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Pollen Resources for the Honey Bee, *Apis mellifera* in Roi-Et Province, Thailand

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Abstract

The objective of this research was to investigated pollen resources for the honey bee, *Apis mellifera* based on pollen morphology analysis. The samples of bee pollen loads and pollen of flowering plants were collected during September – November 2014 in Pathumrat district, Roi-Et province. Acetolysis technique was used for pollen preparation. Pollen morphology analysis was investigated under light microscope (LM). The results showed 22 species of blooming flowers and 14 plant species found in bee pollen loads. There were 8 plant families found among 14 plant species from bee pollen loads, including (1) Arecaceae (2) Asteraceae (3) Bignoniaceae (4) Caricaceae (5) Fabaceae (6) Iridaceae (7) Moringaceae and (8) Rubiaceae. Five plant species can be identified after compared to pollen morphology of blooming flowers, including (1) *Carica papaya* (2) *Helianthus annuus* (3) *Ixora coccinea* (4) *Sesbania grandiflora* (5) *Sphagneticola trilobata* and 9 unknown species. The information from this research will benefit both beekeepers and in crop pollination. There were 2 economic plant species (*C. papaya* and *H. annuus*) found in bee pollen loads, which indicated that *A. mellifera* could be a good pollinator for these crop pollination.

Introduction

The honey bee, *Apis mellifera* plays an important role in the pollination of many crops. They show different preferences for flora surrounding the colony. The bee forages for nectar and pollen by visiting thousands of flowers on their way. During foraging, the bees participate in pollination of crops and do so in large numbers (Bhalchandra et al., 2014; Crane & Walker, 1984; Delaplane & Daniel, 2000; Dukku, 2013). Honey bees consume nectar for energy, whereas consume pollen for proteins, lipids, minerals and vitamins (Herbert & Shimanuki, 1978).

A. mellifera is an introduced species and has been imported to Thailand for research and the beekeeping industry since more than 70 years (Suppasat et al., 2007; Wongsiri et al., 2000). The beekeeping is distributed throughout Thailand, but mostly located in the northern part. This species is used for pollination of many economic crops such as longan, litchi, durian, rambutan and other crops (Suwannapong et al., 2011). The beekeepers need to build up strong bee colonies before honey harvesting season (February-April). The bees need a lot more pollen during build up colony. Food plants of the bees has been observed based on different methods (Hepburn & Radloff, 1998), including direct observation

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of foraging bees (Ayansola & Davies, 2012; Makong, 2016), analysis of pollen loads removed from returning foragers (Köppler et al., 2007; Sopaladawan & Phinyo, 2018; Sopaladawan & Sonyoha, 2018; Sopaladawan et al., 2019), analysis of pollen stores in nests or hives (Ramanujam & Kalpana, 1992), palynological analysis of honey (Adekanmbi & Ogundipe, 2009) and pollen DNA barcoding (Bell et al., 2016).

This research investigated pollen resources for the bees, *A. mellifera* during colony build up period using morphological analysis on pollen from pollen loads of returning foragers. The results could provide information for beekeepers in choosing suitable area for beekeeping, and also benefit on potential economic crop pollination by bees.

Fig. 1 Bee pollen loads collection using pollen trap

Materials and methods

1. Pollen collection

Pollen samples were collected from 2 different sources; (1) bee pollen loads and (2) pollen from blooming flowers. Both types of pollen were sampled once a month during colony built-up time in September-November 2014. Bee pollen were collected from 3 colonies of *A. mellifera* located at a local area of Pathumrat district, Roi-Et province (15º39´57.9´´ N, 103º25´45.9´´ E). A pollen trap was placed at the hive entrances of each colony (Fig. 1) at 7.00-11.00 am (Bhalchandra et al., 2014). Pollen loads from the bees were kept separately in plastic tubes at 4ºC for pollen identification. According to identify plant species from bee pollen, pollen from blooming flowers at the same area were also collected. This type of pollen was used as references of plant species from bee pollen loads.

