Food plant species from pollen load of honey bee (*Apis mellifera*) in Nong Khai Province, Thailand

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ABSTRACT

Diversity of food plants for honey bee (*Apis mellifera*) in Nong Khai Province was studied by comparing morphology of pollen from pollen loads of bees with pollen collected from flowering plant during September – October 2014. Pollen morphology and measurement were investigated under light microscope (LM). There were 43 species of plants blooming at that period. Pollen from bee pollen loads showed 15 food plant species. Among these, 9 species were identified, including 1) *Acmella oleracea*, 2) *Amaranthus viridis*, 3) *Cassia siamea*, 4) *Cleome gynandra*, 5) *Elaeis guineensis*, 6) *Leucaena leucocephala*, 7) *Mimosa pigra*, 8) *M. pucida*, and 9) *Passiflora edulis* and 6 unknown species.

Keywords: Apis mellifera, Food plant, Pollen

INTRODUCTION

Honey bee, *Apis mellifera* is one of the most important pollinators. Worker bees make thousands of flower visiting to collect nectar and pollen, and they pollinate these flowers while doing this (Delaplane and Daniel, 2000; Dukku, 2013; Bhalchandra et al., 2014). The flowers visited by bees enable the reproduction, productivity, and diversification of both wild plants and agricultural crops (Crane and Walker, 1984). Nectar is source of energy for honey bees as the main composition is sugar, whereas pollen is source of proteins, lipids, minerals, and vitamins (Herbert and Shimanuki, 1978).

In Thailand, *A. mellifera* beekeeping distributed throughout the country, but mostly located in the northern part, due to enough food resources for the bees. Various floral plants of the bees has been observed (e.g. Maksong, 2016). The plants visited by honey bees can be determined through different methods (Hepburn and Radloff, 1998), including direct observation of foraging bees (Ayansola and Davies, 2012), analysis of pollen loads removed from returning foragers (Köppler et al., 2007), analysis of pollen stores in nests or hives (Ramanujam and Kalpana, 1992), and palynological analysis of honey (Adekanmbi and Ogundipe, 2009).

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The information on food plant species for the bees is essential in guiding beekeepers in choosing suitable apiary sites. It is also benefit in the identification of crops that may be pollinated by honey bees. Here we investigated species diversity of floral plants of *A. mellifera* using morphological analysis on pollen from bee pollen loads.

MATERIALS AND METHODS

Sample collection

Bee pollen were collected in a bee farm at Tha Bo District, Nong Khai Province (17°52'46.4"N, 102°34'53.0"E) once a month during September-November 2014. We chose to collect pollen in this period because it is the time for colony built-up to get the colonies ready for honey collecting season. Pollen traps were placed at the entrances (Fig. 1) of 3 colonies of *A. mellifera* to collect bee pollen loads at 7.00-11.00 am (Bhalchandra et al., 2014). Samples of bee pollen from each colony were kept separately in a plastic tube at 4ºC for morphological analysis. Moreover, we collected pollen from flowers that blooming in the same study site at the same time. Morphology of pollen collected from flowers was used to identify plant species from bee pollen loads.

Fig. 1 A pollen trap is placed at the hive entrance to collect bee pollen loads.

Plant species identification using pollen morphology analysis

Pollen from the bees and flowers were prepared for morphology analysis using acetolysis method (Erdtman, 1966). Pollen collected from each bee colonies were firstly sorted by color and kept in 1.5 ml microcentrifuge tubes. Added 10% KOH to cover pollen sample in the tube followed by boiling the tube in water bath for 2-3 min. Pollen in suspension was filtered through a thin cloth, transferred the pollen to a new tube and centrifuged at 3,000 rpm for 1 min. Discarded the supernatant, distill water was then added to wash the pollen pellet, centrifuged at 3,000 rpm for 1 min and supernatant liquid is decanted. Repeat the washing step for 2-3 times. Glacial acetic acid was added to the pollen sample, centrifuged for 1 min and discarded the supernatant. Added acetolysis mixture (9:1 acetic anhydride: conc. sulfuric acid) and

placed the tube in warm water for 1 min, centrifuged and discarded the supernatant. Washed the sample using distill water for 2-3 times, followed by dehydrating the sample using 95% and 100% ethanol, respectively. Benzene was added to the sample for the final washing step, centrifuged for 1 min and discarded the supernatant. Added 2-3 drops of silicone oil to the sample and kept it until needed. Pollen from blooming flowers were prepared in the same method.

