Role of Stingless Bee, *Tetragonula pegdeni* and European Honey Bee, *Apis mellifera* in the Pollination of Confectionery Sunflower

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Abstract

The confectionary sunflowers (*Helianthus annuus* L.) are not self- pollination. It needed pollinators for the seeds producing. This experiment aimed to find the suitable pollinators to increase the seed yield. There were two experiments. The first part tried to find the number of stingless bees *(T. pegdeni*) and European honey bees (*A. mellifera,)*, visited the two confectionery sunflowers (Chiang Rai and Hungary) cultivars and one hybrid sunflower (Pacific 77). The number of those two pollinators visiting the sunflowers at every 10 minutes of each hour from 06:00 am to 06:00 pm for 5 days were counted. In the second experiment, the efficiency of the pollination by the selected pollinators on the Chiang Rai cultivar was compared. The RCBD was used with four treatments; pollination by stingless bee, pollination by European honey bee, open- pollination, and close-pollination. The LSD method was used for means comparison of the three replications. The result showed less number of stingless bees visited both Chiang Rai and Hungary varieties than that of the European honey bee. The similar numbers of the two pollinators to visit the hybrid cultivar were found. The better pollination parameters were found from the pollination by European honey bee. In addition, the total seed number, good seed weight and good seed number were statistically different from the other treatments. The results from both experiments confirmed the limitations of the selected pollinators. The visiting time, size and the body anatomy of the bees, the floral structure, the readiness for pollination, were all factors affecting seed production of confectionery sunflower.

Keywords: European honey bee; pollination; pollinator; stingless bee; confectionary sunflower; *Helianthus annuus*

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1. Introduction

Sunflower (*Helianthus annuus* L.) is a composite flower with lots of florets on the flower disc. Each flower unit is a perfect flower but it is a self – incompatible flower because the pistil and stamen are ready for pollination at different times. The experiments showed that the wind had a very low influence on sunflower seeds formation, because the stickiness of the pollen makes it not easy to diffuse (Oz, *et al*., 2009). As a result, the pollination and seeds formation require supporting factors such as insect. The main pollinator of the sunflower is the European honey bee (*Apis mellifera)*, which encourages the cross- pollination and makes better seeds (Free, 1993 and Chambo *et al*., 2011), and also high oil content (Malaipan, 1992). Although the hybrid cultivars are currently produced high yields without the use of insects as pollinator. The experiments of Cimu (1960), confirmed that hybrid sunflowers increased a nearly 40 percent yield if there were insects pollinated. Orostrairat (1993) reported that Hysun 33 hybrid were pollinated by various insects and produced higher yields than the other sunflowers that did not use insect- pollinators at a rate of about 39 percent. The insect pollinators helped to produce three times more than that of non- insect-pollinators in open- pollination sunflower (Boongird, 1990).

Confectionery sunflower is the cultivar, which the seeds are edible. As a non-oil variety, it it a popular snack among Thai health- lover people. However, most of those snack products are imported from foreign countries. Confectionary sunflower seeds in Thailand are open pollination varieties, not the self-pollinated. Therefore, it is necessary to use pollinators to help increasing seed production. Currently, in Thailand there are two types of potential pollinators, i.e. European honey bees, and the specific stingless bees, *Tetragonula, pegdeni* species, which is adapted well with various environments in Thailand. This stingless bee species has been found in all areas of Thailand (Sawatthum, 2004). Currently, this species is popularly used as a pollinator of many economic crops such as longan, rambutan, lychee, etc. The results of the research by Rajasri *et al*., (2012) found a large number of these stingless bees visited the hybrid sunflower.

The objective of this study was to find the suitable insects for pollination that will help increasing the yield of confectionery sunflower seeds. Comparing the pollination potential of both pollinators, i.e. European honey bees species *(A. mellifera)* and the stingless bees of *T. pegdeni* species was also evaluated.

2. Materials and Methods

2.1 Materials

In this experiment, two species of confectionery sunflowers were used. They were Chiang Rai and the Hangari varieties, which showed the potential for commercial production. These two varieties were from the confectionery sunflower seeds development project of Rajamangala University of Technology Lanna.

One of the hybrid sunflowers (Pacific 77) was used to compare with the confectionery

species. Pacific 77 cultivar was a popular oil sunflower cultivar commonly grown in Thailand by Pacific Seed (Thai) Ltd.

2.2 Methods

The experiments were carried out in two steps.