2. Pollen preparation for pollen morphology analysis

Acetolysis method (Erdtman, 1966) was used for pollen morphology analysis, both from the bees and flowers. Firstly, pollen from bee pollen loads were separated by color and kept in 1.5 ml microcentrifuge tubes. Then, 10% KOH was added into the tube until it covered the pollen sample, the purpose of this step was to remove debris from the surface of pollen grains. The suspension was boiled in a water bath for 2-3 min. and filtered through a thin cloth. After filtration, pollen was transferred to a new tube and centrifuged at 3,000 rpm for 1 min., discarded the supernatant. Distilled water was subsequently added for pollen pellet washing, followed by being centrifuged at 3,000 rpm for 1 min. The supernatant was discarded and the washing step was repeated 2-3 times. Next, added glacial acetic acid to remove access water in pollen sample prior to add acetolysis mixture (Jones, 2014), centrifuged for 1 min and decanted the supernatant. Acetolysis mixture (9:1 acetic anhydride: conc. sulfuric acid) was then added to the tube and placed in warm water for 1 min, centrifuged, discarded the supernatant. Distilled water was added to wash the pollen sample, centrifuged and discarded the supernatant. Repeated the washing step for 2-3 times, followed by dehydrating the sample using 95% and 100% ethanol, respectively. For the final washing step, benzene was added to the sample, centrifuged for 1 min and discarded the supernatant. 2-3 drops of silicone oil were added to the pollen sample and it was kept until needed (Jones, 2014). The same method was used for preparation of pollen from blooming flowers.

3. Data analysis and plant species identification based on pollen morphology

Acetolyzed pollen samples were mounted on the slides for light microscopic examination at 40X magnification to perform morphology analysis of pollen. Pictures of 30 pollen grains per plant species were taken (Saensouk, & Saensouk, 2011) under the light microscope for size measurement using program AxioVision AC Rel. 4.1. Pollen symmetry, pollen aperture, and pollen shape were examined. Mean ± standard diviation (SD) of polar axis (P) and equatorial axis (E) of pollen grains from each plant species were calculated to use for comparison between bee pollen and pollen from flowers (Delaplane et al., 2013).

Results and discussion

The study showed totally 22 plant species of blooming flowers found in the study site during September-November 2014 (Table 1). Most of them were planted as human food and for home decorations. The 20, 22 and 22 species had bloomed in September, October and November, respectively. Whereas totally 14 species of plants were observed from bee pollen loads based on pollen morphology analysis (Fig. 2). The 7, 7 and 4 species were found in pollen loads of the bees in September, October and November, respectively (Fig. 3 and Table 2).

Table 1 List of 22 flowering plant species were found during September – November 2014 in Pathumrat district, Roi- Et province

Family name	Scientific name	Common name
Apocynaceae	*Cascabela thevetia*	Yellow oleander
Asteraceae	*Helianthus annuus*	Sunflower
	Sphaneticola trilobata	Singapore daisy/ Trailing daisy
Capparaceae	*Cleome viscosa*	Tickweed/ Asian spiderflower
Caricaceae	*Carica papaya*	Papaya
Convolvulaceae	*Argyreia nervosa*	Baby woodrose
	Ipomoea aquatic	Water morning glory
Cucurbitaceae	*Coccinia grandis*	Ivy gourd
	Cucurbita pepo	Pumpkin
	Luffa cylindrica	Sponge gourd
Fabaceae	*Cassia surattensis*	Scrambled Egg Tree/ Singapore Shower/ Sunshine Tree
	Lablab purpureus	Hyacinth bean
	Neptunia oleracea	Water mimosa
	Senna siamea	Siamese cassia
	Sesbania grandiflora	Corkwood tree
Labiatae	*Ocimum basilicum*	Sweet basil
Malvaceae	*Sida rhombifolia*	Bloom weed
Nyctaginaceae	*Bougainvillea sp.*	Paperflower
Portulacaceae	*Portulaca oleracea*	Flowering purslane
Rubiaceae	*Ixora coccinea*	Jungle geranium
Solanaceae	*Solanum melongena*	Eggplant
Zingiberaceae	*Alpinia galangal*	Siamese ginger

