabricius)  |
| sp.205                   |           | <i>Chlorion</i>      | sp.1              |              |
| sp.206                   |           | <i>Chlorion</i>      | sp.2              |              |
| sp.207                   |           | <i>Episylon</i>      | sp.               |              |
| sp.208                   |           | <i>Liris</i>         | sp.               |              |
| sp.209                   |           | <i>Sceliphron</i>    | <i>javanum</i>    | (Lepeletier) |
| sp.210                   |           | <i>Sphex</i>         | <i>argentatus</i> | Fabricius    |
| sp.211                   |           | <i>Sphex</i>         | <i>sericeus</i>   | Lepeletier   |
|                          |           |                      | <i>lineolus</i>   |              |
| sp.212                   |           | <i>Sphex</i>         | <i>viduatus</i>   | Christ       |
| sp.213                   |           | <i>Sphex</i>         | sp.1              |              |
| sp.214                   |           | <i>Sphex</i>         | sp.2              |              |
| sp.215                   | Vespidae  | <i>Apodynerus</i>    | sp.               |              |
| sp.216                   |           | <i>Auterhynchium</i> | sp.               |              |
| sp.217                   |           | <i>Delta</i>         | <i>esuriens</i>   | Fabricius    |
| sp.218                   |           | <i>Delta</i>         | sp.1              |              |
| sp.219                   |           | <i>Delta</i>         | sp.2              |              |
| sp.220                   |           | <i>Delta</i>         | sp.3              |              |

Appendix Table 1 (Continued)

| Code numbers             | Families | Genera           | Species               | Author name |
|--------------------------|----------|------------------|-----------------------|-------------|
| <b>Order Hymenoptera</b> |          |                  |                       |             |
| sp.221                   | Vespidae | <i>Delta</i>     | sp.4                  |             |
| sp.222                   |          | <i>Delta</i>     | sp.5                  |             |
| sp.223                   |          | <i>Eumenes</i>   | <i>conica</i>         | Fabricius   |
| sp.224                   |          | <i>Eumenes</i>   | sp.1                  |             |
| sp.225                   |          | <i>Eumenes</i>   | sp.2                  |             |
| sp.226                   |          | <i>Eumenes</i>   | sp.3                  |             |
| sp.227                   |          | <i>Phimenes</i>  | sp.1                  |             |
| sp.228                   |          | <i>Phimenes</i>  | sp.2                  |             |
| sp.229                   |          | <i>Polistes</i>  | <i>stigma</i>         | (Fabricius) |
| sp.230                   |          | <i>Polistes</i>  | sp.1                  |             |
| sp.231                   |          | <i>Polistes</i>  | sp.2                  |             |
| sp.232                   |          | <i>Polistes</i>  | sp.3                  |             |
| sp.233                   |          | <i>Polistes</i>  | sp.4                  |             |
| sp.234                   |          | <i>Polistes</i>  | sp.5                  |             |
| sp.235                   |          | <i>Rhynchium</i> | <i>haemorrhoidala</i> | (Fabricius) |
| sp.236                   |          | <i>Rhynchium</i> | <i>quinguecinctum</i> | (Fabricius) |
| sp.237                   |          | <i>Vespa</i>     | <i>affinis</i>        | (Linnaeus)  |
| sp.238                   |          | <i>Vespa</i>     | sp.1                  |             |
| sp.239                   |          | <i>Vespa</i>     | sp.2                  |             |
| sp.240                   |          | <i>Vespa</i>     | sp.3                  |             |
| sp.241                   |          | <i>Vespa</i>     | sp.4                  |             |
| sp.242                   |          | <i>Vespa</i>     | sp.5                  |             |
| sp.243                   |          | <i>Vespa</i>     | sp.6                  |             |

Appendix Table 1 (Continued)

| Code numbers             | Families    | Genera           | Species                                | Author name       |
|--------------------------|-------------|------------------|--|-------------------|
| <b>Order Lepidoptera</b> |             |                  |  |                   |
| sp.244                   | Acraeidae   | <i>Acraea</i>    | <i>violae</i>                          | Fabricius         |
| sp.245                   | Arctiidae   | <i>Amata</i>     | <i>sperbius</i>                        | Fabricius         |
| sp.246                   |             | <i>Amata</i>     | sp.                                    |                   |
| sp.247                   |             | <i>Argina</i>    | sp.                                    |                   |
| sp.248                   |             | <i>Euchromia</i> | <i>elegantissima</i>                   | Wallgram          |
| sp.249                   |             | unidentified     | sp.1                                   |                   |
| sp.250                   |             | unidentified     | sp.2                                   |                   |
| sp.251                   | Danaidae    | <i>Danaus</i>    | <i>chrysippus</i><br><i>chrysippus</i> | (Linnaeus)        |
| sp.252                   |             | <i>Danaus</i>    | <i>genutia genutia</i>                 | (Cramer)          |
| sp.253                   |             | <i>Euploea</i>   | <i>aglae limborgii</i>                 | Moore             |
| sp.254                   |             | <i>Euploea</i>   | <i>core godartii</i><br><i>klugii</i>  | (Lucas)<br>Felder |
| sp.255                   |             | <i>Euploea</i>   | <i>erichsonii</i>                      |                   |
| sp.256                   |             | <i>Euploea</i>   | sp.                                    |                   |
| sp.257                   |             | <i>Ideopsis</i>  | sp.                                    |                   |
| sp.258                   | Gelechiidae | unidentified     | sp.                                    |                   |
| sp.259                   | Geometridae | unidentified     | sp.                                    |                   |
| sp.260                   | Hesperiidae | <i>Caltoris</i>  | <i>bromus</i><br><i>bromus</i>         | Leech             |
| sp.261                   |             | <i>Spialia</i>   | <i>galba</i>                           | (Fabricius)       |
| sp.262                   |             | <i>Telicota</i>  | <i>linna</i>                           | Evans             |
| sp.263                   |             | unidentified     | sp.1                                   |                   |
| sp.264                   |             | unidentified     | sp.2                                   |                   |
| sp.265                   |             | unidentified     | sp.3                                   |                   |
| sp.266                   |             | unidentified     | sp.4                                   |                   |

Appendix Table 1 (Continued)

| Code numbers             | Families     | Genera             | Species                                  | Author name   |
|--------------------------|--------------|--------------------|--|---------------|
| <b>Order Lepidoptera</b> |              |                    |  |               |
| sp.267                   | Lycaenidae   | <i>Amblypodia</i>  | <i>anita anita</i>                       | Hewitson      |
| sp.268                   |              | <i>Cyclosia</i>    | <i>panthona</i>                          | Cramer        |
| sp.269                   | Lycaenidae   | <i>Everes</i>      | <i>lacturnus rileyi</i>                  | Godfrey       |
| sp.270                   |              | <i>Loxura</i>      | <i>atymnus</i><br><i>continentalis</i>   | Fruhstofer    |
| sp.271                   |              | <i>Rapala</i>      | <i>pheretima</i><br><i>petosiris</i>     | (Hewitson)    |
| sp.272                   |              | <i>Spindasis</i>   | <i>syama terana</i>                      | (Fruhstorfer) |
| sp.273                   |              | <i>Surendra</i>    | <i>quercetorum</i><br><i>quercetorum</i> | (Moore)       |
| sp.274                   |              | <i>Zizina</i>      | <i>otis sangra</i>                       | (Moore)       |
| sp.275                   |              | unidentified       | sp.                                      |               |
| sp.276                   | Noctuidae    | unidentified       | sp.                                      |               |
| sp.277                   | Nymphalidae  | <i>Cethosia</i>    | <i>cyane euanthus</i>                    | Fruhstorfer   |
| sp.278                   |              | <i>Cirrochoa</i>   | <i>tyche mithila</i>                     | Moore         |
| sp.279                   |              | <i>Junonia</i>     | sp.                                      |               |
| sp.280                   |              | <i>Neptis</i>      | <i>hylas kamarupa</i>                    | Moore         |
| sp.281                   |              | <i>Tanaecia</i>    | sp.<br><i>agamemnon</i>                  |               |
| sp.282                   |              | unidentified       | sp.                                      |               |
| sp.283                   | Papilionidae | <i>Chilasa</i>     | <i>clytia clytia</i>                     | (Evans)       |
| sp.284                   |              | <i>Graphium</i>    | <i>agamemnon</i><br><i>agamemnon</i>     | Linnaeus      |
| sp.285                   |              | <i>Graphium</i>    | <i>doson axion</i>                       | (Felder)      |
| sp.286                   |              | <i>Lamproptera</i> | <i>meges virescens</i>                   | (Butler)      |

Appendix Table 1 (Continued)

| Code numbers             | Families     | Genera            | Species                                    | Author name  |
|--------------------------|--------------|-------------------|--|--------------|
| <b>Order Lepidoptera</b> |              |                   |  |              |
| sp.287                   | Papilionidae | <i>Pachliopta</i> | <i>aristolochiae</i><br><i>goniopeltis</i> | (Rothschild) |
| sp.288                   |              | <i>Papilio</i>    | <i>demoleus</i><br><i>malayanus</i>        | Wallace      |
| sp.289                   |              | <i>Papilio</i>    | <i>memnon agenor</i>                       | Linnaeus     |
| sp.290                   |              | <i>Papilio</i>    | <i>polytes romulus</i>                     | Cramer       |
| sp.291                   |              | <i>Pathysa</i>    | <i>antiphates</i><br><i>pompilius</i>      | (Fabricius)  |
| sp.292                   |              | <i>Troides</i>    | <i>aeacus aeacus</i>                       | Felder       |
| sp.293                   | Pieridae     | <i>Appias</i>     | <i>albina darada</i>                       |              |
| sp.294                   |              | <i>Appias</i>     | <i>olferna olferna</i><br><i>pomona</i>    |              |
| sp.295                   |              | <i>Catopsilia</i> | <i>pomona</i>                              |              |
| sp.296                   |              | <i>Eurema</i>     | sp.  |              |
| sp.297                   |              | <i>Ixias</i>      | <i>pyrene</i><br><i>yunnanensis</i>        | (Druce)      |
| sp.298                   |              | <i>Leptosia</i>   | <i>nina nina</i>                           | (Fabricius)  |
| sp.299                   | Pyralidae    | unidentified      | sp.1                                       |              |
| sp.300                   |              | unidentified      | sp.2                                       |              |
| sp.301                   | Satyridae    | <i>Mycalesis</i>  | sp.  |              |
| sp.302                   |              | <i>Ypthima</i>    | sp.  |              |
| sp.305                   |              | <i>Melitta</i>    | sp.3                                       |              |
| sp.306                   | Sphingidae   | <i>Cephonodes</i> | <i>hylas hylas</i>                         | (Linnaeus)   |
| sp.307                   | Tortricidae  | unidentified      | sp.  |              |