2.2.1 The first experiment was to find the number of stingless bees *(T. pegdeni*) and European honey bees (*A. mellifera,)*, visited the three sunflowers (Chiang Rai, Hungary, and Pacific 77) cultivars. Each sunflower species was planted in the 10 x 40 square meters area of the Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi. Five flowers of each species were randomly selected. The bees' species and the number of those bees that visited and clung in the five flowers were counted and recorded. The observation was performed at every hour for 10 minutes from 06:00 am to 06:00 pm for 5 days.

2.2.2 Study on the capability of the two pollinators, *Tetragonula pegdeni* stingless bees and European honey bees (*Apis mellifera*) on Chiang Rai confectionery sunflower pollination.

The Chiang Rai confectionery sunflowers were planted on an area of a University in Ayutthaya province. The plot area used was 78 x 29 square meters. The planting distance was 30 x 70 square centimeters, 1 plant per hole.

The randomized complete block design (RCBD) was used. There were three replications and 4 treatments.

(1) Opened pollination: This was a treatment which the natural pollination took place.

(2) European honey bees-pollination: This treatment was done by using 5 x 5 x 4 cubic meters, blue mesh net with 16 channels in 1 square inch cage to cover the sunflower plants. There were 119 plants per cage. One nest of *A. Melliferab* European honey bees was placed in a cage.

(3) Stingless bee-pollination: This treatment was done by using the same size and shape cage as in treatment two to cover the same amount of the sunflowers plants. One nest of *T. pegden*i stingless bees was placed in a cage.

(4) Closed- pollination: The sunflower discs were covered by using the blue mesh net to prevent pollinating by insects.

In all treatments the covering of plants and flowers was stopped when the flowers was at the end of the blooming stage and began to wilt.

The mean comparison was done by the Least Significant Difference (LSD) method. The ten sunflowers from each replication was randomized to collect data as followed; flower disc diameter (cm), number of good seed per flower disc, numbers of abnormal seed per flower disc, numbers of total seed per flower disc, good seed weight, abnormal seed weight, total seed weight, weight of 100 seeds (g) (random), percentages of good seeds, whole peel seeds, and seeds with in the shell.

These parameters were the indicator of the seeds formation efficiency by the

pollination from those two bee types used in the experiment.

3. Results and Discussion

3.1 Results

3.1.1 Diversity of bees in sunflower pollination of the Chiang Rai, the Hungarians and the Pacific 77 cultivars.

European honey bees were found to be the most common insects visiting all three varieties of sunflowers for whole day. They visited the sunflowers in the morning more than the visit in the afternoon. The high numbers of visiting were found between 08:00-09:00 am, and 01:00-02:00 pm on the Chiang Rai variety. European honey bees visited the Pacific 77 in high amounts between 07:00-10:00 am and increased again at 12:00-02:00 pm.

The stingless bees found on the three sunflower varieties between 07:00-10:00 am in a smaller number than European honey bees. They visited the sunflowers again at 02:00 pm in very small quantities. The Pacific 77 cultivar was found to be visited by stingless bees throughout the day.

There were some other insect species visited the flowers such as dwarf honey bees, *A. florea*, vespa and carpenter bees. The result is shown in Figure 1.