Eight families were found among 14 plant species from bee pollen loads, including (1) Arecaceae (2) Asteraceae (3) Bignoniaceae (4) Caricaceae (5) Fabaceae (6) Iridaceae (7) Moringaceae and (8) Rubiaceae. Five species of them could be identified after being compared to the pollen morphology of blooming flowers, including *Carica papaya, Helianthus annuus, Ixora coccinea, Sesbania grandiflora and Sphagneticola trilobata* and 9 unknown species (Fig. 2 and Table 2, 3). Amongst the 9 unknown species, 6 families were identified according to Adekanmbi (2009), Crompton & Wojtas (1993), Harley & Dransfield (2003), Perveen & Qaiser (2009), Rajukar et al. (2018), Rakarcha et al. (2018), Sawani et al. (2014), Sopaladawan & Phinyo (2018), Sopaladawan & Sonyoha (2018) and Sopaladawan et al. (2019) (Table 2, 3). Proportions of food plant species of *A. mellifera* to flowering plant species in September, October, November and in total were 0.35, 0.32, 0.18 and 0.64, respectively (Fig. 3). This suggested that number of food plant species of *A. mellifera* did not correlate to flowering plant species in each month. Whereas, high proportion of food plant species to flowering plant species found in total of 3 months. Earlier report by Sopaladawan et al. (2019) showed lower proportion (0.44) of food plant species to flowering plant species found in Nong Khai Province during the same time of this study, there were 43 flowering plant species, but only 15 species were pollen resources for *A. mellifera.* The results of this study and Sopaladawan et al. (2019) showed the same 7 flowering plant species, including *Cassia siamea, Coccinia grandis, Cucurbita moschata, Ixora coccinea, Ocimum basilicum, Portulaca oleracea* and *Sida rhombifolia.* Interestingly, none of them was found in bee pollen loads, which indicated that these 7 plant species are not good resources of pollen for *A. mellifera* bees.

Table 2 Pollen spectrum of 14 plant species in pollen loads of honey bee (*Apis melllifera*) in Pathumrat district, Roi-Et province during September-November 2014

Family name	Scientific name	September 2014	October 2014	November 2014
Arecaceae	Unknown 1	/	-	-
	Unknown 2	-	-	/
	Unknown 3	-	/	-
	Unknown 4	-	/	-
Asteraceae	*Helianthus annuus*	-	-	/
	Sphagneticola trilobata	/	-	-
	Unknown 5	/	/	-
Bignoniaceae	Unknown 6	-	/	-
Caricaceae	*Carica papaya*	/	-	-
Fabaceae	*Sesbania grandiflora*	-	/	/
	Unknown 7	/	/	/
Iridaceae	Unknown 8	-	/	-
Moringaceae	Unknown 9	/	-	-
Rubiaceae	*Ixora coccinea*	/	-	-

According to 5 identified plant species that were found in the bee pollen loads, 4 species of them had bloomed for the whole 3 months, including *C. papaya, I. coccinea, S. grandiflora* and *S. trilobata.* Whereas another species, *H. annuus* had bloomed only in November. Among 14 species that found in pollen loads of honey bees, only the Unknown 7 was found over 3 months (Table 2). There were 2 species of economic plants found in this study, which were papaya (*C. papaya*) and sunflower (*H. annuus*).