To perform morphology analysis of pollen, acetolyzed pollen were mounted on the slides for light microscopic examination at 40X magnification. Pictures of 30 pollen grains per plant species were taken (Saensouk and Saensouk, 2011) under the light microscope for size measurement using program AxioVision AC Rel. 4.1. Pollen symmetry, pollen aperture, and pollen shape were recorded. Mean ± standard deviation (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.

RESULTS AND DISCUSSION

Here is the first report on floral plant species of *A. mellifera* in Thailand based on pollen morphology analysis. The results showed that there were 43 plant species had bloomed in September-November 2014 at the study site (Table 1). There were 34, 38, and 28 species with blooming flowers in September, October, and November 2014, respectively. Whereas 10, 9, and 6 species were found in pollen load of the bees in September, October, and November 2014, respectively (Table 2 and Fig. 2, 3).

Table 1 List of 43 flowering plant species found during September – November 2014 in Tha Bo District, Nong Khai Province

Family	Scientific name	Thai name
Amaranthaceae	*Amaranthus viridis*	ผักโขมป่า
	Gomphrena celosioides	บานไม่รู้โรยป่า
	G. globosa	บานไม่รู้โรย
Lamiaceae	*Ocimum basilicum*	โหระพา
	O. canum	แมงลัก
	O. sanctum	กระเพรา
Apocynaceae	*Alstonia scholaris*	สัตบรรณ
	Catharanthus roseus	แพงพวย
	Plumeria obtusa	ลั่นทมขาว
	Tabernaemontana pandacaqui	พุด
Arecaceae	*Elaeis guineensis*	ปาล์มน้ำมัน

Table 1 (Cont.) List of 43 flowering plant species found during September – November 2014 in Tha Bo District, Nong Khai Province

Family	Scientific name	Thai name
Asteraceae	*Acmella oleracea*	ผักคราดหัวแหวน
	Ageratum conyzoides	สาบแร้งสาบกา
	Cosmos sulphureus	ดาวกระจาย
	Eupatorium odoratum	สาบเสือ
	Tagetes erecta	ดาวเรือง
	Tridax procumbens	ตีนตุ๊กแก
	Verononia cinerea	หมอน้อย
Bignoniaceae	*Oroxylum indicum*	เพกา/ลิ้นฟ้า
Capparaceae	*Cleome gynandra*	ผักเสี้ยนขน
Convolvulaceae	*Ipomoea aquatica*	ผักทอดยอด,
	Merremia umbellata	ผักบุ้งแดงจิงจ้อขาว
Commelinaceae	*Commelina bengalensis*	ผักปลาบ
Cucurbitaceae	*Coccinia grandis*	ตำลึง
	Cucurbita moschata	ฟักทอง
	Momordica charantia	มะระขี้นก
Fabaceae	*Cassia siamea*	ขี้เหล็ก
	Centrosema pubescens	ถั่วลาย
	Crotalaria juncea	ปอเทือง
	Leucaena leucocephala	กระถิน
	Mimosa pigra	ไมยราบยักษ์
	M. pucida	ไมยราบ
	Neptunia javanica	กาเสดโคก
	Tamarindus indica	มะขาม
Malvaceae	*Gossypium herbaceum*	ฝ้าย
	Hibiscus rosa-sinensis	ชบา
	H. sabdariffa	กระเจี๊ยบแดง
	Sida rhombifolia	หญ้าขัด
Tiliaceae	*Muntingia calabura*	ตะขบฝรั่ง
Passifloraceae	*Passiflora edulis*	เสาวรส
Portulacaceae	*Portulaca oleracea*	คุณนายตื่นสาย
Rubiaceae	*Ixora coccinea*	เซ็มแดง

