

Diversity, Distribution and Behavior of Bee Pollinators (Hymenoptera: Apoidea) Effect the Amount of Pollen Grains on Physic Nut (*Jatropha curcas* L.) in Thailand

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ABSTRACT: A survey was made on the diversity and distribution of bee pollinators visiting physic nut (*Jatropha curcas* L.) flowers, and on flower sex variation, covering sample plantation sites in 20 provinces in Thailand from April to August, 2009. Sixty-one species of bee in the superfamily Apoidea were found visiting flowers of the physic nut. The Indian honey bee (*Apis cerana indica* F.), in the social bees, had the most widespread distribution, followed by the dwarf honey bee (*A. florea* F.), the giant honey bee (*A. dorsata* F.), and a stingless bee (*Tetragonula pagdeni* Schwarz). Bee pollinators preferred female to male flowers. Physic nut flowers in all locations were mainly pollinated by bees. Physic nut plants were found to be androgynous, polygamous and/or gynoeceious. A single gynoeceious plant, producing only female flowers, was observed, which was the first such plant to be reported in Thailand. In the wet season, the flower sex ratio among the 5 regions was significantly different. The ratios in the south, north and east regions (1 female per 10.00 ± 1.38 , 10.33 ± 3.76 and 10.50 ± 1.10 male flowers, respectively) were higher than those in the central and northeast regions (1 female per 13.67 ± 7.80 and 15.00 ± 5.51 male flowers). The polygamous plants in Suphan Buri province exceptionally showed 9 females: 23 males: 7 perfect flowers in non-toxic variety.

KEY WORDS: diversity, distribution, bee pollinators, Apoidea, physic nut

INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a small tree or large shrub of the family Euphorbiaceae. Non-edible oil of

physic nut can be used for the production of biodiesel (Kumar and Sharma, 2005). Physic nut is generally monoecious with the female and male flowers on the same plant and in the same in-

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florescence. The number of female and male flowers per inflorescence varies widely among reported observations (1-19 female per 25-238 male) (Raju and Ezradanam, 2002; Bhattacharya *et al.*, 2005; Chang-wei *et al.*, 2007). Insect pollinators effect successful pollination of the physic nut flower, resulting in complete fruit setting, and in turn these pollinators take some food, such as nectar and pollen, from physic nut flowers (Delaplane and Mayer, 2000).

In Asia, there have been only limited investigations of the diversity of insect pollinators visiting the physic nut. In a pioneer work 13 species were reported in India by Raju and Ezradanam (2002). Later, Bhattacharya *et al.* (2005) also surveyed this plant in India but found just six species of insect pollinators. More recently more than 50 species of insect pollinators were observed in Thailand (Malaipan *et al.*, 2007), and Inson and Malaipan (2011) recorded that 311 insect species were found on the female flowers in 20 provinces of Thailand. Raju and Ezradanam (2002) reported that bees were numerous and effective pollinators on different species of plants. With regard to floral structure and adaptive features of visitors, it was stated that honey bees (*A. dorsata*, *A. florea* and *A. mellifera*) were effective pollinators. Vespid wasps (*Eumenes*, *Vespa*) and beetles are not considered to be effective pollinators as the structural and behavioral characteristics of these visitors do not match with physic nut flowers. The bees mostly collect-

ed floral rewards on different plants of physic nut. These bees were effective geitonogamous and xenogamous pollinators. Malaipan *et al.* (2007) suggested that insect pollinators were divided into two groups, major and minor. The major group consisted of social bees, blow fly and syrphid flies, and the minor group included other insects in Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Thysanoptera.

This study focuses on all the kinds of bees observed, with an aim to suggest how best to utilize them, because bees are the most common, important insect pollinators. The study also seeks to assess whether insect pollinators are adequate for physic nut pollination in each region of Thailand. The results will provide baseline data on bees in Thailand that can be used to monitor bee populations in physic nut plantations, and a guideline for both conservation strategies and pollination techniques in managing bees on physic nut flowers.