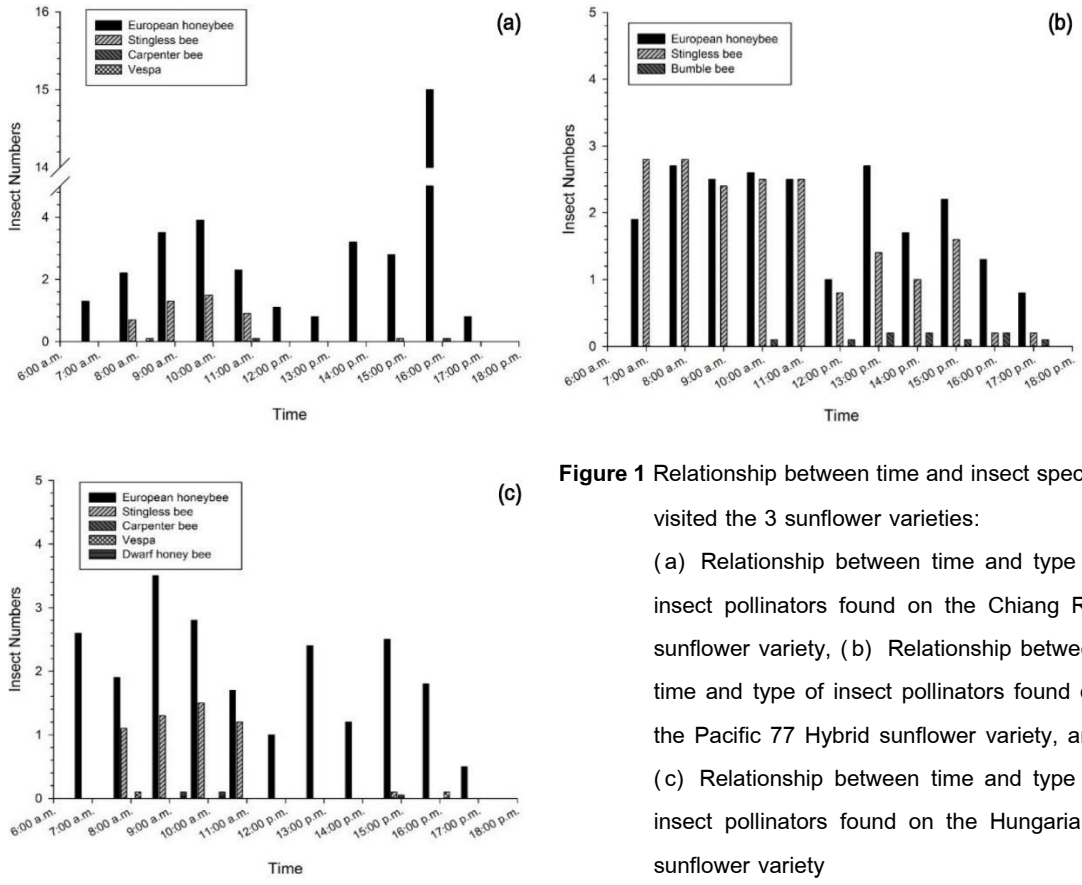

Figure 1 Relationship between time and insect species visited the 3 sunflower varieties:
(a) Relationship between time and type of insect pollinators found on the Chiang Rai sunflower variety, (b) Relationship between time and type of insect pollinators found on the Pacific 77 Hybrid sunflower variety, and (c) Relationship between time and type of insect pollinators found on the Hungarians sunflower variety

3.1.2 Comparison of the pollination efficiency by European honey bees and stingless bees on the confectionery sunflower, Chiang Rai variety

The results of this experiment showed the components of sunflower yield (Table 1) as follow. The diameter of the flower disc showed no differences in all treatments. The good seed numbers were significantly different in all treatments. The pollination by European honey bees showed the highest good seed number, followed by the open- pollination treatment, close- pollination and the stingless bee pollination of 774.10, 474.37, 39.83 and 21.97 seeds, respectively.

The number of abnormal seeds in the open- pollination and the European honey bees pollination was not significantly different, 424.68 seeds and 338.63 seeds, respectively. However, the number of abnormal seeds in the close- pollination and the stingless bees pollination showed significant difference of 1026.95 seeds and 1117.13 seeds, respectively.

Good seed weight of all experiments was significantly different. The European honey bees pollination treatments was the highest good seed weight, followed by the open- pollination with 77.52 g and 49.57 g, respectively. It was a significant difference in the good seed weight of the close-pollination and the stingless bees pollination (5.49 g and 2.51 g, respectively).

The abnormal seed weight of the stingless bee pollination and the close-pollination experiment was not significantly different, 44.73 and 37.13 g, respectively. But these abnormal seeds were statistically different from those of the open- pollination and the European honey bees pollination. The abnormal seed weight of the open- pollination and the European honey bees pollination were 7.74 and 8.93 g, respectively.

The total seed weight of the open-pollination, the stingless bees pollination and the close- pollination experiments were not significantly different with 57.31, 47.24 and 42.62 g, respectively. The total seed weight of these three experiments displayed significant difference from that of the European honey bees pollination. The total seed weight of European honey bee pollination was 86.45 g.

Weight of 100 seeds of the open-pollination, the European honey bees pollination and the close- pollination was not significantly different, 11.43, 10.55 and 9.60 g, respectively. The weight of 100 seeds of these three treatments showed significant difference from that of stingless bees pollination which was found at 7.85 g.

The seed length after removing the shell of the close- pollination, the European honey bees pollination and the open- pollination was not significantly different. They were 1.14, 1.11 and 1.07 cm, respectively. The seed lengths of the open-pollination and the stingless bees pollination did not show a significant difference. The seed length of the stingless bee pollination was 1.02 cm.