Table 2 Pollen spectrum of 13 plant species in pollen load of honey bee (*Apis melllifera*) in Tha Bo District, Nong Khai Province during September-November 2014

Family	Scientific name	September 2014	October 2014	November 2014
Amaranthaceae	*Amaranthus viridis*	-	/	-
Arecaceae	*Elaeis guineensis*	/	-	-
Asteraceae	*Acmella oleracea*	-	/	-
Capparaceae	*Cleome gynandra*	/	/	/
Fabaceae	*Cassia siamea*	/	/	-
	Mimosa pigra	/	/	-
	M. pucida	/	/	/
	Leucaena leucocephala	/	/	/
Passifloraceae	*Passiflora edulis*	/	-	-
	Unknown 1	/	/	-
	Unknown 2	-	-	/
	Unknown 3	/	-	-
	Unknown 4	-	/	-
	Unknown 5	/	-	/
	Unknown 6	-	-	/

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

Family name	Scientific name	Symmetry	Pollen aperture	Pollen shape (P/E)	P (µm) ± S.D.	E (µm) ± S.D.
Amaranthaceae	*Amaranthus viridis*	Radial	Periporate	Prolate-spheroidal	18.82±1.36	18.32±1.38
Arecaceae	*Elaeis guineensis*	Radial	Tricolpate	Prolate-spheroidal	26.53±1.56	25.81±1.66
Asteraceae	*Acmella oleracea*	Radial	Periporate	Prolate-spheroidal	26.53±1.56	25.81±1.66
Capparaceae	*Cleome gynandra*	Radial	Pericolpate	Prolate	16.07±1.36	10.63±0.61
Fabaceae	*Cassia siamea*	Radial	Tricoporate	Prolate-spheroidal	35.79±1.98	33.87±1.76
	Mimosa pigra	Radial	Pericolporate	Subprolate	20.69±0.89	16.30±0.88
	M. pucida	Radial	Tricolporate	Prolate-spheroidal	8.74±0.57	7.99±0.46
	Leucaena leucocephala	Radial	Tricolporate	Prolate-spheroidal	43.89±1.89	42.30±1.96
Passifloraceae	*Passiflora edulis*	Radial	Periporate	Prolate	45.81±1.54	32.23±1.82
	Unknown 1	Radial	Periporate	Prolate-spheroidal	33.32±1.37	31.57±1.15
	Unknown 2	Radial	Tricolporate	Prolate	34.21±1.98	24.26±1.50
	Unknown 3	Radial	Periporate	Prolate-spheroidal	35.18±1.74	34.79±1.81
	Unknown 4	Bilateral	Tricolpate	Prolate-spheroidal	33.78±1.67	32.98±1.60
	Unknown 5	Radial	Tricolporate	Prolate-spheroidal	18.37±0.63	17.69±0.53
	Unknown 6	Radial	Tricolporate	Prolate-spheroidal	58.08±1.58	57.09±1.84