MATERIALS AND METHODS

Study sites

Surveys of insect pollinators and flowers were undertaken at physic nuts plantations in 20 provinces located in five major regions in Thailand as follows: 1) North region: Chiang Rai, Chiang Mai, Lamphun, Lampang, Phayao and Nan; 2) Central region: Kamphaeng Phet, Chai Nat, Suphan Buri and Nakhon Pathom; 3) Northeast region: Nakhon Ratchasima, Khon Kaen, Kalasin,

Maha Sarakham, Udon Thani and Sakon Nakhon; 4) East region: Chon Buri and Rayong; and 5) South region: Chumphon and Phang Nga. At each site, at least 100 m² was covered in sampling.

Sampling methods

The sampling methods followed Malaipan *et al.* (2007) and Kwaiser and Hendrix (2008). Twenty quadrants (each of 100 m²) were selected from a large area in each province. Pollinator behavior was observed, and the number and species of insects were recorded during the period 08.00-12.00 h, from April to August 2009. Insects not identified visually were caught with a sweep-net and later examined in the laboratory. These insects were identified to families, or species where possible. Identifications were performed by employing the taxonomic keys from Triplehorn and Johnson (2005), Michener (2000), Sakagami *et al.* (1985), Schwarz (1937, 1939), Tadauchi and Tasen (2009). All specimens were preserved and maintained in the insect museum of the Entomology Department, Kasetsart University, Bangkok, Thailand.

The sex ratio of 500 sample physic nut flowers in each province was recorded. The numbers of pollen grains on stigmas of the female flowers was counted at 17.00-18.00 h by taking photos, and the results were classified into four grade levels: A (>1,500 pollen grains/stigma), B (300-1,500), C (< 300), and F (very few or almost no pollen grains) (Figure 1).

Data analyses

The number of bee species per family was calculated and compared among Apoidea. The frequency of occurrence of bees was used to depict distribution patterns of bees and to identify major groups. The bee density (the number of bee foragers visiting 100 male and 100 female flowers), amount of pollen grains on stigmas and flower sex ratios were compared among 20 provinces in Thailand.

RESULTS AND DISCUSSION

Diversity of Apoidea

Hymenopteran specimens were collected from 20 provinces of Thailand during April to August 2009, amounting to 140 species, 47 genera and 11 families, namely Apidae (24 spp., 17.27%), Chrysididae (2 spp., 1.44%), Evaniidae (1 sp., 0.72%), Formicidae (18 spp., 12.95%), Halictidae (14 spp., 10.07%), Megachilidae (23 spp., 16.55%), Mutillidae (1 sp., 0.72%), Pompilidae (2 spp., 1.44%), Scoliidae (14 spp., 10.07%), Sphecidae (11 spp., 7.91%), and Vespidae (29 spp., 20.86%). Among the superfamily Apoidea, 61 species belonged to the Apiformes and 11 to the Spheciformes. Apiformes or true bees were chosen for analyses and discussion, while Spheciformes or wasps in Sphecidae *s. lato* such as *Chalybion bengalense*, *Chlorion lobatum*, *Chlorion* spp. (2 spp.), *Liris* sp., *Sceliphron javanum*, *Sphex argentatus*, *S. sericeus lineolus*, *S. viduatus*, *Sphex* spp. (2 spp.) (see Table 1) were omitted because of their minor role in pollination. Even

though adult sphecids gathered on floral nectar and extra-floral nectaries, 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).

Distribution of bees (Apiformes)

Sixty-one species of bees had different distributions among the 20 provinces. Among the social bees, the Indian honey bee (*A. cerana indica*) was found most frequently (11 provinces), followed by the dwarf honey bee (*A. florea*) (9), giant honey bee (*A. dorsata*) (7), and a stingless bee (*T. pagdeni*) (6). The other social bees were found in 1-5 provinces (see Table 2).