**Appendix Table 1** (Continued)

| Code numbers            | Families       | Genera        | Species          | Author name |
|-------------------------|----------------|---------------|------------------|-------------|
| <b>Order Mantodea</b>   |                |               |                  |             |
| sp.308                  | Mantidae       | <i>Mantis</i> | <i>religiosa</i> | Linnaeus    |
| sp.309                  |                | unidentified  | sp.              |             |
| <b>Order Orthoptera</b> |                |               |                  |             |
| sp.310                  | Acrididae      | unidentified  | sp.              |             |
| sp.311                  | Tettrigoniidae | unidentified  | sp.              |             |

**Appendix Table 2** Distribution of insect pollinators on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |    |    |         |    |    |    |      |    |       |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|----|----|---------|----|----|----|------|----|-------|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    |    |    | Central |    |    |    | East |    | South |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN | UT | CN      | KP | NP | SB | CB   | RY | CP    | PNG |                    |
| sp.1             | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 2                  |
| sp.2             | /   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.3             | -   | /  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.4             | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 1                  |
| sp.5             | -   | /  | /  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 3                  |
| sp.6             | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.7             | -   | -  | /  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.8             | -   | /  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.9             | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.10            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 1                  |
| sp.11            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | /   | 1                  |
| sp.12            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.13            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.14            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -   | 1                  |
| sp.15            | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.16            | /   | -  | /  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 3                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |    |    |         |    |    |    |      |    |       |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|----|----|---------|----|----|----|------|----|-------|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    |    |    | Central |    |    |    | East |    | South |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN | UT | CN      | KP | NP | SB | CB   | RY | CP    | PNG |                    |
| sp.17            | -   | /  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.18            | /   | /  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 3                  |
| sp.19            | -   | -  | -  | -   | /         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.20            | -   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.21            | -   | -  | -  | -   | -         | -   | -  | -  | -  | /  | /  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |
| sp.22            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.23            | -   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.24            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.25            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | /  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.26            | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.27            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.28            | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.29            | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.30            | -   | -  | /  | /   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 3                  |
| sp.31            | -   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.32            | -   | -  | /  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 2                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |         |    |    |    |      |    |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|---------|----|----|----|------|----|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    | Central |    |    |    | East |    |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR      | SN | UT | CN | KP   | NP | SB | CB    | RY | CP | PNG |                    |
| sp.33            | -   | -  | -  | /   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.34            | -   | -  | -  | /   | -         | /   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 2                  |
| sp.35            | /   | /  | -  | -   | /         | -   | -  | -  | -  | -       | /  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 4                  |
| sp.36            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 2                  |
| sp.37            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.38            | /   | -  | -  | /   | -         | /   | -  | -  | -  | -       | /  | /  | /  | /    | -  | /  | -     | /  | -  | -   | 9                  |
| sp.39            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.40            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | /    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.41            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.42            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.43            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | /     | -  | -  | -   | 1                  |
| sp.44            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | /    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.45            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | /     | -  | -  | -   | 1                  |
| sp.46            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.47            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | /     | -  | -  | -   | 1                  |
| sp.48            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |    |    |         |    |    |    |      |    |       |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|----|----|---------|----|----|----|------|----|-------|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    |    |    | Central |    |    |    | East |    | South |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN | UT | CN      | KP | NP | SB | CB   | RY | CP    | PNG |                    |
| sp.49            | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.50            | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.51            | -   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.52            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.53            | -   | -  | -  | -   | -         | -   | -  | /  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.54            | -   | /  | -  | /   | /         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 5                  |
| sp.55            | -   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.56            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 1                  |
| sp.57            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 1                  |
| sp.58            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -   | 1                  |
| sp.59            | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.60            | -   | /  | -  | /   | -         | /   | -  | -  | -  | -  | /  | -  | /       | /  | /  | -  | -    | /  | -     | -   | 8                  |
| sp.61            | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.62            | -   | /  | /  | -   | -         | /   | -  | -  | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | -     | -   | 4                  |
| sp.63            | -   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |
| sp.64            | -   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |    |           |    |    |    |    |         |    |    |    |      |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|----|-----------|----|----|----|----|---------|----|----|----|------|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     |    | Northeast |    |    |    |    | Central |    |    |    | East |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA | PHY       | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB    | RY | CP | PNG |                    |
| sp.65            | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.66            | /   | /  | /  | /   | /  | /         | -  | -  | -  | /  | -       | -  | /  | /  | /    | /  | /     | /  | -  | -   | 13                 |
| sp.67            | /   | /  | -  | -   | -  | /         | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 4                  |
| sp.68            | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.69            | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.70            | -   | /  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.71            | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | /   | 1                  |
| sp.72            | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.73            | /   | /  | -  | /   | -  | -         | -  | -  | /  | -  | -       | -  | -  | -  | -    | /  | -     | -  | -  | -   | 5                  |
| sp.74            | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.75            | -   | /  | /  | -   | -  | /         | -  | -  | -  | -  | -       | /  | -  | /  | -    | -  | -     | -  | /  | -   | 6                  |
| sp.76            | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.77            | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.78            | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.79            | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -  | -   | 1                  |
| sp.80            | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -       | -  | -  | -  | -    | /  | -     | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB    | RY | CP | PNG |                    |
| sp.81            | -   | /  | -  | /   | -         | /   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | /  | -  | -   | 4                  |
| sp.82            | -   | -  | -  | -   | -         | -   | -  | -  | /  | -  | -       | -  | -  | /  | -    | -  | /     | -  | -  | -   | 3                  |
| sp.83            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.84            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.85            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | /  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.86            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.87            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.88            | /   | /  | /  | -   | -         | -   | -  | -  | -  | -  | -       | /  | /  | -  | -    | -  | -     | -  | -  | -   | 5                  |
| sp.89            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.90            | -   | -  | -  | -   | -         | -   | -  | /  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.91            | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.92            | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -  | -   | 1                  |
| sp.93            | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.94            | -   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.95            | -   | -  | -  | -   | -         | -   | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.96            | -   | -  | -  | -   | -         | -   | -  | -  | /  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |         |    |    |    |      |    |       |    |    |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|---------|----|----|----|------|----|-------|----|----|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    | Central |    |    |    | East |    | South |    |    |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS      | NR | SN | UT | CN   | KP | NP    | SB | CB | RY | CP | PNG |                    |
| sp.97            | /   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 1                  |
| sp.98            | -   | -  | -  | -   | -         | /   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 1                  |
| sp.99            | /   | -  | -  | -   | -         | /   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 2                  |
| sp.100           | -   | -  | -  | -   | -         | -   | /  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 1                  |
| sp.101           | /   | /  | /  | -   | -         | -   | -  | -  | -       | -  | /  | /  | -    | /  | -     | -  | /  | -  | -  | -   | 7                  |
| sp.102           | -   | /  | /  | -   | /         | /   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 4                  |
| sp.103           | /   | -  | -  | -   | -         | -   | /  | /  | -       | /  | /  | -  | /    | /  | /     | -  | -  | -  | -  | -   | 8                  |
| sp.104           | -   | /  | /  | -   | -         | -   | /  | -  | -       | /  | -  | -  | /    | -  | -     | -  | -  | -  | -  | -   | 5                  |
| sp.105           | -   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | /   | 1                  |
| sp.106           | /   | /  | /  | /   | -         | /   | /  | -  | -       | -  | -  | -  | -    | /  | /     | -  | /  | /  | /  | /   | 11                 |
| sp.107           | -   | /  | /  | -   | -         | /   | -  | -  | -       | -  | -  | -  | /    | /  | /     | -  | -  | /  | -  | -   | 7                  |
| sp.108           | -   | /  | /  | /   | /         | /   | /  | -  | -       | -  | -  | -  | -    | /  | /     | -  | /  | -  | -  | -   | 9                  |
| sp.109           | /   | -  | -  | -   | /         | -   | -  | -  | -       | -  | -  | -  | -    | /  | /     | -  | -  | -  | -  | -   | 4                  |
| sp.110           | /   | /  | /  | -   | /         | /   | /  | -  | /       | /  | /  | -  | -    | -  | -     | /  | -  | /  | -  | -   | 11                 |
| sp.111           | -   | /  | /  | /   | /         | /   | -  | -  | /       | /  | -  | -  | -    | -  | -     | -  | -  | /  | -  | -   | 8                  |
| sp.112           | -   | -  | -  | /   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB    | RY | CP | PNG |                    |
| sp.113           | -   | /  | /  | /   | -         | -   | -  | -  | -  | /  | -       | -  | -  | -  | /    | /  | -     | -  | -  | -   | 6                  |
| sp.114           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -  | -  | -   | 1                  |
| sp.115           | -   | /  | /  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.116           | /   | -  | /  | -   | -         | /   | /  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -  | -   | 5                  |
| sp.117           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -  | -   | 1                  |
| sp.118           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | /  | -   | 1                  |
| sp.119           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | /     | /  | /  | /   | 6                  |
| sp.120           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.121           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | /  | -  | -   | 2                  |
| sp.122           | -   | /  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.123           | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.124           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | /   | 1                  |
| sp.125           | -   | -  | /  | -   | -         | -   | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.126           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.127           | -   | -  | -  | -   | -         | -   | -  | -  | -  | /  | /       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.128           | -   | /  | /  | -   | -         | -   | /  | -  | /  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 4                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |    |       |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|----|-------|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    |    | South |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB | RY    | CP | PNG |                    |
| sp.129           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | /  | -  | -     | -  | -   | 2                  |
| sp.130           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -  | -     | -  | /   | 2                  |
| sp.131           | /   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.132           | /   | /  | -  | /   | /         | -   | -  | /  | -  | /  | -       | -  | /  | /  | -    | /  | /  | -     | /  | 11  |                    |
| sp.133           | /   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 3                  |
| sp.134           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.135           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | /   | 2                  |
| sp.136           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.137           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.138           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.139           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.140           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.141           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.142           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | /  | -  | -     | /  | 2   |                    |
| sp.143           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.144           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |         |    |    |    |      |    |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|---------|----|----|----|------|----|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    | Central |    |    |    | East |    |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR      | SN | UT | CN | KP   | NP | SB | CB    | RY | CP | PNG |                    |
| sp.145           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | /  | -  | -     | -  | -  | -   | 1                  |
| sp.146           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | /  | -  | -  | /    | -  | /  | -     | -  | -  | -   | 3                  |
| sp.147           | -   | -  | /  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | /   | 2                  |
| sp.148           | -   | -  | -  | -   | -         | -   | -  | /  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.149           | /   | /  | -  | /   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 3                  |
| sp.150           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | /   | 1                  |
| sp.151           | -   | -  | -  | -   | -         | -   | -  | -  | -  | /       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.152           | /   | -  | /  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 2                  |
| sp.153           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.154           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.155           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 1                  |
| sp.156           | -   | /  | /  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -  | -    | -  | /  | -     | -  | -  | -   | 3                  |