Note: P = polar axis, E = equatorial axis

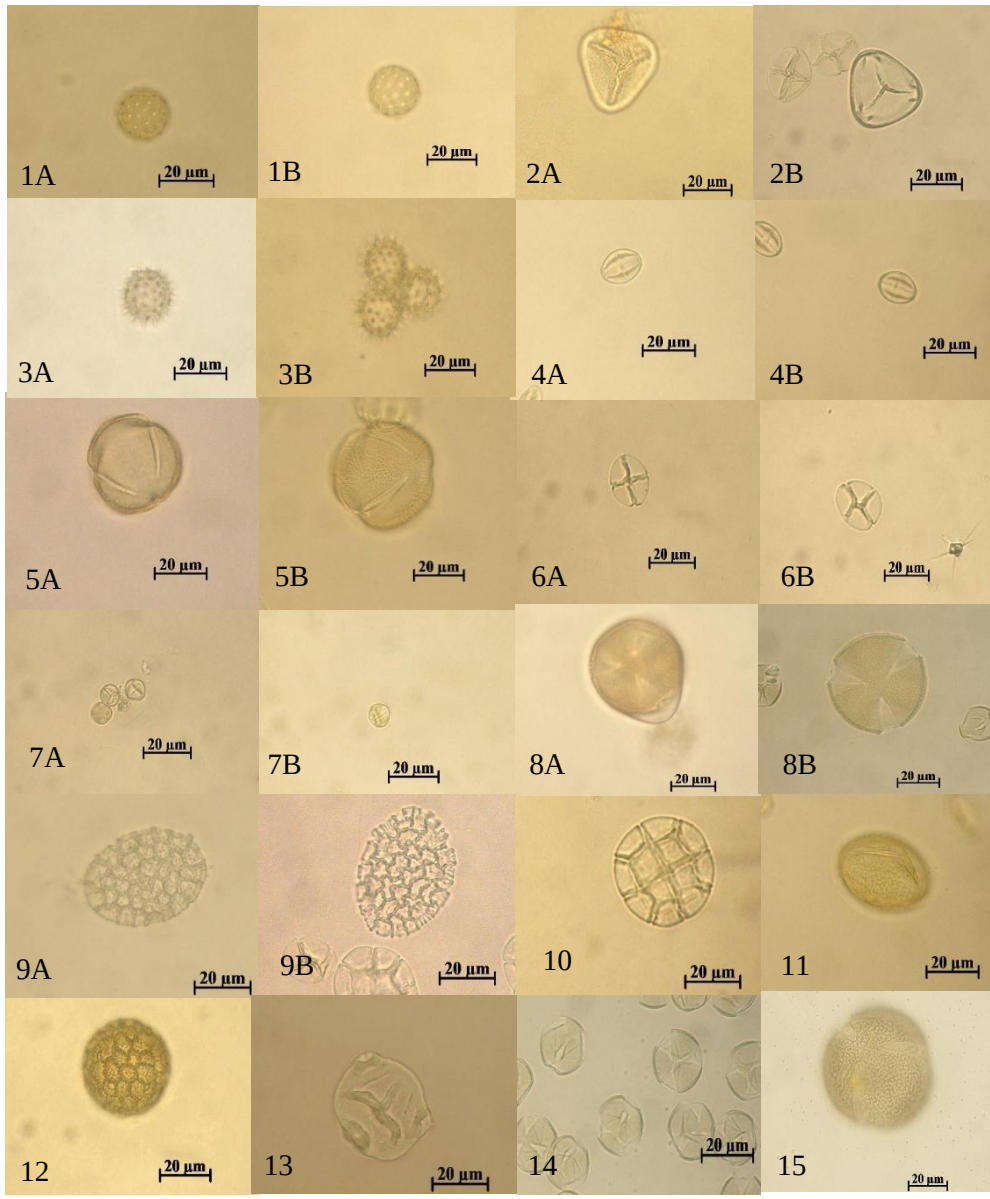

Fig. 2 Pollen grain morphology of food plant species for honey bee (*Apis mellifera*);
A = pollen from flowers, B = pollen from pollen loads
1= *Amaranthus viridis*, 2 = *Elaeis guineensis*, 3 = *Acmella oleracea*,
4 = *Cleome gynandra*, 5 = *Cassia siamea*, 6 = *Mimosa pigra*, 7 = *M. pucida*
8 = *Leucaena leucocephala*, 9 = *Passiflora edulis*, 10 = Unknown 1
11 = Unknown 2, 12 = Unknown 3, 13 = Unknown 4, 14 = Unknown 5
15 = Unknown 6

Morphology analysis of pollen from 2 sources; bee pollen and pollen from flowers, indicated that there were 15 plant species found in pollen load of the bees. Among these, 9 species can be identified and 6 unknown species (Table 2, 3 and Fig. 2). In September and November, number of plant species found in bee pollen loads were correlated to number flowering plant species (Fig. 3). Correlation in September indicated that the more species of flowering plant observed, the more plant species found in bee pollen loads and *vice versa* in November. Proportions of number of food plant species of *A. mellifera* to number of flowering plant species in September, October, November and in total were 0.29, 0.26, 0.18, and 0.44, respectively (Fig. 3).