The length of the whole seed of all experiments was significantly different. The

length from the close-pollination seed was the longest one at 1.54 cm, followed by the seed from European honey bees pollination, open-pollination and the stingless bees pollination (1.48, 1.44 and 1.40 cm, respectively).

Diameter of seed, total number of seeds in sunflower, width of whole shell seed, seed thickness and the seed width and seed thickness after crusted of all treatments were not significantly different. Percentages of seed in the experiments using bees to pollinate and the open-pollination were almost similar with 69.35 and 52.66 %, respectively.

Table 1 Elements of sunflower yield,Chiang Rai variety from the 4 different pollination methods

Treatments	Diameter of the flower disc	Good seed number (seed/ flower)	Abnormal seed number (seed/ flower)	Total seed number (seed/ flower)	Good seed weight (G/ flower)	Abnormal seed weight (G/flower)	Total seed weight (G/flower)	Weight of 100 seeds (G)	Seed whole peel			Seed within the shell			Good seed percentage (%)
									Width (cm.)	Length (cm.)	Thickness (cm.)	Width (cm.)	Length (cm.)	Thickness (cm.)	
Close-pollination	14.18	39.83^{c}	1026.95^{a}	1066.78	5.49^{c}	37.13^{a}	42.62^{b}	9.60^{a}	0.71	1.54^{a}	0.51	0.46	1.14^{a}	0.23	3.75^{c}
Open-pollination	16.13	474.37^{b}	424.68^{b}	899.05	49.57^{b}	7.74^{b}	57.31^{b}	11.43^{a}	0.65	1.44^{bc}	0.46	0.42	1.07^{ab}	0.21	52.66^{b}
Bee-pollination	15.42	774.10^{a}	338.63^{b}	1112.73	77.52^{a}	8.93^{b}	86.45^{a}	11.55^{a}	0.65	1.48^{ab}	0.46	0.46	1.11^{a}	0.22	69.35^{a}
Stingless bee-pollination	17.09	21.97^{c}	1117.13^{a}	1139.1	2.51^{c}	44.73^{a}	47.24^{b}	7.85^{b}	0.66	1.40^{c}	0.47	0.42	1.02^{b}	0.21	2.05^{c}
F-test	ns	**	**	ns	**	**	**	*	ns	*	ns	ns	*	ns	**
C.V. (%)	15.27	17.46	16.24	10.39	13.34	34.96	15.87	28.56	5.67	2.28	9.23	9.16	3.76	9.39	13.53

The mean difference with each letter in each column was different at the confidence level of 95 %; Using the least significant difference (LSD) method at confidence level 95 %

* significant difference at 95 % confidence level; ** significant difference at 99 % confidence level.

3.2 Discussion

In general, it is said that confectionery sunflowers is a self-incompatible crop, fully dependent upon insect visitation for the seed production (Dag *et al*. 2002). Sawatthum *et al*. (2015) did the preliminary study of floral biology of Chiang Rai confectionery sunflower. It is found that the flower disc of this cultivar consists of sub-flowers arranged in a circle, stacked from the largest outer circle to the smallest inner circle. These flowers bloomed from the outermost circle, then went inside. Each floret began to bloom at the pollen shed stage, which was usually found in the early morning to before noon. A small number of florets found to bloom in the afternoon. Most florets that have gone through this stage would enter to the stigma receptive stage in the afternoon and wilt on the next day. Then the flowers in the next loop of flower disc would enter the blooming cycle. This would continue until no more florets to bloom. This cycle took around 5-7 days. It was also found a high percentage of seed set on the outer round of the flower disc and the percentage of seeds reduced gradually into the inner rounds.

Sawatthum *et al*. (2015) also studied

the pollination biology of Chiang Rai confectionery sunflower. The pollination biology examination looked for the seed production by the florets pollination from the same flowers and from different flowers, and from different dishes. It is found that there was none of any seed on the florets using pollen from the same florets. Nevertheless, the percentage of seeds was improved by using pollen from different florets in the same dish. The highest percentage of seeds was found when using pollen from different florets from different discs. These results confirmed that the Chiang Rai confectionery sunflowers are completely self- incompatible crop.

Based on the comparison of pollination efficiency of stingless bees and European honey bees on Chiang rai confectionary sunflower in this study, European honey bees had the higher pollination efficiency. The stingless bees showed the poor pollination performance. Various researchers confirmed the performance of the European honey Bees as the maximum seed production bees (Free, 1993; Morgado *et al*., 2002). Oz. *et al*. (2009) reported that European honey bees in cages was capable to pollinate the hybrid sunflower and increased seed yield per flower disc up to 206 percentage. This is because European honey bees need to store and accumulate large amounts of nutrients in their hive. Therefore, the amount of food required is more substantial. Other insect pollinator groups only need the food for themselves (Müller *et al*., 2006).