| sp.157           | -   | /  | -  | -   | -         | -   | -  | -  | /  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -   | 2                  |
| sp.158           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -  | -   | 2                  |
| sp.159           | -   | -  | -  | -   | -         | /   | -  | -  | -  | /       | -  | -  | /  | /    | -  | -  | -     | -  | -  | -   | 4                  |
| sp.160           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | /  | /    | -  | -  | -     | -  | -  | -   | 3                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |         |    |    |      |    |    |       |    |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|---------|----|----|------|----|----|-------|----|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    | Central |    |    | East |    |    | South |    |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR      | SN | UT | CN   | KP | NP | SB    | CB | RY | CP | PNG |                    |
| sp.161           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | /  | -     | -  | -  | -  | -   | 1                  |
| sp.162           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.163           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | /  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.164           | -   | -  | -  | -   | -         | -   | -  | /  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.165           | -   | /  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.166           | -   | -  | -  | -   | -         | -   | -  | /  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.167           | -   | /  | /  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.168           | -   | -  | -  | -   | -         | -   | -  | -  | /  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.169           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.170           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.171           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.172           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.173           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.174           | -   | /  | -  | -   | /         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.175           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.176           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |         |    |    |      |    |    |       |    |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|---------|----|----|------|----|----|-------|----|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    | Central |    |    | East |    |    | South |    |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR      | SN | UT | CN   | KP | NP | SB    | CB | RY | CP | PNG |                    |
| sp.177           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.178           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.179           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.180           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.181           | -   | -  | -  | -   | -         | -   | /  | -  | /  | /       | -  | -  | -    | /  | -  | -     | -  | -  | -  | -   | 4                  |
| sp.182           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.183           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.184           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.185           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.186           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | -  | /  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.187           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.188           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -       | /  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.189           | /   | /  | -  | -   | /         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 3                  |
| sp.190           | -   | -  | /  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.191           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.192           | -   | -  | -  | -   | -         | /   | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |    |           |    |    |    |    |    |         |    |    |    |      |    |    |       |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|----|-----------|----|----|----|----|----|---------|----|----|----|------|----|----|-------|-----|--------------------|
|                  | North                                     |    |    |     |    | Northeast |    |    |    |    |    | Central |    |    |    | East |    |    | South |     |                    |
|                  | CM  | CR | LP | LPH | NA | PHY       | KS | KK | MS | NR | SN | UT      | CN | KP | NP | SB   | CB | RY | CP    | PNG |                    |
| sp.193           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -  | -     | -   | 1                  |
| sp.194           | /   | -  | /  | /   | -  | /         | -  | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | /  | -     | -   | 6                  |
| sp.195           | /   | -  | /  | -   | /  | /         | -  | -  | -  | -  | /  | -       | /  | -  | -  | /    | -  | /  | -     | -   | 8                  |
| sp.196           | -   | -  | /  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.197           | -   | /  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.198           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.199           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | /  | -  | -  | -    | -  | -  | -     | -   | 2                  |
| sp.200           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.201           | -   | -  | -  | -   | -  | -         | /  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.202           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | /     | -   | 1                  |
| sp.203           | -   | /  | -  | -   | /  | -         | -  | -  | -  | -  | /  | /       | -  | -  | -  | -    | -  | -  | -     | -   | 4                  |
| sp.204           | /   | -  | -  | /   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /  | -     | -   | 3                  |
| sp.205           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.206           | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.207           | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.208           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | /  | -  | -  | -    | -  | -  | -     | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |         |    |    |    |      |    |    |       |    |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|---------|----|----|----|------|----|----|-------|----|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    | Central |    |    |    | East |    |    | South |    |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS      | NR | SN | UT | CN   | KP | NP | SB    | CB | RY | CP | PNG |                    |
| sp.209           | -   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.210           | -   | /  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.211           | -   | -  | -  | -   | /         | /   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.212           | /   | /  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 2                  |
| sp.213           | -   | -  | /  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.214           | -   | /  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.215           | -   | -  | -  | -   | /         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | /  | -   | 2                  |
| sp.216           | /   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.217           | -   | -  | /  | /   | -         | -   | -  | /  | -       | /  | -  | -  | /    | -  | -  | -     | -  | -  | -  | -   | 5                  |
| sp.218           | /   | -  | /  | /   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | /  | -  | -   | 4                  |
| sp.219           | -   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | /    | /  | -  | /     | -  | /  | -  | -   | 4                  |
| sp.220           | /   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | /    | -  | -  | /     | -  | -  | -  | -   | 3                  |
| sp.221           | /   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.222           | -   | -  | -  | -   | /         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |
| sp.223           | /   | -  | /  | -   | -         | /   | -  | -  | -       | -  | -  | -  | /    | -  | -  | /     | -  | -  | -  | -   | 5                  |
| sp.224           | /   | -  | -  | -   | -         | -   | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |    |       |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|----|-------|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    |    | South |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB | RY    | CP | PNG |                    |
| sp.225           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.226           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.227           | -   | /  | -  | -   | /         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.228           | /   | -  | -  | -   | -         | /   | -  | -  | -  | -  | -       | /  | -  | -  | -    | -  | -  | -     | -  | -   | 3                  |
| sp.229           | /   | /  | -  | -   | /         | -   | /  | /  | -  | /  | /       | /  | /  | /  | -    | /  | -  | -     | -  | -   | 11                 |
| sp.230           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.231           | -   | -  | -  | -   | -         | -   | /  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.232           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.233           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.234           | /   | -  | /  | -   | /         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 3                  |
| sp.235           | -   | -  | -  | -   | -         | -   | -  | -  | /  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.236           | /   | /  | /  | -   | /         | /   | /  | -  | -  | /  | -       | -  | /  | /  | /    | -  | -  | -     | -  | -   | 10                 |
| sp.237           | -   | /  | -  | /   | -         | -   | /  | /  | /  | /  | /       | /  | /  | /  | -    | /  | -  | -     | /  | -   | 11                 |
| sp.238           | /   | -  | -  | -   | -         | -   | -  | -  | /  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.239           | /   | -  | -  | -   | /         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.240           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |    |           |    |    |    |    |    |    |         |    |    |      |    |    |       |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|----|-----------|----|----|----|----|----|----|---------|----|----|------|----|----|-------|-----|--------------------|
|                  | North                                     |    |    |     |    | Northeast |    |    |    |    |    |    | Central |    |    | East |    |    | South |     |                    |
|                  | CM  | CR | LP | LPH | NA | PHY       | KS | KK | MS | NR | SN | UT | CN      | KP | NP | SB   | CB | RY | CP    | PNG |                    |
| sp.241           | -   | /  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.242           | -   | /  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | /  | -    | -  | -  | -     | -   | 2                  |
| sp.243           | -   | /  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.244           | -   | -  | /  | -   | -  | -         | -  | /  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 2                  |
| sp.245           | -   | /  | /  | -   | /  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 3                  |
| sp.246           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | /  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.247           | /   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.248           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.249           | -   | /  | -  | /   | /  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 3                  |
| sp.250           | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.251           | -   | -  | /  | -   | -  | -         | -  | /  | -  | -  | -  | /  | -       | -  | -  | -    | -  | -  | -     | -   | 3                  |
| sp.252           | -   | -  | -  | -   | -  | -         | /  | -  | -  | -  | -  | /  | -       | -  | -  | -    | -  | -  | -     | -   | 2                  |
| sp.253           | -   | -  | -  | -   | -  | -         | -  | /  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |
| sp.254           | /   | -  | -  | /   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 2                  |
| sp.255           | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | /  | -  | -     | -   | 2                  |
| sp.256           | -   | -  | /  | -   | -  | -         | -  | -  | -  | -  | -  | -  | -       | -  | -  | -    | -  | -  | -     | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |    |           |    |    |    |    |    |         |    |    |    |      |    |       |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|----|-----------|----|----|----|----|----|---------|----|----|----|------|----|-------|----|-----|--------------------|
|                  | North                                     |    |    |     |    | Northeast |    |    |    |    |    | Central |    |    |    | East |    | South |    |     |                    |
|                  | CM  | CR | LP | LPH | NA | PHY       | KS | KK | MS | NR | SN | UT      | CN | KP | NP | SB   | CB | RY    | CP | PNG |                    |
| sp.257           | /   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 2                  |
| sp.258           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.259           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -   | 1                  |
| sp.260           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | /   | 1                  |
| sp.261           | -   | -  | -  | -   | -  | -         | /  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.262           | -   | -  | /  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.263           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | /     | -  | -   | 1                  |
| sp.264           | -   | -  | -  | /   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.265           | -   | -  | -  | -   | -  | -         | -  | -  | -  | -  | -  | /       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.266           | -   | -  | /  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.267           | -   | -  | /  | -   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.268           | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.269           | -   | -  | -  | -   | -  | /         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | /   | 2                  |
| sp.270           | -   | /  | -  | /   | -  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 2                  |
| sp.271           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |
| sp.272           | -   | -  | -  | -   | /  | -         | -  | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |    |       |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|----|-------|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    |    | South |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB | RY    | CP | PNG |                    |
| sp.273           | /   | /  | /  | /   | /         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | /     | -  | -   | 6                  |
| sp.274           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.275           | -   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.276           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.277           | -   | -  | /  | -   | -         | /   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.278           | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.279           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | /     | -  | -   | 2                  |
| sp.280           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.281           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | /   | 1                  |
| sp.282           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | /   | 1                  |
| sp.283           | /   | -  | /  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.284           | /   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.285           | /   | -  | /  | -   | -         | /   | -  | -  | -  | -  | -       | -  | -  | /  | -    | -  | -  | -     | -  | -   | 4                  |
| sp.286           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 1                  |
| sp.287           | -   | -  | -  | -   | -         | -   | -  | /  | -  | -  | -       | -  | /  | -  | -    | -  | -  | -     | -  | -   | 2                  |
| sp.288           | /   | -  | -  | -   | -         | -   | /  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -  | -     | -  | -   | 2                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup> | Twenty provinces in Thailand <sup>2</sup> |    |    |     |           |     |    |    |    |    |         |    |    |    |      |    |       |    |    |     | Total <sup>3</sup> |
|------------------|---|----|----|-----|-----------|-----|----|----|----|----|---------|----|----|----|------|----|-------|----|----|-----|--------------------|
|                  | North                                     |    |    |     | Northeast |     |    |    |    |    | Central |    |    |    | East |    | South |    |    |     |                    |
|                  | CM  | CR | LP | LPH | NA        | PHY | KS | KK | MS | NR | SN      | UT | CN | KP | NP   | SB | CB    | RY | CP | PNG |                    |
| sp.289           | -   | /  | -  | -   | -         | -   | /  | -  | /  | -  | -       | -  | -  | /  | -    | -  | -     | -  | -  | -   | 4                  |
| sp.290           | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | /  | -   | 2                  |
| sp.291           | /   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.292           | /   | -  | -  | -   | -         | -   | /  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.293           | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.294           | /   | -  | /  | /   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | /  | -     | -  | -  | -   | 5                  |
| sp.295           | -   | /  | /  | /   | -         | /   | /  | -  | /  | /  | -       | /  | /  | /  | -    | -  | -     | /  | -  | /   | 12                 |
| sp.296           | -   | -  | /  | -   | -         | -   | -  | -  | -  | /  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.297           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | /  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.298           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | /  | /   | 2                  |
| sp.299           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.300           | -   | /  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |
| sp.301           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | /   | 1                  |
| sp.302           | -   | /  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 2                  |
| sp.303           | -   | -  | -  | -   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | /    | -  | -     | -  | -  | -   | 1                  |
| sp.304           | -   | -  | -  | /   | -         | -   | -  | -  | -  | -  | -       | -  | -  | -  | -    | -  | -     | -  | -  | -   | 1                  |