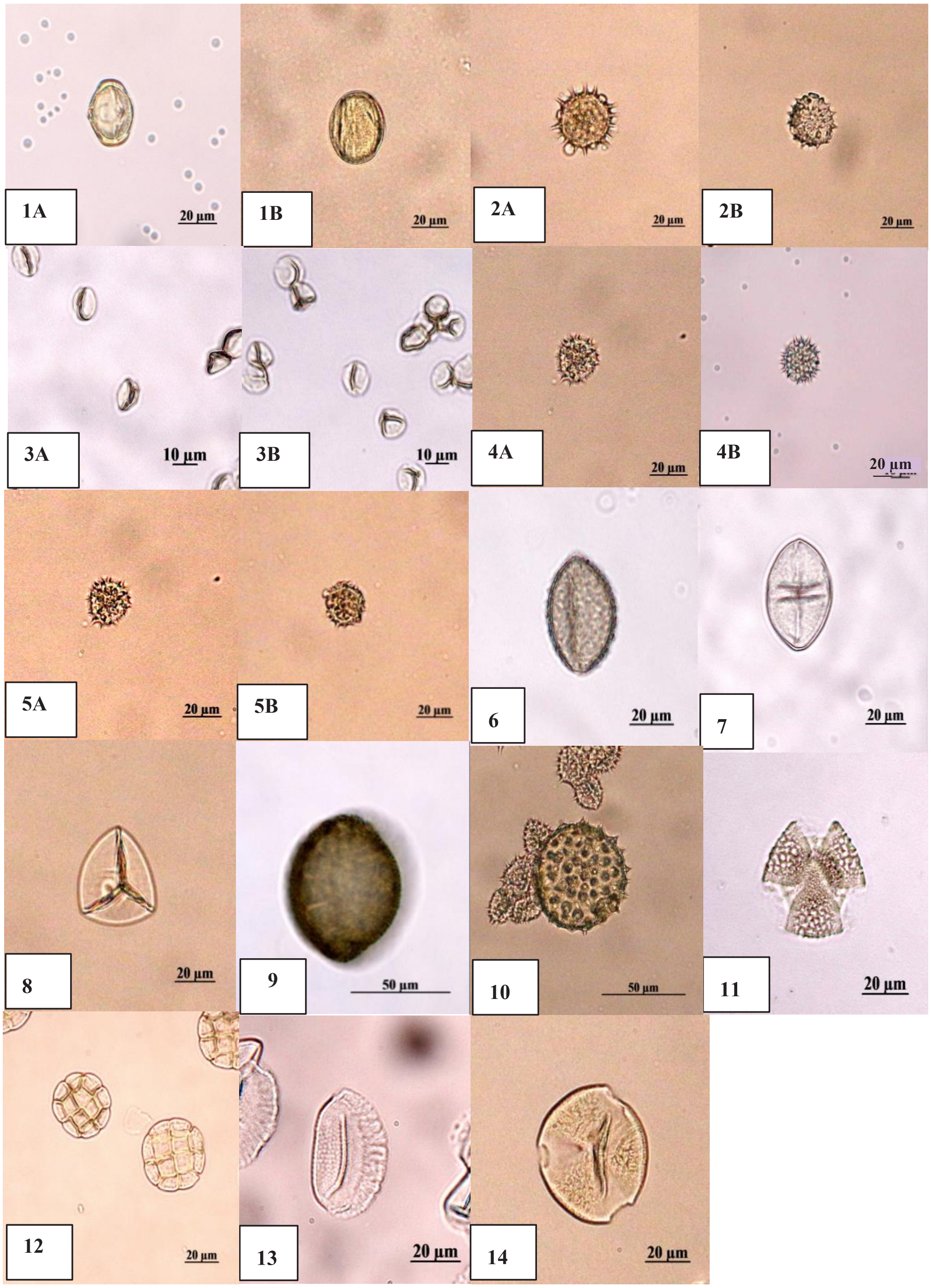

Fig. 2 Pollen grain morphology of food plant species for honey bee (*Apis mellifera*);
A = pollen from flowers, B = pollen from pollen loads
1= *Carica papaya,* 2 = *Helianthus annuus,* 3 = *Ixora coccinea,*
4 = *Sesbania grandiflora,* 5 = *Sphagneticola trilobata,* 6 = Unknown 1, 7 = Unknown 2
8 = Unknown 3, 9 = Unknown 4, 10 = Unknown 5, 11 = Unknown 6, 12 = Unknown 7, 13 = Unknown 8, 14 = Unknown 9

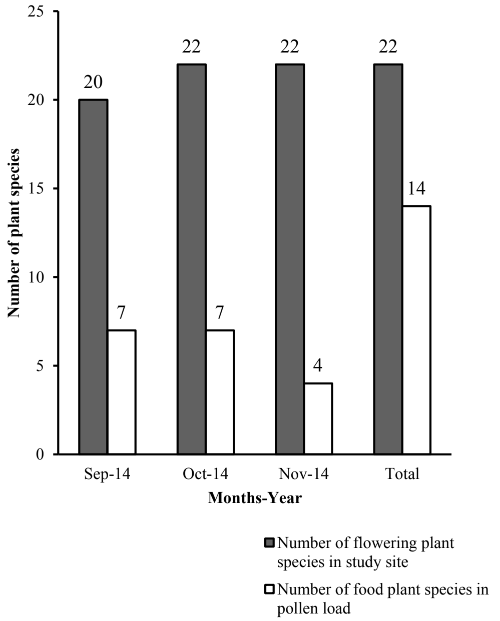

Fig. 3 Proportions of flowering plant species and food plant species in pollen loads of honey bee (*Apis mellifera*) were found during September – November 2014 in Pathumrat district, Roi-Et province