The relatively widespread occurrence on physic nut of several species of social bees is consistent with the results of Bhattacharya *et al.* (2005), who suggested that *A. dorsata*, *A. florea* and *A. mellifera* were the most common and effective pollinators. Of the 61 bee species, *A. cerana indica*, *A. florea*, *A. mellifera ligustica* and *T. pagdeni* would be the most appropriate target for more detailed studies to determine pollination efficiency in physic nut plantations.

Density of insect pollinators on physic nut flowers

The number of bee pollinators (Apiformes) visiting female and male physic nut flowers is shown in Figures 2 and 3. The range was 1-75 bees/100 female flowers (mean 30 bees) and 1-28

bees/100 male flowers (mean 6 bees). The highest number of bees per 100 female flowers was found in Phang Nga Province (75 bees), that per 100 male flowers was found in Nan Province (28 bees), while the lowest number was found in three provinces i.e. Sakon Nakhon, Udon Thani and Chai Nat (1 bee/100 female flowers), and Udon Thani (1 bee/100 male flowers). The number of bees was higher on female flowers than on male flowers.

Amount of pollen grains on stigmas of physic nut flowers The amount of pollen grains on stigmas was classified into four levels as mentioned in "Materials and Methods". Among the 2,000 stigmas observed, 44.0% belonged to the grade A, 37.1% to C, and 18.9% to B (Figure 3). Low bee densities on flowers in Sakon Nakhon (1 bee/100 female flowers, 2 bees/100 male flowers), Udon Thani (1 bee/100 female, 1 bee/100 male), Kalasin (3 bees/100 male), and Khon Kaen (25 bees/100 female, 2 bees/100 male) can explain the small amounts of pollen grains on stigmas in Sakon Nakhon (B=66.67%, C=33.33%), Udon Thani (B=50%, C=50%), Kalasin (C=100%), and Khon Kaen (C=100%).

Sex ratio variation in physic nuts

Ten-thousand flowers of physic nut plants in 20 provinces were classified into three categories i.e. androgynous, gynoeceous, and polygamous (Figure 4). Androgynous or monoecious plants have female and male flowers on the same plant and in the same in-

florescence; polygamous plants have female, male, and perfect (hermaphrodite) flowers in the same inflorescence; and gynoeceious plants have only female flowers. Only one plant of the last category was found in Nan province, representing a first reported case in Thailand; the plant produced only female or pistillate flowers in all inflorescences. Gynoeceious plants are caused by a mutation and are not attractive to insect pollinators, so usually no pollen grains are present on the stigma and no fertilization occurs. Because of the flowers of this type do not secrete nectar. If nectar is available various kinds of insect should be attracted. Fruits of pistillate flowers, if any, do not develop completely.

In the wet season, the flower sex ratio among the 5 regions differed significantly. The ratios were higher in the south, north and east regions (1 female per 10.00 ± 1.38 , 10.33 ± 3.76 and 10.50 ± 1.10 male flowers, respectively) than in the central and northeast (1 female per 13.67 ± 7.80 and 15.00 ± 5.51 male flowers) (Table 3). This difference may be caused by differences in cumulative rainfall. The south, north and east regions have higher annual cumulative rainfall (1,190.3-2,822.5 mm), while the central and northeast regions have lower annual cumulative rainfall (1,447.3-1,475.7 mm). In the same way, the flower sex ratio among 19 locations showed significant differences; the highest flower sex ratio (female: male) was found in Chiang Rai and Lamphun (1:6), followed by Kam-

phaeng Phet and Nakhon Pathom (1:9), Chiang Mai, Sakon Nakhon, Chon Buri, Chumphon and Phang Nga (1:10), Udon Thani and Rayong (1:11), Nan, Phayao and Maha Sarakham (1:12), Khon Kaen (1:15), Lampang and Kalasin (1:16), while the lowest flower sex ratios were found in Chai Nat (1:23) and Nakhon Ratchasima (1:26). Polygamous plants in Suphan Buri had 9 females: 23 males: 7 perfect flowers and some abnormal fruits.