**Appendix Table 2** (Continued)

| No. <sup>1</sup>   | Twenty provinces in Thailand <sup>2</sup> |     |     |     |     |           |     |     |     |     |     |         |     |     |     |      |     |     |       |     | Total <sup>3</sup> |
|--------------------|---|-----|-----|-----|-----|-----------|-----|-----|-----|-----|-----|---------|-----|-----|-----|------|-----|-----|-------|-----|--------------------|
|                    | North                                     |     |     |     |     | Northeast |     |     |     |     |     | Central |     |     |     | East |     |     | South |     |                    |
|                    | CM  | CR  | LP  | LPH | NA  | PHY       | KS  | KK  | MS  | NR  | SN  | UT      | CN  | KP  | NP  | SB   | CB  | RY  | CP    | PNG |                    |
| sp.305             | -   | -   | -   | -   | /   | -         | -   | -   | -   | -   | -   | -       | -   | -   | -   | -    | -   | -   | -     | -   | 1                  |
| sp.306             | -   | /   | -   | -   | -   | -         | -   | -   | -   | -   | -   | -       | -   | -   | /   | -    | -   | -   | -     | -   | 2                  |
| sp.307             | /   | -   | -   | -   | -   | -         | -   | -   | -   | -   | -   | -       | -   | -   | -   | -    | -   | -   | -     | -   | 1                  |
| sp.308             | -   | -   | -   | -   | -   | /         | -   | -   | -   | -   | -   | -       | -   | /   | -   | -    | /   | -   | -     | -   | 3                  |
| sp.309             | -   | -   | -   | -   | -   | -         | -   | -   | -   | -   | -   | -       | -   | /   | -   | -    | -   | -   | -     | -   | 1                  |
| sp.310             | -   | -   | -   | -   | -   | -         | -   | -   | -   | -   | -   | -       | -   | -   | -   | -    | -   | /   | -     | -   | 1                  |
| sp.311             | -   | -   | -   | -   | /   | -         | -   | -   | -   | -   | -   | -       | -   | -   | -   | -    | -   | -   | -     | -   | 1                  |
| Total <sup>4</sup> | 73  | 88  | 59  | 43  | 44  | 45        | 23  | 12  | 12  | 18  | 17  | 17      | 29  | 31  | 35  | 19   | 21  | 27  | 12    | 18  |                    |
| H <sup>5</sup>     | 4.3                                       | 4.5 | 4.1 | 3.8 | 3.8 | 3.8       | 3.1 | 2.5 | 2.5 | 2.9 | 2.8 | 2.8     | 3.4 | 3.4 | 3.6 | 2.9  | 3.0 | 3.3 | 2.5   | 2.9 |                    |

<sup>1</sup> No. column or species code numbers were described in Appendix Table 2.

<sup>2</sup> CM = Chiang Mai, CR = Chiang Rai, LP = Lampang, LPH = Lamphun, NA = Nan, PHY = Phayao, KS = Kalasin, KK = Khon Kaen, MS = Maha Sarakham, NR = Nakhon Ratchasima, SN = Sakon Nakhon, UT = Udon Thani, CN = Chai Nat, KP = Kamphaeng Phet, NP = Nakhon Pathom, SB = Suphan Buri, CB = Chon Buri, RY = Rayong, CP = Chumphon, and PNG = Phangnga; “/” = presence and “-” = absence insect pollinators on physic nut flowers.

**Appendix Table 3** Behavior of insect pollinators on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Grouping   | Behavior observation   |
|--|--|
| <b>Major</b>   |  |
| 1. Hymenoptera: Apidae; <i>Apis</i> spp. and <i>Trigona</i> spp.)  | <ul style="list-style-type: none"> <li>- They and their broods depended on both nectar and pollen.</li> <li>- They usually collected nectar all day (08.00-18.00 h), and often collected pollen in the morning (07.30-10.30 h).</li> <li>- Advanced eusocial.</li> <li>- Cross-pollination and xenogamy.</li> </ul>  |
| 2. Diptera: Calliphoridae; <i>Chrysomya megacephala</i> and Syrphidae; <i>Eristalis arvorum</i> , <i>E. obscuritarsis</i> , <i>Helophilus bengaliensis</i> and <i>Rhingia</i> spp. | <ul style="list-style-type: none"> <li>- They usually ate mixed nectar and pollen on male flowers, and visited female flowers for nectar source.</li> <li>- When high air temperature not found them.</li> <li>- Solitary insect.</li> <li>- Most self-pollination or geitonogamy, and sometimes xenogamy.</li> </ul>  |
| <b>Minor</b>   |  |
| 1. Hymenoptera: other Apidae, Chrysididae, Evaniidae, Formicidae, Halictidae, Megachilidae, Pompilidae, Scoliidae, Sphecidae and Vespidae  | <ul style="list-style-type: none"> <li>- They not so often encountered on flowers.</li> <li>- Both nectar and pollen forager, either nectar or pollen forager.</li> <li>- Various social levels such as solitary, communal, quasisocial, subsocial, parasocial, and primitive eusocial.</li> <li>- Different functions in ecology; predator, and parasite.</li> <li>- Cross-pollination, and self-pollination</li> </ul> |

Appendix Table 3 (Continued)

| Grouping  | Behavior observation  |
|---|---|
| Minor   |   |
| 2. Other insects in Blattodea, Coleoptera, Diptera, Hemiptera, Lepidoptera, and Orthoptera                    | <ul style="list-style-type: none"> <li>- They not so often encountered on flowers.</li> <li>- Both nectar and pollen forager, either nectar or pollen forager.</li> <li>- Solitary insect.</li> <li>- Most self-pollination or geitonogamy, and sometimes xenogamy.</li> </ul>  |
| 3. Hemiptera: Scutelleridae; <i>Callidea</i> sp., <i>Chrysocoris grandis</i> , and <i>Chrysocoris stollii</i> | <ul style="list-style-type: none"> <li>- They often visited on flowers.</li> <li>- Sucking nectar, sap from fruit, stalk sucking stem.</li> <li>- Solitary insect, but aggregation of nymph.</li> <li>- Most self-pollination or geitonogamy, and rare xenogamy.</li> </ul>   |
| 4. Hemiptera: Reduviidae, Mantodea: Mantidae, and spider  | <ul style="list-style-type: none"> <li>- Pest of physic nuts</li> <li>- They rarely found on flowers.</li> <li>- Nectar were sucked by Reduviidae, and sometimes they caught major pollinators .</li> <li>- Mantidae and spider were stayed on flowers for catching prey.</li> <li>- Indirect self-pollination</li> <li>- Pest of plants and predator of insect pollinators.</li> </ul> |

**Appendix Table 4** Number of species and percentage of insect pollinators in Hymenoptera, that visited on *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Superfamilies | Number of Species | %     |
|---------------|-------------------|-------|
| Apoidea       | 72                | 51.43 |
| Vespoidea     | 65                | 46.43 |
| Chrysoidea    | 2                 | 1.43  |
| Evanoidea     | 1                 | 0.71  |
| Total         | 140               | 100   |

**Appendix Table 5** Types and sex ratios of *Jatropha curcas* L. flowers in 20 provinces from April to August 2009

| Thailand Regions | Provinces         | Average of flower sex ratio |      |         |
|------------------|-------------------|-----------------------------|------|---------|
|                  |                   | female                      | male | perfect |
| North            | Chiang Mai        | 1                           | 10   | -       |
|                  | Chiang Rai        | 1                           | 6    | -       |
|                  | Lampang           | 1                           | 17   | -       |
|                  | Lamphun           | 1                           | 6    | -       |
|                  | Nan               | 1                           | 12   | -       |
|                  | Phayao            | 1                           | 12   | -       |
| Northeast        | Kalasin           | 1                           | 16   | -       |
|                  | Khon Kaen         | 1                           | 15   | -       |
|                  | Maha Sarakham     | 1                           | 12   | -       |
|                  | Nakhon Ratchasima | 1                           | 26   | -       |
|                  | Sakon Nakhon      | 1                           | 10   | -       |
|                  | Udon Thani        | 1                           | 11   | -       |
| Central          | Chai Nat          | 1                           | 23   | -       |
|                  | Kamphaeng Phet    | 1                           | 9    | -       |
|                  | Nakhon Pathom     | 1                           | 7    | -       |
|                  | Suphan Buri       | 9                           | 23   | 7       |
|                  | Chon Buri         | 1                           | 10   | -       |
| East             | Rayong            | 1                           | 11   | -       |
|                  | Chumphon          | 1                           | 10   | -       |
| South            | Phangnga          | 1                           | 10   | -       |

**Appendix Table 6** Metrodology of air temperature, accumulative rainfall, and relative humidity from four locations in 2009-2011

| Locations   | Months    | Air temperature (°C) |      | Average               | Average accumulative rainfall (mm) |
|---|-----------|----------------------|------|-----------------------|------------------------------------|
|   |           | Max.                 | Min. | relative humidity (%) |                                    |
| Kamphaeng Saen (KPS) and Aviation School (AS) location, 2009      | January   | 29.3                 | 14.4 | 69                    | 0.1                                |
|   | February  | 34.5                 | 18.7 | 68                    | 0.0                                |
|   | March     | 35.5                 | 21.8 | 68                    | 15.9                               |
|   | April     | 35.9                 | 23.8 | 70                    | 39.3                               |
|   | May       | 33.9                 | 24.0 | 75                    | 343.2                              |
|   | June      | 33.4                 | 24.6 | 77                    | 47.9                               |
|   | July      | 32.9                 | 23.8 | 76                    | 90.9                               |
|   | August    | 34.4                 | 23.9 | 74                    | 76.0                               |
|   | September | 33.9                 | 24.0 | 75                    | 22.6                               |
|   | October   | 32.6                 | 23.1 | 76                    | 313.5                              |
|   | November  | 31.5                 | 18.6 | 70                    | 5.2                                |
|   | December  | 31.5                 | 16.7 | 70                    | 0.0                                |
| Agriculture Research and Development Center (ARDC) location, 2009 | January   | 29.8                 | 15.6 | 70                    | 0.0                                |
|   | February  | 34.7                 | 20.4 | 73                    | 3.0                                |
|   | March     | 35.6                 | 23.3 | 72                    | 10.4                               |
|   | April     | 36.8                 | 24.4 | 67                    | 101.5                              |
|   | May       | 34.1                 | 23.5 | 79                    | 146.5                              |
|   | June      | 33.8                 | 22.9 | 77                    | 101.4                              |
|   | July      | 33.1                 | 22.5 | 77                    | 68.3                               |
|   | August    | 34.6                 | 22.3 | 75                    | 68.6                               |
|   | September | 34.0                 | 21.9 | 78                    | 253.4                              |
|   | October   | 32.9                 | 20.9 | 83                    | 116.2                              |
|   | November  | 31.7                 | 19.6 | 75                    | 5.2                                |
|   | December  | 32.1                 | 18.9 | 74                    | 0.0                                |