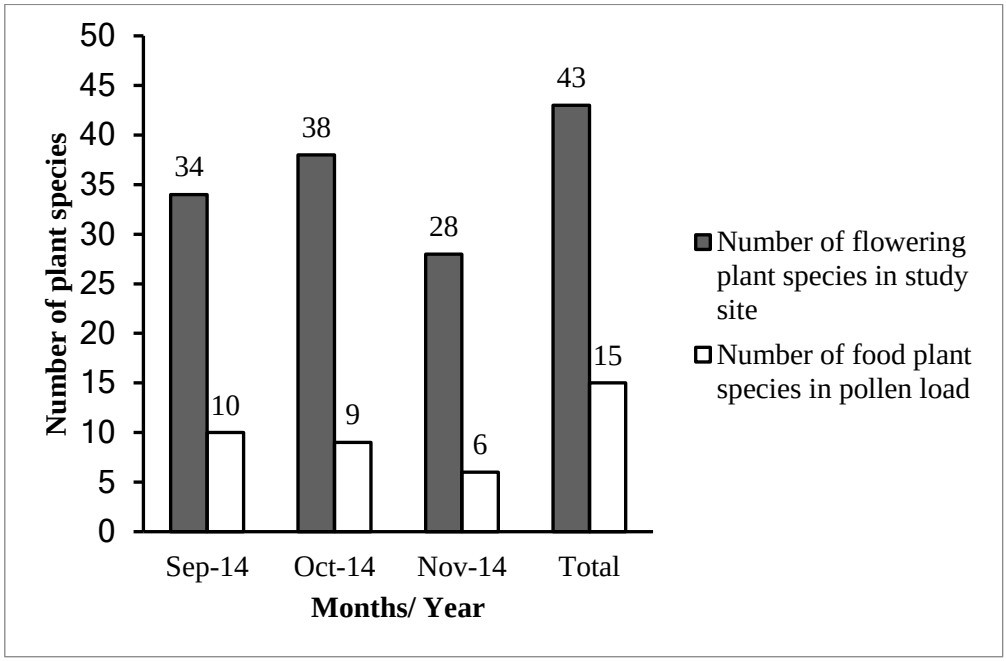

Fig. 3 Comparison of number of flowering plant species and food plant species in pollen load of honey bee (*Apis mellifera*) were found during September – November 2014 in Tha Bo District, Nong Khai Province.

There were 5 out of 9 identified plant species had bloomed for the whole 3 months of the study period, including *A. oleracea*, *C. siamea*, *E. guineensis*, *L. leucocephala*, and *P. edulis*. The other 4 species; *A. viridis*, *C. gynandra*, *M. pigra*, and *M. pucida* bloomed for 2 months (September and October). Among 15 species that found in pollen loads of honey bee, there were 3 species, *C. gynandra* *M. pucida*, and *L. leucocephala* that occurring in 3 months (Table 2). This indicated that these 3 species are good pollen resources for *A. mellifera*. Most of the food plant species reported in this study are wild, except *E. guineensis* and *P. edulis*.

These 2 species are economic plants, and the results suggested that *A. mellifera* could be good pollinators for them.

There were some reports on food plant species of stingless bees in Thailand (Jongjitvimol and Wattanachaiyingcharoen, 2006, Sopaladawan and Sonyoha, 2017). Sopaladawan and Sonyoha (2017) found that *P. edulis* is one of food plant species of stingless bee (*Trigona laeviceps*).