CONCLUSIONS

Sixty-one species of Apiformes (bees) were indentified among the samples obtained in 20 provinces in Thailand. Indian honey bee (*A. cerana indica*) and small carpenter bee (*Ceratina* sp.1) were found in 11 provinces, showing the widest distribution among the bees. Among social bees, the Indian honey bee had the highest frequency of occurrence (11 provinces), followed by the dwarf honey bee (*A. florea*) (9 provinces), the giant honey bee (*A. dorsata*) (7 provinces), and a stingless bee (*T. pagdeni*) (6 provinces). Density of bee pollinators/100 physic nut flowers was found in great ranges (1-75 individuals/100 female flowers, and 1-28 individuals/100 male flowers). Bee density affected the amount of pollen grains on stigmas, and physic nut flowers were evidently pollinated by insect pollinators so that all stigmas were covered with pollen grains. There are three types of physic nut plants, namely androgynous, gynoeceious and polygamous plants; and three types of flow-

ers (female, male, and perfect flowers) occur. Flower sex ratios were found to be variable, 1:6-1:26 (female: male) at most locations, except an atypical flower sex ratio of 9: 23: 7 (female: male: perfect) was found in Suphan Buri. Different climatic conditions of the locations may have had some effect on the flower sex ratio and type of flowers.

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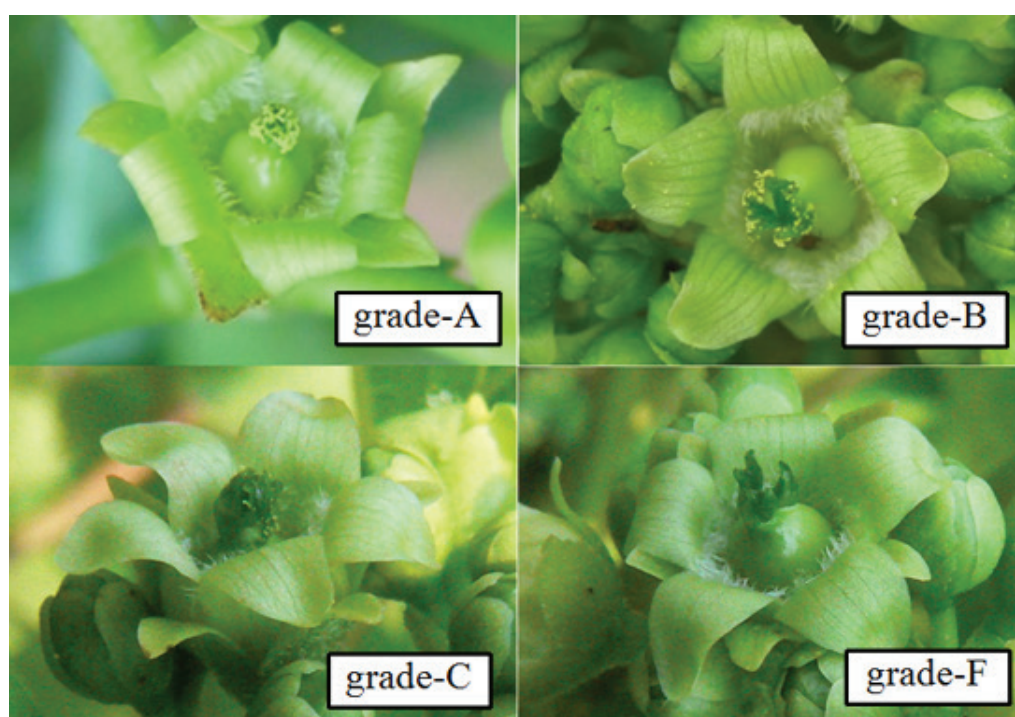


Figure 1. Evaluation of amount of pollen grains on stigmas of physic nut flowers during 17.00-18.00 h.

Remark: grade-A: > 1,500 pollen grains/stigma
 grade-B: 300-1,500 pollen grains/stigma
 grade-C: < 300 pollen grains/stigma
 grade-F means no pollen grain on stigma.
 Stigma of physic nut flower has three lobes.