Appendix Table 6 (Continued)

| Locations                    | Months    | Air temperature (°C) |      | Average               | Average                    |
|------------------------------|-----------|----------------------|------|-----------------------|----------------------------|
|                              |           | Max.                 | Min. | relative humidity (%) | accumulative rainfall (mm) |
| Bangkhen (BK) location, 2011 | January   | 32.4                 | 22.6 | 59                    | 0.2                        |
|                              | February  | 34.1                 | 25.3 | 72                    | 21.8                       |
|                              | March     | 31.5                 | 24.1 | 75                    | 173.3                      |
|                              | April     | 34.2                 | 26.1 | 77                    | 134.7                      |
|                              | May       | 34.9                 | 26.5 | 77                    | 296.5                      |
|                              | June      | 33.4                 | 26.7 | 78                    | 411.5                      |
|                              | July      | 33.3                 | 25.6 | 79                    | 317.6                      |
|                              | August    | 33.2                 | 25.8 | 80                    | 297.0                      |
|                              | September | 33.1                 | 25.7 | 81                    | 223.6                      |
|                              | October   | 33.0                 | 25.3 | 80                    | 362.0                      |
|                              | November  | 34.2                 | 25.6 | 64                    | 0.9                        |
|                              | December  | 32.0                 | 22.8 | 60                    | 0.7                        |

**Source:** Nakhon Pathom Meteorological Station (2009);  
Thailand Meteorological Department (2009, 2011)

**Appendix Table 7** Anthesis and receptive time on *Jatropha curcas* L. flowers during 07.00-11.00 h on April 16<sup>th</sup> (wet season) and December 27<sup>th</sup> (dry season) in 2009 at Kamphaeng Saen (KPS) location

| Times of day | Event   |  |
|--------------|---|--|
|              | Wet season (April 16 <sup>th</sup> )                                | Dry season (December 27 <sup>th</sup> )                        |
| 07.00 h      | - Male and female flowers were closed.                              | - Male and female flowers were closed.                         |
| 07.30 h      | - Male flowers started blooming.                                    |  |
| 08.00 h      | - Anthesis began<br>- Honey bees collected pollen.; 31.1°C          | 20.2°C of air temperature.                                     |
| 08.30 h      | - Female flowers started blooming.                                  | - Male flowers started blooming.                               |
| 09.00 h      | - Receptive time  | - Anthesis began<br>- Honey bees visited and collected pollen. |
| 09.30 h      | - Honey bees collected pollen and nectar.                           | - Female flowers started blooming.                             |
| 10.00 h      | - Honey bees collected nectar from male and female flowers.; 35.0°C | - Receptive time   |
| 10.30 h      | - Female flowers were pollinated by honey bees.                     | - Honey bees collected pollen and nectar. ; 23.1°C             |
| 11.00 h      |   | - Female flowers were pollinated by honey bees.                |

**Appendix Table 8** Pollen germination percentage comparison in intra-variety (toxic) in different seasons of year at Kamphaeng Saen (KPS) in 2009, inter-variety (toxic and non-toxic) in the dry season at Bangkhen (BK) in 2011, and pollen grains from male and perfect flowers of *Jatropha curcas* L. at Aviation School (AS) location in 2009

| Comparisons                           | Types of flowers | Seasons <sup>1</sup> | Variety <sup>2</sup> sources | Pollen <sup>3</sup> germination (%) | Temperature <sup>4</sup> (°C) |
|---------------------------------------|------------------|----------------------|------------------------------|-------------------------------------|-------------------------------|
| Intra-variety (KPS location, 2009)    | male             | summer               | KPSt                         | 70.50 <sup>a</sup>                  | 35.0                          |
|                                       |                  | rainy                | KPSt                         | 60.33 <sup>b</sup>                  | 32.8                          |
|                                       |                  | winter               | KPSt                         | 55.60 <sup>c</sup>                  | 23.0                          |
| Inter-variety (BK location, 2011)     | male             | rainy                | KPSt                         | 62.00 <sup>b</sup>                  | 33.0                          |
|                                       |                  | rainy                | ARDCnt                       | 21.00 <sup>c</sup>                  | 33.0                          |
|                                       |                  | rainy                | ASnt                         | 19.00 <sup>c</sup>                  | 33.0                          |
|                                       |                  | rainy                | SSnt                         | 85.00 <sup>a</sup>                  | 33.0                          |
| Different flowers (AS location, 2009) | male             | winter               | ASnt                         | 34.00 <sup>ns</sup>                 | 23.5                          |
|                                       | perfect          | winter               | ASnt                         | 35.50 <sup>ns</sup>                 | 23.5                          |

<sup>1</sup> Rainy season = June-October, Winter season = November-February, Summer season = March-May

<sup>2</sup> KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

<sup>3</sup> Average of pollen germination percentages were to be significantly different among three seasons (df = 29, p = 0.05), inter-variety (df = 39, p = 0.05), non significantly (ns) different in between male and perfect flowers (df = 19, p = 0.05).

<sup>4</sup> Average temperature were measured at 10.00-11.00 h

**Appendix Table 9** Comparison of pollen germination percentage from pollen grains on the bodies of three species of honey bees in the nature, in the summer season (April) 2009 at Kamphaeng Saen (KPS) location, at 35.5°C

| Species of bees           | Pollen germination <sup>1</sup> (%) |
|---------------------------|-------------------------------------|
| <i>Apis cerana indica</i> | 80.17 <sup>a</sup>                  |
| <i>Apis dorsata</i>       | 29.67 <sup>b</sup>                  |
| <i>Apis florea</i>        | 21.67 <sup>b</sup>                  |

<sup>1</sup> Average of pollen germination percentages were to be significantly different in among bees (df = 29, p = 0.05), that pollen grains from the bodies of three species of honey bees (except pollen grains from corbicular) were studied pollen germination. Average temperature air was 35.5°C.

**Appendix Table 10** Average frequency of visiting of *Apis florea* on stigmas, average of fresh fruits, and dry seed weight (g) of *Jatropha curcas* L. flowers, in each amount of pollen grains grade, in the summer season (April) 2009 at Kamphaeng Saen (KPS) location

| Average                                    | Amount of pollen grains grade <sup>2</sup> |                   |                   |
|--|--|-------------------|-------------------|
|  | A  | B                 | C                 |
| Frequency of visiting (times) <sup>1</sup> | 18.20 <sup>a</sup>                         | 9.00 <sup>b</sup> | 5.60 <sup>c</sup> |
| Weight (g)                                 |  |                   |                   |
| Fresh fruit <sup>3</sup>                   | 10.54                                      | 10.30             | 10.25             |
| Dry seed <sup>4</sup>                      | 0.78                                       | 0.71              | 0.79              |

<sup>1</sup> Average frequency of visiting of *Apis florea* were to be significantly different in among grade level of amount of pollen grains (df = 29, p = 0.05).

<sup>2</sup> A = very fulfillment of pollen grains (>1,500 pollen grains/stigma)  
 B = moderated pollen grains (300-1,500 pollen grains/stigma)  
 C = slight pollen grains (< 300 pollen grains/stigma)

<sup>3</sup> Average of fresh fruit weight (g) were non significantly different in among pollen grade (df = 29, p = 0.05). Fresh fruit was the yellow fruits.

<sup>4</sup> Average of dry seed weight (g) were non significantly different in among pollen grade (df = 29, p = 0.05). Dry seeds were dried with 50°C-60°C about 48 hours.

**Appendix Table 11** Characteristics of fruit set from female and male flowers of *Jatropha curcas* L. in non-toxic variety on August 25<sup>th</sup> 2009 at Agriculture Research and Development Center (ARDC) location

| Characteristic of fruit set | Fruit set (%)  |  |
|-----------------------------|--|--|
|                             | Female flower  | Perfect flower   |
| Complete fruits             | 100<br>(65% black, 30% yellow,<br>and 5% green fruits) | 17.39<br>(5% black, 10.39% yellow,<br>and 2% green fruits) |
| Dwarf fruits                |  | 30.43  |
| Failed set fruits           |  | 52.18  |
| Total                       | 100  | 100  |

**Appendix Table 12** Frequency of occurrence of fruit numbers per inflorescence of *Jatropha curcas* L. in toxic variety, on October, 2008 at Kamphaeng Saen (KPS) location

| Rang of fruit number/inflorescence | Frequency of occurrence <sup>1</sup> (%) |
|------------------------------------|--|
| 0-5                                | 16.96 <sup>c</sup>                       |
| 6-10                               | 52.68 <sup>a</sup>                       |
| 11-15                              | 25.00 <sup>b</sup>                       |
| 16-20                              | 4.46 <sup>d</sup>                        |
| 21-25                              | 0.89 <sup>e</sup>                        |

<sup>1</sup> Frequency of occurrence were to be significantly different in among vary rages of fruits number per inflorescence (df = 99, p = 0.05).

**Appendix Table 13** Effect of fruit numbers per inflorescence to size of fruits, weight of fruit and seed, and oil content of *Jatropha curcas* L. at Kamphaeng Saen (KPS) location in 2009

| Parameter                 | Number of fruits/inflorescence |                   |                   |
|---------------------------|--------------------------------|-------------------|-------------------|
|                           | 5 fruits                       | 10 fruits         | 15 fruits         |
| Size                      |                                |                   |                   |
| Diameter (cm)             | 2.24                           | 2.07              | 2.01              |
| Length (cm)               | 2.41                           | 2.29              | 2.23              |
| Weight of fresh fruit (g) | 8.20 <sup>a</sup>              | 5.22 <sup>b</sup> | 5.21 <sup>b</sup> |
| Weight of seed (g)        |                                |                   |                   |
| Fresh seed                | 1.03                           | 0.87              | 0.87              |
| Dry seed                  | 0.61                           | 0.56              | 0.55              |
| Kernel                    | 0.42                           | 0.41              | 0.41              |
| Oil content (%)           | 25.87                          | 25.87             | 25.48             |

<sup>1</sup> Weight of fresh fruit were to be significantly different in among vary rages of fruit number/inflorescence (df = 29, p = 0.05).

<sup>2</sup> Dry seeds were dried with 50°C-60°C about 48 hours.