Maksong (2016) reported from direct observation, found that *E. guineensis* and *M. pucida* are pollen resources for 3 honey bee species; *A. florea*, *A. cerana*, and *A. dorsata* in Thailand which related to this study.

CONCLUSION

The study on diversity of food plant species for *A. mellifera* in September-November 2014 at Tha Bo District, Nong Khai Province using morphology analysis on pollen from pollen loads of the bees showed 43 flowering plant species. Fifteen plant species were found in bee pollen. Nine species of them can be identified, whereas 6 unknown species were investigated. The results could be benefit to beekeepers in Thailand in which wild plant species are suitable resources of pollen for the bees during colony building up. Moreover, *A. mellifera* could be able to be a good pollinator for economic plants, *A. viridis* and *E. guineensis*.

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REFERENCES

Adekanmbi, O. and Ogundipe, O. (2009). Nectar Sources for the honey bee (*Apis mellifera adansonii*) revealed by pollen content. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2),* 211-217.

Ayansola, A.A. and Davies, B.A. (2012). Honeybee floral resources in southwestern Nigeria. *Journal of Biology and Life Science, 3(1),*127-138.

Bhalchandra, W., Baviskar, R.K. and Nikam, T.B. (2014). Diversity of nectariferous and polleniferous bee flora at Anjaneri and Dugarwadi hills of Western Ghats of Nasik district (M.S.) India. *Journal of Entomology and Zoology study, 2(4),* 244-249.

Crane, E. and Walker, P. (1978). *Pollination directory for World Crops*. International Bee Research Association, London.

Delaplane, K.S. and Daniel, F.M. (2000). *Crop pollination by bees*. CABI Publishing, New York.

Dukku, U.H. (2013). Identification of plants visited be honeybee, *Apis mellifera* L. in the Sudan Savanna zone of northeastern Nigeria. *African Journal of Plant Science, 7(7),* 273-284.

Erdtman, G. (1966). *Pollen morphology and plant taxonomy angiosperms (an introduction to palynology I)*. London: Heffner Publishing.

Hepburn, H.R. and Radloff, S.E. (1998). *Honeybees of Africa*. Springer-Verlag, Berlin Hedelberg.

Herbert, E.W. and Shimanuki, H. (1978). Chemical composition and nutritive value of bee-collected and bee-stored pollen. *Apidologie, 9(1)*, 33-40.

Jongjitvimol, T. and Wattanachaiyingcharoen, W. (2006). Pollen Food Sources of Stingless Bees *Trigona apicalis* Smith, 1857, *Trigona collina* Smith, 1857 and *Trigona fimbriata* Smith, 1857 (Apidae, Meliponiae) in Thailand. *The Natural History Journal of Chulalongkorn University, 6(2)*: 75-82.

Köppler, K., Vorwohl, G. and Koeniger, N. (2007). Comparison of pollen spectra collected by four different subspecies of the honey bee *Apis mellifera*. *Apidologie, 38,* 341-353.

Maksong, S. (2016). Diversity of bee flora at Tipuya, Chalae, Thong Pha Phum, Kanchanaburi (in Thai). *Journal of Science and Technology, 24(1),* 76-86.

Ramanujam, C.G.K. and Kalpana, T.P. (1992). *Tamarindus indica* L.: An important forage plant for *Apis florea* in south central India. *Apidologie, 23*, 401-413.

Saensouk, P. and Saensouk, S. (2011). Palynology of Some Bignoniaceae Species in Northeastern Thailand. *KKU Research Journal, 16(2),* 187-195.

Sopaladawan, P.N. and Sonyoha, J. (2017). Food plant species from pollen load of stingless bee (*Trigona laeviceps* Smith) in area of Agricultural Occupation Promotion and Development Center, Khon Kaen Province. *Journal of Science and Technology Mahasarakham Unversity: the 13rd Mahasarakham University Research Conference*, 618-629.