Source: Malaipan *et al.* (2007)

Table 1. Species list of insect pollinators (Hymenoptera) on physic nut flowers in 20 provinces from April to August 2009.

Orders	Families	Scientific Name
Hymenoptera	Apidae	<i>Amegilla</i> sp., <i>Apis andreniformis</i> Smith, <i>A. cerana indica</i> - <i>F.</i> , <i>A. dorsata</i> F., <i>A. florea</i> F., <i>A. mellifera ligustica</i> L., <i>Ceratina</i> spp.(3 spp.), <i>Pithitis smaragdula</i> F., <i>Podalirius crocea</i> Bingham, <i>Thyreus</i> sp., <i>Lepidotrigona ventralis</i> Smith, <i>Tetragonilla collina</i> Smith, <i>Tetragonula laeviceps</i> Smith, <i>Tetragonula pagdeni</i> Schwarz, <i>Tetragonula</i> spp. (4 spp.), <i>Tetrigona melanoleuca</i> Cockerell, <i>Xylocopa aestuans</i> (L.), <i>X. collaris</i> Cockerell, <i>X. latipes</i> (Drury)
	Chrysididae	<i>Stilbum cyanurum</i> (Förster), <i>Stilbum</i> sp.
	Evaniidae	<i>Evania</i> sp.
	Formicidae	<i>Anoplolepis gracilipes</i> (Smith), <i>Camponotus</i> spp.(4spp.), <i>Iridomyrmex</i> sp., <i>Meranoplus</i> sp., <i>Monomorium</i> spp. (2 spp.), <i>Oecophylla smaragdina</i> F., <i>Nylanderia</i> spp. (3 spp.), <i>Soleopsis geminata</i> (Fabricius), <i>Tetraponera rufonigra</i> (Jerdon) Unidentified (3 spp.)
	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>Euaspid</i> spp. (2 spp.), <i>Lithurge</i> sp., <i>Megachile hera</i> Bingham, <i>M. disjuncta</i> (F.), <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.)
	Scoliidae	<i>Campsomeris collaris quadrifasciata</i> F., <i>Campsomeris phalerata</i> Saussure, <i>Liacos</i> sp., <i>Megascolia azurea rubiginosa</i> F., <i>Scolia quadripustulata humeralis</i> Saussure, <i>Scolia</i> spp. (4 spp.), Unidentified (5 spp.)
	Sphecidae(s. lato)	<i>Chalybion bengalense</i> (Dahlbom), <i>Chlorion lobatum</i> (F.), <i>Chlorion</i> spp. (2 spp.), <i>Liris</i> sp., <i>Sceliphron javanum</i> (Lepeletier), <i>Sphex argentatus</i> F., <i>Sphex sericeus lineolus</i> Lepelletier, <i>Sphex viduatus</i> Christ, <i>Sphex</i> spp. (2 spp.)
	Vespidae	<i>Apodynerus</i> sp., <i>Anterhynchium</i> sp., <i>Delta esuriens</i> (Fabricius), <i>Delta</i> spp. (5 spp.), <i>Eumenes conica</i> Fabricius, <i>Eumenes</i> spp.(3 spp.), <i>Phimenes</i> spp.(2 spp.), <i>Polistes stigma</i> (F.), <i>Polistes</i> spp.(5 spp.), <i>Rhynchium haemorrhoidale</i> (F.), <i>R. quinquecinctum</i> (F.), <i>Vespa affinis</i> (Linnaeus), <i>Vespa</i> spp. (6 spp.)

Table 2. Distribution of major social bees on physic nut flowers in 20 provinces from April to August 2009.