<sup>3</sup> Kernels = Dry seeds were extracted seed coats and kernels.

**Appendix Table 14** Plant canopy diameter, plant height, and growth development of *Jatropha curcas* L. among four original sources, on plot-B in the rainy season 2011, 90 days after planting at Bangkhen (BK) location

| Original Sources | Plant canopy diameter (cm) | Plant height (cm) | Vegetative period (days) | Flowering period (days) | Fruit Ripening period (days) |
|------------------|----------------------------|-------------------|--------------------------|-------------------------|------------------------------|
| KPSt             | 49.15                      | 47.10             | 68                       | 16                      | 45                           |
| ARDCnt           | 42.78                      | 45.72             | 65                       | 16                      | 44                           |
| ASnt             | 40.40                      | 51.98             | 57                       | 15                      | 45                           |
| SSnt             | 41.78                      | 43.78             | 72                       | 17                      | 45                           |

KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

**Appendix Table 15** Weight of fruit, seed and kernel, and oil content of *Jatropha curcas* L. on plot-B in the rainy season 2011 at Bangkhen (BK) location

| Original sources <sup>1</sup> | Weight of fruit (g) | Weight of seed (g) <sup>4</sup> |                   |                     | Moisture (%) | Kernel (%) | Oil content (%)    |
|-------------------------------|---------------------|---------------------------------|-------------------|---------------------|--------------|------------|--------------------|
|                               |                     | Fresh                           | Dry <sup>2</sup>  | Kernel <sup>3</sup> |              |            |                    |
| KPSt                          | 8.13 <sup>a</sup>   | 1.02 <sup>a</sup>               | 0.60 <sup>a</sup> | 0.41                | 41.18        | 68.33      | 22.01 <sup>b</sup> |
| ARDCnt                        | 10.09 <sup>a</sup>  | 0.93 <sup>b</sup>               | 0.53 <sup>b</sup> | 0.35                | 43.01        | 66.04      | 30.10 <sup>a</sup> |
| ASnt                          | 9.50 <sup>a</sup>   | 0.73 <sup>b</sup>               | 0.70 <sup>a</sup> | 0.35                | 4.11         | 50.00      | 28.20 <sup>a</sup> |
| SSnt                          | 5.74 <sup>b</sup>   | 0.80 <sup>b</sup>               | 0.52 <sup>b</sup> | 0.35                | 35.00        | 67.31      | 29.31 <sup>a</sup> |

<sup>1</sup> KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

<sup>2</sup> Dry seeds from only fruit were dried with 50-60°C about 48 hour.

<sup>3</sup> Kernels = Dry seed were extracted seed coat and kernel.

<sup>4</sup> Weight of fresh fruit, fresh seed, dry seed, and oil content were significantly different among four original sources, but weight of kernel were non significantly different in four original sources (df = 11, p = 0.05).

**Appendix Table 16** Species list and number (individuals) of insect pollinators on *Jatropha curcas* L. flowers in four original sources on plot-B in the rainy season 2011 at Bangkhen (BK) location

| Orders      | Families      | Species                    | Number of insect pollinators (individuals) |        |      |      |
|-------------|---------------|----------------------------|--|--------|------|------|
|             |               |                            | KPSt                                       | ARDCnt | ASnt | SSnt |
| Hymenoptera | Apidae        | <i>Apis florea</i>         | 3  | 4      | 1    | 0    |
|             |               | <i>A. cerana indica</i>    | 5  | 3      | 3    | 0    |
|             |               | <i>Trigona pagdeni</i>     | 1  | 6      | 4    | 2    |
| Diptera     | Fomicidae     | <i>Camponotus</i> sp.      | 7  | 1      | 6    | 4    |
|             | Vespidae      | <i>Polites</i> sp.         | 0  | 0      | 1    | 0    |
|             | Calliphoridae | <i>Chrysomya</i>           |  |        |      |      |
|             |               | <i>megacephala</i>         | 4  | 3      | 1    | 0    |
|             | Drosophilidae | <i>Drosophila</i> sp.      | 10   | 0      | 0    | 0    |
|             | Syrphidae     | <i>Eristalis</i>           |  |        |      |      |
|             |               | <i>obscuritarsis</i>       | 4  | 2      | 0    | 1    |
|             |               | <i>Helophilus</i> sp.      | 4  | 0      | 1    | 4    |
|             |               | <i>Rhingia</i> sp.         | 0  | 1      | 0    | 0    |
| Hemiptera   | Scutelleridae | <i>Chrysocoris stollii</i> | 2  | 1      | 4    | 0    |

KPSt = toxic variety from Kamphaeng Saen location

ARDCnt = non-toxic variety from Agriculture Research and Development Center location

ASnt = non-toxic variety from Aviation School location

SSnt = non-toxic variety from Mr. Suksan Suttipunphiboon

**Appendix Table 17** Species list, species diversity index ( $H'$ ), and number of insect pollinators (Hymenoptera) on *Jatropha curcas* L. flowers in the wet and dry season 2008-2009 at Kamphaeng Saen (KPS) location

| Insect pollinator species <sup>1</sup> | Number of insect pollinator/area <sup>2</sup> |       |                         |        |
|--|---|-------|-------------------------|--------|
|  | Wet season <sup>3</sup>                       |       | Dry season <sup>4</sup> |        |
|  | Individuals                                   | %     | Individuals             | %      |
| <b>Apidae</b>                          |   |       |                         |        |
| <i>Apis cerana indica</i>              | 3   | 2.35  | 8                       | 1.21   |
| <i>A. dorsata</i>                      | 108   | 75.88 | 24                      | 3.66   |
| <i>A. florea</i>                       | -   | -     | 511                     | 77.28  |
| <i>Xylocopa aestuans</i>               | -   | -     | 1                       | 0.14   |
| <b>Megachilidae</b>                    |   |       |                         |        |
| <i>Megachile</i> sp.                   | -   | -     | 3                       | 0.51   |
| <b>Formicidae</b>                      |   |       |                         |        |
| <i>Camponotus</i> sp.                  | 9   | 6.46  | 20                      | 3.04   |
| <i>Crematogaster</i> sp.               | 21  | 14.73 | 45                      | 6.82   |
| <b>Vespidae</b>                        |   |       |                         |        |
| <i>Eumenis</i> sp.                     | -   | -     | 15                      | 2.28   |
| <i>Polites</i> sp.                     | -   | -     | 8                       | 1.27   |
| <i>Vespa affinis</i>                   | 1   | 0.59  | 15                      | 2.28   |
| <i>V. tropica</i>                      | -   | -     | 10                      | 1.51   |
| Total of individuals                   | 170   | 100   | 661                     | 100.00 |
| Total of species                       | 5   |       | 11                      |        |
| Species diversity index ( $H'$ )       | 0.79  |       | 0.98                    |        |

<sup>1</sup> Insect pollinators visited on both female and male flowers of physic nuts

<sup>2</sup> Individual of insect pollinators/10 rais; 1 rai=1,600 m<sup>2</sup>

<sup>3</sup> Wet season during April-September 2009

<sup>4</sup> Dry season during October 2008-March 2009

**Appendix Table 18** Number of insect pollinators in five directions of plant canopy of *Jathapha curcas* L. in toxic variety, every hour during 07.00- 18.00 h, on October 2008 at Kamphaeng Saen (KPS) location

| Number of insect pollinators (individuals) |                            |                  |                  |                 |                 |                    |
|--|----------------------------|------------------|------------------|-----------------|-----------------|--------------------|
| Times                                      | Directions of plant canopy |                  |                  |                 |                 | Total <sup>1</sup> |
|  | East                       | North            | West             | South           | Central         |                    |
| 07.00-08.00h                               | 15                         | 3                | 2                | 5               | 0               | 8 <sup>d</sup>     |
| 08.00-09.00h                               | 12                         | 7                | 2                | 6               | 5               | 18 <sup>c</sup>    |
| 09.00-10.00h                               | 5                          | 6                | 11               | 7               | 4               | 17 <sup>c</sup>    |
| 10.00-11.00h                               | 14                         | 12               | 10               | 9               | 6               | 27 <sup>bc</sup>   |
| 11.00-12.00h                               | 11                         | 17               | 4                | 8               | 5               | 30 <sup>bc</sup>   |
| 12.00-13.00h                               | 9                          | 11               | 4                | 9               | 5               | 25 <sup>bc</sup>   |
| 13.00-14.00h                               | 17                         | 19               | 9                | 11              | 12              | 42 <sup>a</sup>    |
| 14.00-15.00h                               | 10                         | 12               | 10               | 13              | 4               | 29 <sup>bc</sup>   |
| 15.00-16.00h                               | 12                         | 10               | 16               | 14              | 8               | 32 <sup>b</sup>    |
| 16.00-17.00h                               | 8                          | 8                | 18               | 11              | 10              | 29 <sup>bc</sup>   |
| 17.00-18.00h                               | 1                          | 1                | 13               | 2               | 3               | 6 <sup>d</sup>     |
| Total <sup>2</sup>                         | 114 <sup>a</sup>           | 106 <sup>b</sup> | 99 <sup>bc</sup> | 95 <sup>c</sup> | 62 <sup>d</sup> | 263                |

<sup>1</sup> Number of insect pollinators was to be significantly different in every hour during 07.00-17.00 h (df = 32, p = 0.05).

<sup>2</sup> Number of insect pollinators were to be significantly different among five directions of plant canopy (df = 14, p = 0.05).