Papaya (*C. papaya*) is an important economic fruit crop in the tropical and subtropical countries. In 2008, papaya production in 20 countries over the world was over 9.1 million metric tons. India was the first ranked in papaya production, while Thailand was the 8th ranked. Annual papaya production in Thailand was about 215,000 tons in 2013, the largest papaya planting area in Thailand is in the eastern part. Thai people consume papaya both unripe and ripe forms (Janthasri & Chaiayaboon, 2016; Somsri, 2014). However, to increase papaya production, insect pollinators such as the hawk moth, stingless bees and honey bees had been considered to use for papaya pollination in Australia (Garrett, 1995; McGregor, 1976). There was no report on insect pollinators in papaya in Thailand. Another economic plant that could be identified from bee pollen loads was sunflower (*H. annuus*), which some beekeepers normally keep the bees in sunflowers field to collect honey (Andrada et al., 2004). Honey bee pollination activity is very important in seed production of hybrid sunflower (DeGrandi-Hoffman & Watkins, 2000; Sawatthum, 2020). So, this research could provide information on potential papaya and sunflower pollination services by *A. mellifera.*

Moreover, the study showed that honey bees also feed on non-crop flowers. The information of which plants are the bees foraged could be benefit on having those plants surround the fields or orchards to keep the pollinators close to the cropping system (Jones, 2014).

Conclusion

In summary, the study on pollen resources of the honey bee, *A. mellifera* in September-November 2014 at Pathumrat District, Roi-Et Province using morphology analysis on pollen from pollen loads of the bees showed 22 flowering plant species. Fourteen plant species were found in bee pollen loads. Five species of them can be identified based on pollen morphology, whereas 9 unknown species were observed. The results could be of benefit to beekeepers in which they get more information on pollen resources for the bees during the time of colony build up. Moreover, the results also provided more information on potential pollinators for economic crop pollination in Thailand.

Table 3 Pollen characteristics from pollen loads of honey bee (*Apis mellifera*)

Family name	Scientific name	Symmetry	Pollen aperture	Pollen shape (P/E)	P (µm) ± S.D.	E (µm)± S.D.
Arecaceae	Unknown 1	Bilateral	Monocolporate	Prolate	43.70±3.89	27.02±3.70
	Unknown 2	Bilateral	Monocolporate	Prolate	46.75±2.81	27.02±2.88
	Unknown 3	Radial	Tricolporate	Prolate spheroidal	39.61±2.86	35.43±2.82
	Unknown 4	Bilateral	Monocolporate	Prolate spheroidal	69.28±3.65	58.70±3.98
Asteraceae	*Helianthus annuus*	Radial	Periporate	Prolate spheroidal	29.79±1.31	28.12±1.23
	Sphagneticola trilobata	Radial	Periporate	Prolate spheroidal	20.09±0.95	18.55±0.88
	Unknown 5	Radial	Periporate	Prolate spheroidal	50.79±3.29	48.22±2.94
Bignoniaceae	Unknow 6	Radial	Tricolporate	Oblate spheroidal	35.83±1.64	34.07±1.77
Caricaceae	*Carica papaya*	Radial	Tricolpate	Subprolate	27.66±1.71	20.86±1.53
Fabaceae	*Sesbania grandiflora*	Radial	Periporate	Prolate spheroidal	19.62±0.73	18.99±0.86
	Unknown 7	Radial	Periporate	Prolate spheroidal	39.92±1.84	37.57±1.88
Iridaceae	Unknown 8	Bilateral	Monocolporate	Prolate	40.80±1.94	22.89±1.31
Moringaceae	Unknown 9	Radial	Tricolporate	Prolate spheroidal	41.13±2.31	39.08±2.14
Rubiaceae	*Ixora coccinea*	Radial	Tricolporate	Prolate spheroidal	12.79±0.87	10.23±0.93

Remark: P = Polar axis, E = Equatorial axis

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