Sp. ¹	Twenty provinces in Thailand ²																				Total
	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	
<i>A. c.</i>	/	/	/	/	-	/	/	-	-	-	-	-	-	-	/	/	-	/	/	/	11
<i>A. d.</i>	-	/	/	-	-	/	-	-	-	-	-	-	/	/	/	-	-	/	-	-	7
<i>A. f.</i>	-	/	/	/	/	/	/	-	-	-	-	-	-	-	/	/	-	/	-	-	9
<i>A. m.</i>	/	-	-	-	/	-	-	-	-	-	-	-	-	-	/	/	-	-	-	-	4
<i>L. v.</i>	-	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>T. c.</i>	/	-	/	-	-	/	/	-	-	-	-	-	-	-	-	-	/	-	-	-	5
<i>T. l.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	/	-	-	-	1
<i>T. p.</i>	/	-	-	-	-	-	-	-	-	-	-	-	-	-	/	-	/	/	/	/	6
<i>T. m.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	/	-	1

¹ *A. c.* = *Apis cerana indica*, *A. d.* = *A. dorsata*, *A. f.* = *A. florea*, *A. m.* = *A. mellifera ligustica*, *L. v.* = *Lepidotrigona ventralis*, *T. c.* = *Tetragonilla collina*, *T. l.* = *Tetragonula laeviceps*, *T. p.* = *Tetragonula pagdeni*, *T. m.* = *Tetrigona melano-leuca*

² 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 of insect pollinators on physic nut flowers.

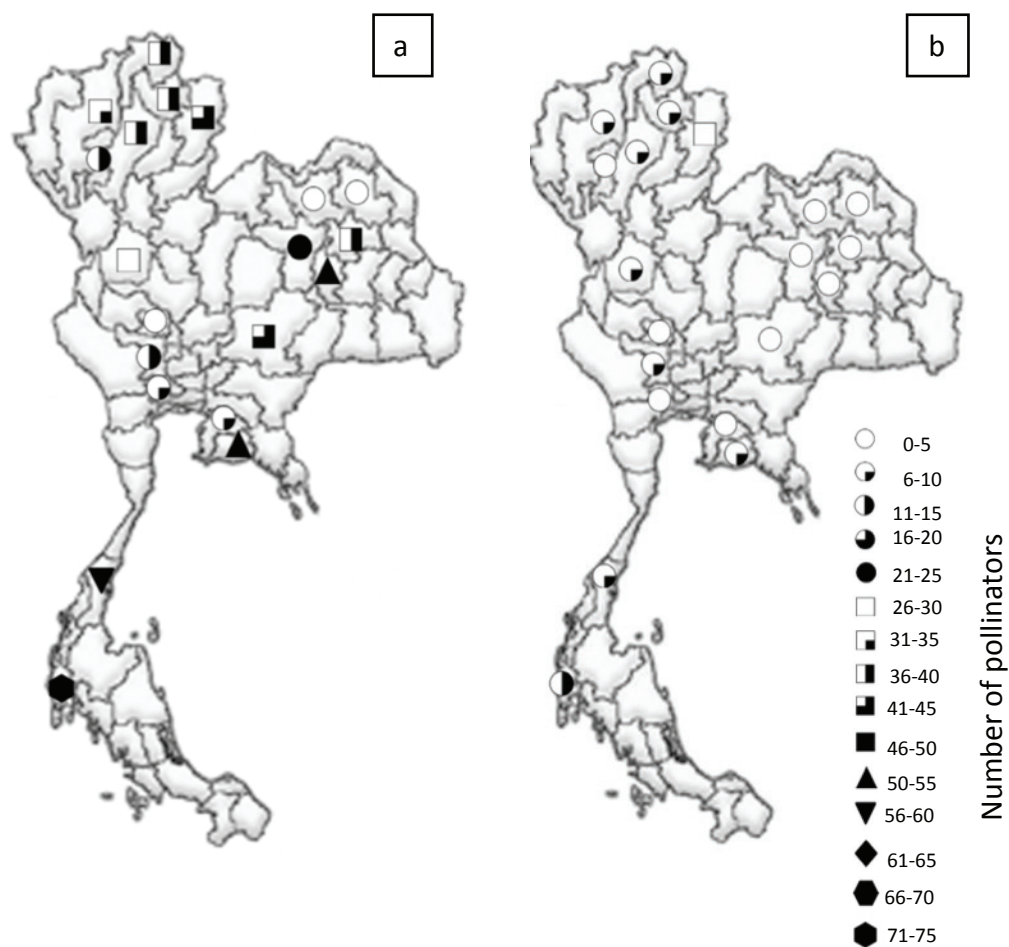


Figure 2. The density of bee pollinators (Apiformes) on physic nut flowers during 08.00-12.00 hrs in 20 provinces from April to August 2009.