**Appendix Table 19** Percentage of Hymenopteran in five positions of *Jathapha curcas* L. plot in toxic variety on October 2008 at Kamphaeng Saen (KPS) location

| Position of area | Flower (%) |       | Hymenopteran visiting on flowers (%) |       |
|------------------|------------|-------|--------------------------------------|-------|
|                  | Female     | Male  | Female                               | Male  |
| Northeast        | 21.80      | 19.90 | 25.00                                | 21.21 |
| Northwest        | 17.29      | 20.15 | -                                    | -     |
| Central          | 18.04      | 20.11 | 75.00                                | 27.27 |
| Southeast        | 12.78      | 20.40 | -                                    | 24.24 |
| Southwest        | 30.09      | 19.44 | -                                    | 27.28 |
| Total            | 100        | 100   | 100                                  | 100   |

**Appendix Table 20** Abundance of insect pollinators on *Jatropha curcas* L. in toxic variety during March to June 2007 at Kamphaeng Saen (KPS) location

| Specie of insect pollinators   | Abundance of insect pollinators (%) |        |        |        |
|--------------------------------|-------------------------------------|--------|--------|--------|
|                                | March                               | April  | May    | June   |
| <i>Apis cerana indica</i>      | 0.41                                | 4.00   | 27.00  | 31.58  |
| <i>Apis dorsata</i>            | 0.43                                | -      | -      | -      |
| <i>Apis florea</i>             | 89.84                               | 81.00  | 60.00  | 10.53  |
| <i>Crematogaster</i> sp.       | 4.54                                | 13.00  | -      | 21.05  |
| <i>Chrysomyia megacephala</i>  | 0.42                                | -      | 9.00   | 21.05  |
| <i>Eristaris obscuritarsis</i> | 4.36                                | -      | 1.00   | -      |
| <i>Helophilus bengaliensis</i> | -                                   | -      | 1.00   | -      |
| <i>Rhingia</i> sp.             | -                                   | 2.00   | -      | 15.79  |
| <i>Ateva</i> sp.               | -                                   | -      | 2.00   | -      |
| Total                          | 100.00                              | 100.00 | 100.00 | 100.00 |
| Total of species               | 6.00                                | 4.00   | 6.00   | 5.00   |
| Maximum air temperature (°C)   | 36.20                               | 35.90  | 32.00  | 34.00  |
| Minimum air temperature (°C)   | 20.50                               | 22.70  | 27.40  | 23.90  |
| Accumulative rainfall (mm)     | 16.90                               | 151.30 | 206.90 | 234.20 |
| Average relative humidity (%)  | 66.50                               | 70.50  | 81.50  | 72.50  |

**Appendix Table 21** Number, preference and species ratio of ants on *Jatropha curcas* L. flowers during 10.00-13.00 h on December 2008 at Kamphaeng Saen (KPS) location, at 18.1 °C, 0.1 mm of accumulative rainfall, and 72% RH

| Species of ants                      | Number of ants<br>(individuals) on flowers |                 |       | Preference of<br>visitors on<br>flowers (%) |                 | Species ratio of<br>ants on flowers<br>(%) |                 |
|--------------------------------------|--|-----------------|-------|---|-----------------|--|-----------------|
|                                      | Female<br>flowers                          | Male<br>flowers | Total | Female<br>flowers                           | Male<br>flowers | Female<br>flowers                          | Male<br>flowers |
|                                      | <i>Camponotus</i> sp.1                     | 5               | 12    | 17  | 29.41           | 70.59                                      | 3.68            |
| <i>Camponotus</i> sp.2               | 4  | 14              | 18    | 22.22                                       | 77.78           | 2.94                                       | 2.21            |
| <i>Iridomyrmex</i> sp.               | 2  | 10              | 12    | 16.67                                       | 83.33           | 1.47                                       | 1.58            |
| <i>Meranoplus</i> sp.                | 3  | 6               | 9     | 33.33                                       | 66.67           | 2.21                                       | 0.95            |
| <i>Monomorium</i> sp.1               | 10   | 20              | 30    | 33.33                                       | 66.67           | 7.35                                       | 3.15            |
| <i>Monomorium</i> sp.2               | 5  | 23              | 28    | 17.86                                       | 82.14           | 3.68                                       | 3.63            |
| <i>Ochetellus</i> sp.1               | 12   | 26              | 38    | 31.58                                       | 68.42           | 8.82                                       | 4.10            |
| <i>Ochetellus</i> sp.2               | 8  | 15              | 23    | 34.78                                       | 65.22           | 5.88                                       | 2.37            |
| <i>Paratrechina</i> sp.1             | 25   | 155             | 180   | 13.89                                       | 86.11           | 18.38                                      | 24.45           |
| <i>Paratrechina</i> sp.2             | 12   | 23              | 35    | 34.29                                       | 65.71           | 8.82                                       | 3.63            |
| <i>Paratrechina</i> sp.3             | 2  | 8               | 10    | 20.00                                       | 80.00           | 1.47                                       | 1.26            |
| <i>Solenopsis</i><br><i>geminata</i> | 48   | 322             | 370   | 12.97                                       | 87.03           | 35.29                                      | 50.79           |
| Total of ant                         | 136  | 634             | 770   | 17.66                                       | 82.34           | 100  | 100             |
| Total of flowers                     | 12   | 496             | 508   | 2.36  | 97.64           | -  | -               |

**Appendix Table 22** Dwarf bees (*Apis florea*) preference on visiting of *Jatropha curcas* L., female or male flowers in toxic variety of during 08.00-19.00 h on April 18<sup>th</sup> 2007 at Kamphaeng Saen (KPS) location, at 30.85 °C, 6.0 mm of accumulative rainfall, and 64% RH

| Times         | Average number of dwarf bee visiting/hour (individuals) |                     |
|---------------|---|---------------------|
|               | Female  | Male                |
| 08.00-09.00 h | 18.95 <sup>de</sup>                                     | 8.70 <sup>de</sup>  |
| 09.00-10.00 h | 18.95 <sup>de</sup>                                     | 12.00 <sup>de</sup> |
| 10.00-11.00 h | 25.58 <sup>cd</sup>                                     | 22.20 <sup>d</sup>  |
| 11.00-12.00 h | 22.58 <sup>cd</sup>                                     | 50.70 <sup>c</sup>  |
| 12.00-13.00 h | 70.10 <sup>a</sup>                                      | 57.30 <sup>bc</sup> |
| 13.00-14.00 h | 69.79 <sup>a</sup>                                      | 72.32 <sup>ab</sup> |
| 14.00-15.00 h | 66.95 <sup>a</sup>                                      | 84.40 <sup>a</sup>  |
| 15.00-16.00 h | 60.63 <sup>a</sup>                                      | 54.00 <sup>c</sup>  |
| 16.00-17.00 h | 55.58 <sup>ab</sup>                                     | 50.80 <sup>c</sup>  |
| 17.00-18.00 h | 42.32 <sup>bc</sup>                                     | 24.00 <sup>d</sup>  |
| 18.00-19.00 h | 7.89 <sup>e</sup>                                       | 1.29 <sup>e</sup>   |

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**Appendix Table 23** Nectar concentration in the honey stomach of dwarf bees (*Apis florea*) visited on *Jatropha curcas* L. flowers during 07.00-18.00 h, on April 19<sup>th</sup> 2007 at Kamphaeng Saen (KPS) location, at 29.7 °C, 6.4 mm of accumulative rainfall, and 75.5% RH

| Times         | Average of nectar concentration (% Brix)<br>from honey bees stomach |
|---------------|---|
| 07.00-19.00 h | 20.00 <sup>c</sup>  |
| 09.00-11.00 h | 30.67 <sup>b</sup>  |
| 11.00-13.00 h | 28.00 <sup>b</sup>  |
| 13.00-15.00 h | 37.67 <sup>a</sup>  |
| 15.00-17.00 h | 41.33 <sup>a</sup>  |
| 17.00-18.00 h | 38.67 <sup>a</sup>  |

**Appendix Table 24** The preference of the Hymenopteran visiting on female and male *Jatropha curcas* L. flowers during 10.00-13.00 h on December 2008 at Kamphaeng Saen (KPS) location, At 18.1 °C, 0.1 mm of accumulative rainfall, and 72% RH

| Species of Insect<br>Pollinators | Female flowers      |          |                                      | Male flowers        |          |                                      |
|----------------------------------|---------------------|----------|--------------------------------------|---------------------|----------|--------------------------------------|
|                                  | Number <sup>1</sup> |          | Number                               | Number <sup>1</sup> |          | Number                               |
|                                  | Flowers             | Visitors | Visitors/100<br>flowers <sup>2</sup> | Flowers             | Visitors | Visitors/100<br>flowers <sup>2</sup> |
| Ants (12 spp.)                   | 77                  | 79       | 103                                  | 306                 | 184      | 60                                   |
| <i>Apis cerana indica</i>        | 3                   | 2        | 67                                   | 25                  | 4        | 16                                   |
| <i>A. mellifera</i>              |                     |          |                                      |                     |          |                                      |
| <i>ligustica</i>                 | 1                   | 1        | 100                                  | 77                  | 7        | 9                                    |
| <i>Trigona laeviceps</i>         | 16                  | 4        | 25                                   | 89                  | 6        | 7                                    |
| <i>T. pagdeni</i>                | 10                  | 3        | 30                                   | 53                  | 4        | 8                                    |

<sup>1</sup> Number (individuals) of observed flowers, and visited flowers by bees and ants, in each female and male flowers.

<sup>2</sup> Percentage of visitors on 100 flowers in each female and male flowers were calculated and approximated the individuals of visitors.

**Appendix Table 25** Bees speed visiting on flowers/minute of *Jatropha curcas* L. during 10.00-13.00 h on February 2009 at Kamphaeng Saen (KPS) location, at 21.8 °C, no rainfall, and 67.5% RH

| No. individual of bees    | Number of flowers/minute |                    |                     |                   |
|---------------------------|--------------------------|--------------------|---------------------|-------------------|
|                           | <i>Apis cerana</i>       |                    | <i>A. mellifera</i> | <i>Trigona</i>    |
|                           | <i>indica</i>            | <i>A. florea</i>   | <i>ligustica</i>    | <i>pagdeni</i>    |
| 1                         | 14                       | 14                 | 15                  | 10                |
| 2                         | 21                       | 9                  | 26                  | 9                 |
| 3                         | 12                       | 10                 | 11                  | 9                 |
| 4                         | 14                       | 12                 | 18                  | 11                |
| 5                         | 11                       | 8                  | 12                  | 10                |
| 6                         | 11                       | 12                 | 15                  | 13                |
| 7                         | 14                       | 17                 | 19                  | 6                 |
| 8                         | 14                       | 10                 | 14                  | 7                 |
| 9                         | 15                       | 8                  | 16                  | 7                 |
| 10                        | 15                       | 7                  | 18                  | 14                |
| Average <sup>1</sup>      | 14.10 <sup>a</sup>       | 10.70 <sup>b</sup> | 16.40 <sup>a</sup>  | 9.60 <sup>b</sup> |
| Average size of bees (mm) |                          |                    | 15 <sup>a</sup>     |                   |
| <sup>2</sup>              | 12 <sup>ab</sup>         | 10 <sup>b</sup>    |                     | 4 <sup>c</sup>    |

<sup>1</sup> Number of bees were to be significantly different among four species (df = 39, p = 0.05).

<sup>2</sup> Size of bees just measure from the tip of its head, ignoring the antennae, to the end of its abdomen.