Even though the stingless bees store food (nectar and pollen) in the hive for colony members, similar to that of the European honey bees, it was found the low pollination efficiency of the stingless bees on the Chiang rai confectionery sunflower variety. It is probably because of the times of days when the stingless bees came to visit the sunflowers. The stingless bees often visited the sunflowers in the morning when there were a lot of pollen but in the afternoon, when the flowers gave off the nectar (stigma receptive stage), there was less numbers of stingless bees visited the flowers, which was found only by 02 : 00 pm. In the afternoon most of the florets are in the stigma receptive stage. The nectars are ready for insects that come down to the nectar gland's position. But the nectar glad might be too deep for the styled of the stingless bees to be reached. This is a difference from the European honey bees, which the styled are longer and long enough to reach the nectar in the floret, which makes most of the European honey bees visiting the flowers all day. Saez *et al*. (2012) also reported that European honey bees are the dominant flower visitors of sunflower. In addition, Teixeira and Zampieron (2008) emphasized that the nectar collected bees would result in better seeds than the pollen collected bees. From these observations, the stingless bee visited the Pacific 77 hybrid cultivar throughout the day at a number close to that of the European honey bees might because the number of florets in hybrid cultivars were more than those of the confectionery variety. According to Rajasri *et al*., (2012) lots of stingless bees *(Tetragonula*

irridipenis) visited sunflower of the CMS line and the Restorer line in both morning and afternoon. However, the effects of the visits from stingless bees on the seed formation of the hybrid sunflower have not been reported.

Some studies found good pollination by stingless bees. Heard (1999) stated that stingless bees are general insects for plant pollination, particularly in tropical and subtropical areas. *Lepidotrigona terminata* was found to be a good pollinator for coffee (Klein *et al.*, 2003), strawberry in green house (Malagodi-Braga and Kleinert, 2004), and tomato (del Sarto *et al.*, 2005).

Some researchers found less capability of stingless bees for pollination. Occhiuzzi (2000) reported that in the sweet pepper pollination, smaller stingless bees were significantly low performance pollinators. Roubik (1995) also found that small stingless bees had less capacity in pollination of sweet pepper. This corresponded to the experiment of Boonthai and Sawatthum (2014) which indicated that the smaller stingless bees were less effective pollinator in pollination of rambutans.

This less efficiency in pollination of stingless bees might affect by the anatomy of the flowers such as the floral structure of stigma and the position of the anther. The body of the stingless bees is small and cannot touch the reproductive part of both sexes of the flower. *Tetragonisca angustula*, one of the stingless bees, collected the nectar from the side of the flowers of radish without touching the stigma (Thai, 2001), resulting in no pollination from the visit. In addition, the body size of the stingless bees also results in pollination of some plants, as reported by Sawatthum *et al*. (2017). It was found that the stingless bees, *Lepidotrigona terminata* visited both the male and female of cucurbit flowers. However, no pollination was found which was totally different from the other bees. There was more effectiveness in cucurbit pollination from the other bees. This may be because of the small size of the stingless bees which has the potential to collect and transport food in small quantities. When they had the food from one sex flower as needed, then they would return to the nest without visiting any other flowers. So, there was no transmission of pollen that caused seed formation.

Various disadvantages of the stingless bees made them not helpful in pollination of confectionery sunflowers, which were differed from the European honey bees. The European honey bees were able to facilitate seed formation of confectionery sunflowers well in a single swarm (Pisanty *et al*., 2013). With the appropriately visited times, the body size of the European species of bees is larger than the stingless bees, resulting in an ease to touch and carry reproductive parts of the flowers. As a result, European honey bees were good pollinators suitable for confectionery sunflowers' pollination.

Therefore, the matching between the plants and the pollinators should take into account in various constraints on both sides such as pollinator morphology, size and foraging arragement on focal plant (Sahli and conner,

2007), including the floral morphology and bio-physiological variation between flowers, for the effective pollination.

4. Conclusions

European honey bees exhibited the potential as effective pollinators better than stingless bee to increase the seed yield of the confectionery sunfowers. This is because the European bee visited the sunflowers throughout the day, and the European honey bee pollination had statistical differences of total seed number, good seed weight and good seed number, compared to those of stingless bee pollination and other treatments.

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