Note: a, b = Numbers of bee pollinators (Apiformes) visited 100 female and 100 male flowers.

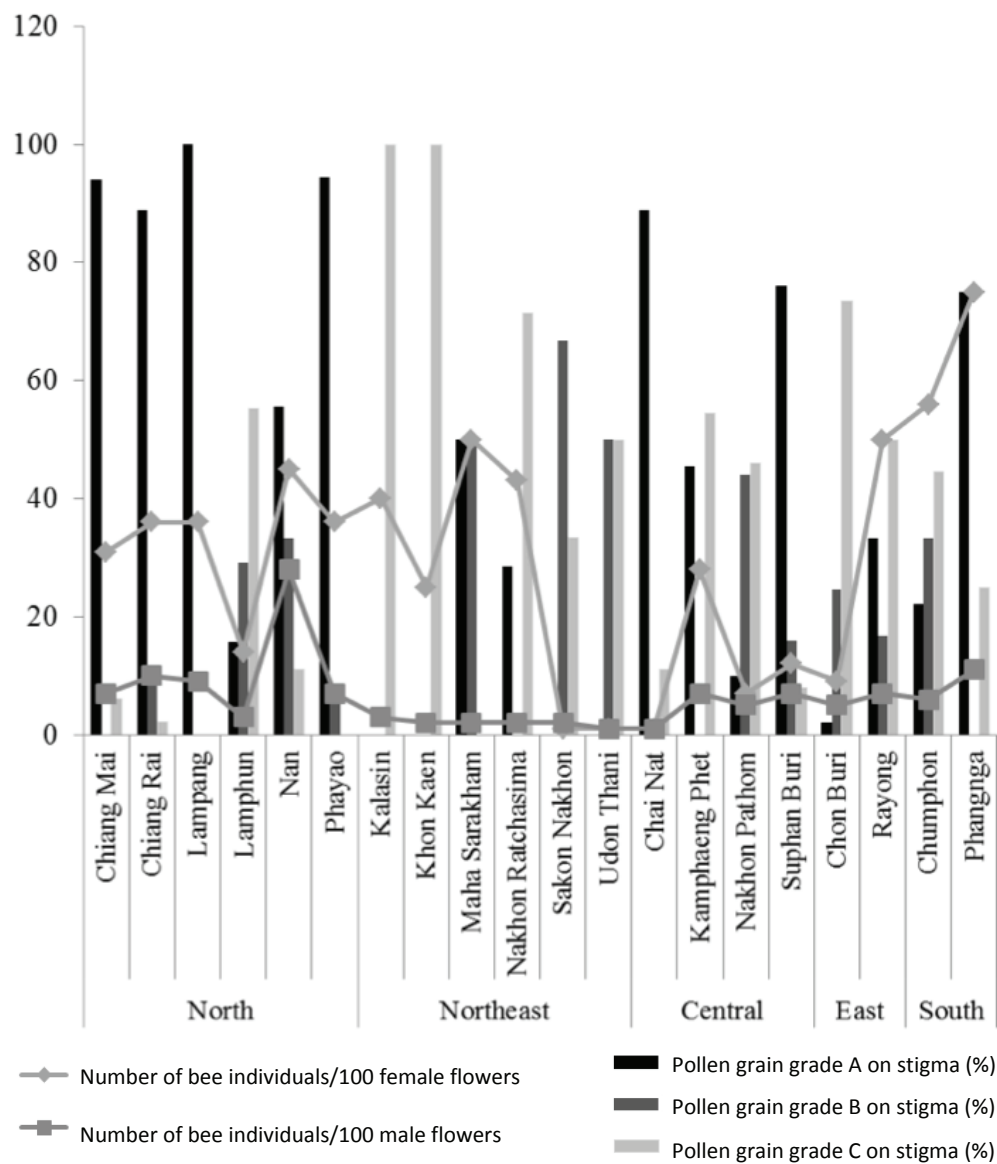
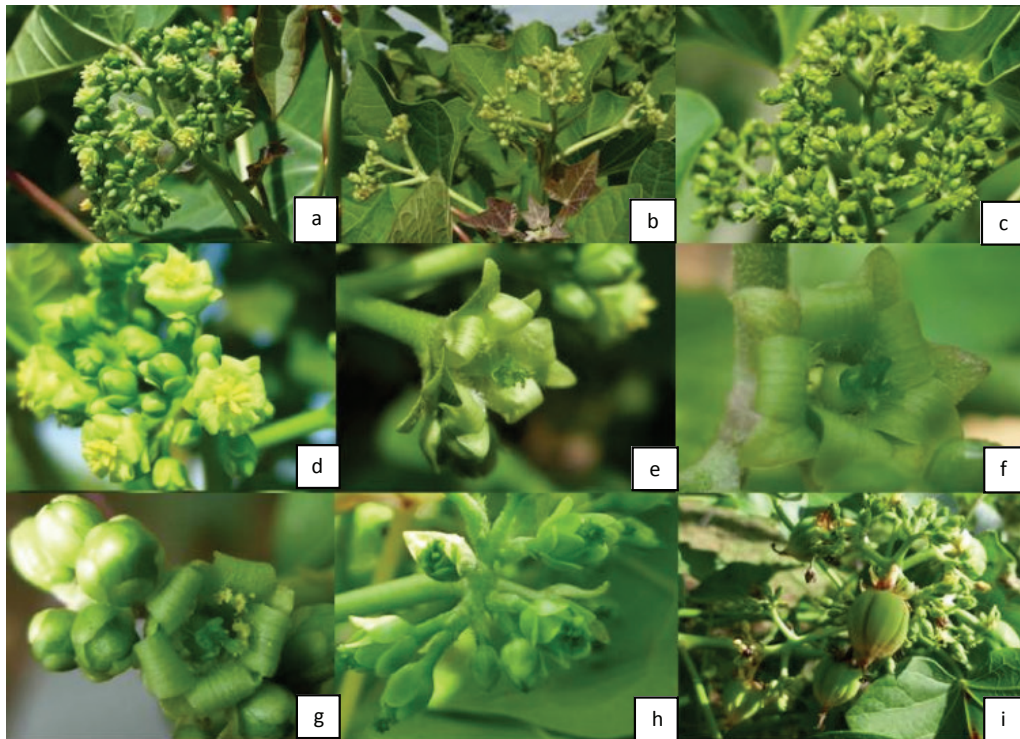


Figure 3. Relation between the density of bee pollinators (Apiformes) on flower and amount of pollen grains (%) on stigma of physic nut in 20 provinces from April to August 2009.

Table 3. Types and sex ratios of physic nut flowers in 5 regions from April to August 2009.

Thailand Regions	Average of flower sex ratio		
	female	male	perfect
North	1	$10.33^b \pm 3.76$	-
Northeast	1	$15.00^a \pm 5.51$	-
Central	1	$13.67^a \pm 6.80$	-
East	1	$10.50^b \pm 1.10$	-
South	1	$10.00^b \pm 1.38$	-
Suphan Buri (Central)	1	23	7

Note: 10,000 flowers were used to measure the sex ratio. Perfect flowers of the non-toxic variety were found only in Suphan Buri Province in Central Thailand.

**Figure 4.** Types of *Jatropha curcas* L. plants and flowers observed in 2009 at 20 provinces, Thailand.

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 cm

e = General female flower; diameter of female flowers is 0.9 cm

f, g = Variation in anther number on perfect flowers

h = Female flowers from gynoecious plant in Nan

i = Abnormal fruits from gynoecious plant