**Appendix Table 26** Number of foragers of *Apis cerana indica*, and *A. mellifera ligustica* during 07.00-18.00 h on November 21<sup>st</sup> 2008 at Kamphaeng Saen (KPS) location, at 20.2°C, no accumulative rainfall, and 75.5% RH

| Times                  | Number of foragers (individual) |        |                                 |        |
|------------------------|---------------------------------|--------|---------------------------------|--------|
|                        | <i>Apis cerana indica</i>       |        | <i>Apis mellifera ligustica</i> |        |
|                        | Nectar                          | Pollen | Nectar                          | Pollen |
| 07.00-08.00 h          | 61                              | 7      | 56                              | 6      |
| 08.00-09.00 h          | 33                              | 20     | 29                              | 15     |
| 09.00-10.00 h          | 12                              | 27     | 10                              | 25     |
| 10.00-11.00 h          | 3                               | 9      | 2                               | 7      |
| 11.00-12.00 h          | 1                               | 5      | -                               | -      |
| 12.00-13.00 h          | -                               | -      | -                               | -      |
| 13.00-14.00 h          | 2                               | 1      | 1                               | -      |
| 14.00-15.00 h          | -                               | -      | -                               | -      |
| 15.00-16.00 h          | 1                               | -      | 1                               | -      |
| 16.00-17.00 h          | 32                              | -      | 28                              | -      |
| 17.00-18.00 h          | 16                              | -      | 12                              | -      |
| Total (individuals)    | 161                             | 69     | 139                             | 53     |
| Number of foragers (%) | 70                              | 30     | 72                              | 28     |

**Appendix Table 27** Number of pollen (9 species of plants) foragers of *Apis cerana indica* and *A. mellifera ligustica*, at the hive entrances every 2 hours during 08.30-16.30 h on November 22<sup>nd</sup> 2008 at Kamphaeng Saen (KPS) location, at 20.7°C, no accumulative rainfall, and 75.5%RH

| Families        | Species of plants            | Numbers of pollen foragers |      |                       |      |
|-----------------|------------------------------|----------------------------|------|-----------------------|------|
|                 |                              | <i>Apis cerana</i>         |      | <i>Apis mellifera</i> |      |
|                 |                              | <i>indica</i>              |      | <i>ligustica</i>      |      |
|                 |                              | Individual                 | %    | Individual            | %    |
| Asteraceae      | <i>Tithonia diversifolia</i> | -                          | -    | 2                     | 5.0  |
| Asteraceae      | <i>Tridax procumbens</i>     | 1                          | 2.5  | 3                     | 7.5  |
| Caesalpinoideae | <i>Delonix regia</i>         | 8                          | 20.0 | 3                     | 7.5  |
| Convolvulaceae  | <i>Ipomoea aquatic</i>       | 1                          | 2.5  | 2                     | 5.0  |
| Mimosaceae      | <i>Mimosa pudica</i>         | 4                          | 10.0 | 6                     | 15.0 |
| Mimosaceae      | <i>Leuceana</i>              |                            |      |                       |      |
|                 | <i>leucocephala</i>          | 8                          | 20.0 | 6                     | 15.0 |
| Mimosaceae      | <i>Leuceana</i> sp.          | 2                          | 5.0  | 2                     | 5.0  |
| Poaceae         | <i>Zea mays</i>              | 9                          | 22.5 | 9                     | 22.5 |
| Poaceae         | <i>Saccharum sinense</i>     | 7                          | 17.5 | 7                     | 17.5 |
| Total           |                              | 40                         | 40   | 100                   | 100  |

**Appendix Table 28** Effect of various botanical factors to weight of fruit and seed, and oil content during June to August 2009 at Agriculture Research and Development Center (ARDC) location

| Treatment <sup>1</sup> | Fruit of weight (g) <sup>2</sup> | Weight of seed (g) |      |        | Moisture (%) | Kernel (%) | Oil content (%) <sup>2</sup> |
|------------------------|----------------------------------|--------------------|------|--------|--------------|------------|------------------------------|
|                        |                                  | Fresh              | Dry  | Kernel |              |            |                              |
| YfOpFf                 | 10.52 <sup>a</sup>               | 0.936              | 0.54 | 0.39   | 42.84        | 72.71      | 42.8 <sup>a</sup>            |
| YfHpFf                 | 8.83 <sup>b</sup>                | 0.878              | 0.48 | 0.26   | 45.33        | 54.79      | 39.0 <sup>a</sup>            |
| YfOpPf                 | 8.03 <sup>b</sup>                | 0.98               | 0.64 | 0.40   | 34.90        | 62.38      | 34.0 <sup>b</sup>            |
| BfOpFf                 | 3.02 <sup>c</sup>                | -                  | 0.65 | 0.39   | -            | 59.63      | 26.4 <sup>c</sup>            |

<sup>1</sup> YfOpFf = control, yellow fruit, open-pollination, on female flower

YfHpFf = yellow fruit, hand-pollination, stigma pollen A-grade, on female flower

YfOpPf = yellow fruit, open-pollination, on perfect flower

BfOpFf = black fruit, open-pollination, on female flower

<sup>2</sup> Fruit weight and percentage of oil contents were to be significantly different among variable pollination experiments (df = 11, p = 0.05)

**Appendix Table 29** Effect of self- and cross-pollination to weight of fruit and seed, and oil content of *Jatropha curcas* L. during November 2009 to January 2010 at Aviation School (AS) location

| Treatment <sup>1</sup> | Fruit Weight (g) <sup>2</sup> | Weight of seed (g) |      |        | Moisture (%) | Kernel (%) | Oil content (%) <sup>2</sup> |
|------------------------|-------------------------------|--------------------|------|--------|--------------|------------|------------------------------|
|                        |                               | Fresh              | Dry  | Kernel |              |            |                              |
| CrHpFf                 | 4.13 <sup>b</sup>             | 0.89               | 0.67 | 0.42   | 25.31        | 62.52      | 28.00 <sup>ab</sup>          |
| OpFf                   | 2.73 <sup>c</sup>             | 0.75               | 0.74 | 0.48   | 1.99         | 65.31      | 34.40 <sup>a</sup>           |
| OpPf                   | 7.57 <sup>a</sup>             | 0.93               | 0.60 | 0.39   | 35.81        | 65.33      | 15.00 <sup>c</sup>           |
| SHpOFF                 | 2.86 <sup>c</sup>             | 0.67               | 0.47 | 0.28   | 30.80        | 60.86      | 32.72 <sup>a</sup>           |
| SHpIFf                 | 2.52 <sup>c</sup>             | 0.62               | 0.55 | 0.34   | 10.08        | 61.48      | 26.50 <sup>b</sup>           |

<sup>1</sup> OpFf = open- pollination, on female flower

SHpOFF = self-hand-pollination, outside plant, same clone, on female flower

SHpIFf = self-hand-pollination, inside plant, on female flower

CrHpFf = cross-hand-pollination, on female flower

OpPf = open-pollination, on perfect flower

<sup>2</sup> Fruit weight and percentage of oil contents were to be significantly different between self- and cross-pollination (df = 11, p = 0.05)

**Appendix Table 30** Amount of pollen grains percentage on *Jatropha curcas* L. flowers in different level, were visited by ants and four bees observed during 17.00-18.00 h on November 2008 at Kamphaeng Saen (KPS) location

| Percentage of amount of pollen grains in different visitors | Grades of pollen on stigma <sup>1</sup> |      |       |       | Total |
|---|---|------|-------|-------|-------|
|   | A                                       | B    | C     | F     |       |
| Ants  | -                                       | 4.50 | 34.00 | 61.50 | 100   |
| <i>Apis cerana indica</i>                                   | 14.79                                   | 6.92 | 37.12 | 41.17 | 100   |
| <i>Apis mellifera ligustica</i>                             | 25.00                                   | 8.33 | 41.67 | 25.00 | 100   |
| <i>Trigona laeviceps</i>                                    | -                                       | 7.14 | 55.36 | 37.50 | 100   |
| <i>Trigona pagdeni</i>                                      | 2.51                                    | 5.53 | 46.54 | 45.42 | 100   |
| Mean  | 8.46                                    | 6.48 | 42.94 | 42.12 | 100   |

<sup>1</sup> A-grade > 1,500 pollen grains/stigma

B-grade = 300-1,500 pollen grains/stigma

C-grade < 300 pollen grains/stigma

F-grade is none pollen grain on stigma.

<sup>2</sup> Percentage of pollen grains grades were to be significantly different among ants and four bees ( $p = 0.05$ ).

**Appendix Table 31** Fruit sets percentage after female flowers of *Jatropha curcas* L. visited by various Hymenopteran pollinators, weight of dry seed and kernel (g), and oil content (%) during November to December 2008 at Kamphaeng Saen (KPS) location

| Species of<br>Hymenoptera<br>pollinators | Number<br>(individuals) |               | Fruit<br>set<br>(%) <sup>1</sup> | Weight of seed<br>(g) |        | Kernel<br>(%) | Oil<br>content<br>(%) |
|--|-------------------------|---------------|----------------------------------|-----------------------|--------|---------------|-----------------------|
|  | Female<br>flowers       | Fruit<br>sets |                                  | Dry                   | Kernel |               |                       |
|  | Ants                    | 169           | 160                              | 94.67                 | 0.76   | 0.49          | 63.89                 |
| <i>Apis cerana indica</i>                | 160                     | 152           | 95.00                            | 0.66                  | 0.42   | 64.47         | 34.65                 |
| <i>A. mellifera ligustica</i>            | 58                      | 55            | 94.83                            | 0.71                  | 0.45   | 63.64         | 38.23                 |
| <i>Trigona laeviceps</i>                 | 164                     | 155           | 94.51                            | 0.77                  | 0.49   | 63.64         | 38.15                 |
| <i>Trigona pagdeni</i>                   | 146                     | 138           | 94.52                            | 0.72                  | 0.46   | 63.38         | 34.98                 |

<sup>1</sup> Percentage of fruit set were to be non significantly different among ants and four bees.

**Appendix Table 32** Effect of various pollination methods to weight of fruit and seed, and oil content of *Jatropha curcas* L. during September to November in 2009 at Kamphaeng Saen (KPS) location

| Pollination methods                       | Fresh fruit (g) <sup>1</sup> | Weight of seed (g) |      |        | Moisture (%) | Kernel (%) | Oil content (%) <sup>1</sup> |
|---|------------------------------|--------------------|------|--------|--------------|------------|------------------------------|
|   |                              | Fresh              | Dry  | Kernel |              |            |                              |
| Full pollinated by <i>Trigona pagdeni</i> | 5.99 <sup>cd</sup>           | 1.03               | 0.69 | 0.43   | 33.01        | 62.32      | 40.03 <sup>a</sup>           |
| Full pollinated by ants                   | 4.69 <sup>d</sup>            | 0.70               | 0.47 | 0.40   | 32.86        | 85.11      | 26.42 <sup>b</sup>           |
| Full pollinated by <i>Apis florea</i>     | 6.95 <sup>c</sup>            | 0.90               | 0.68 | 0.44   | 24.44        | 64.71      | 41.28 <sup>a</sup>           |
| One time visited by <i>Apis florea</i>    | 6.23 <sup>c</sup>            | 0.90               | 0.69 | 0.45   | 23.33        | 65.22      | 39.70 <sup>ab</sup>          |
| Hand-pollinated (A-grade)                 | 11.58 <sup>a</sup>           | 1.01               | 0.70 | 0.43   | 30.69        | 61.43      | 41.20 <sup>a</sup>           |
| Open pollinated                           | 8.20 <sup>b</sup>            | 1.04               | 0.62 | 0.42   | 40.38        | 67.74      | 25.87 <sup>b</sup>           |

<sup>1</sup> Weight of fresh fruit (g) and percentage of oil contents were to be significantly different among variable pollination method (df = 17, p = 0.05).

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