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Effect of Different Beehives Size and Daily Activity of Stingless Bee *Tetragonula Laeviceps* on Bee-Pollen Production

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ABSTRACT

Bee activities and colony may affect the production of bee products. Bee-pollen is one of the important products of honeybees, besides of honey. *Tetragonula laeviceps* is a group member of stingless bees found in tropical region. In Indonesia, *Tetragonula laeviceps* mostly create a nest in bamboos that make unfortunately difficult for harvesting of its products. The aim of this study was to investigate the effect of different beehives size and the daily activity (morning vs. afternoon) of stingless bee *Tetragonula laeviceps* on bee-pollen production. This study was conducted in Ngrandu, Katongan Village, Sub-district of Nglipar, Gunungkidul, Yogyakarta. Forty colonies of *Tetragonula laeviceps* were obtained from bamboos (similar in diameter and length) were transferred into four different group of beehives size (BS) made from dried wood, respectively: 35x17.5x13.5 (BS1); 35x20x17.5 (BS2); 37.5x20x20 (BS3) and 40x20x20 cm (BS4). Each group of beehives size had ten colonies/beehives as replicates. The colonies consisted of a queen bee, eggs, worker bees, and drones were transferred into wood beehives, and they were kept for two months for the study. The bee-pollen was harvested from beehives, separated from the propolis then weighed. The production of bee-pollen in the four different beehives size (BS1: 2.34±2.48 g/colony; BS2: 4.56±3.94 g/colony; BS3: 1.30±1.22 g/colony, BS4: 1.02±0.63 g/colony) was not different (ns) among the groups. The daily activity of bee bring the pollen to the nest in the morning (07.00 to 11.00) was significantly different ($P<0.05$) among the groups, but not significant (ns) different in the afternoon (14.00 to 17.00). The frequency of bringing in pollen into the hives at the morning is higher than at the afternoon (21.75 vs. 12.43 times/5 minutes). It could be concluded that the colony in beehive size of BS2 (35x20x17.5 cm) has higher daily activities and thus higher bee-pollen production, compared to the other beehives groups.

Keywords: Bee-pollen, Stingless bees, *Tetragonula laeviceps*, Tropical bee

Introduction

Between honeybees including stingless bees and flowering plants have a special relationship in which both of them get the benefit. Honeybees obtain nectar and pollen as a food from flowers and the plants are pollinated by honeybees. Honeybees do visit flowers to collect pollen and nectar, as a source of protein, fats, vitamins, and minerals which are essential for honeybee's growth and development, repairing of worn out tissue and stimulating the development of hypopharyngeal glands (Abrol, 2011; Malerbo-Souza, 2011). The production of honey, wax and royal jelly are directly related to the amount of pollen needed to feed the colony (Malerbo-Souza, 2011). Foraging labour's bee is divided into some of individuals forage for nectar, some for pollen, some carrying both and some for fewer with water. Foraging activity requires information about locations, distances between them and the availability of food by guide bees (Abrol, 2011; Sihombing, 2005).

Tetragonula laeviceps is a group of stingless bees that can be found in the tropics areas. The *Tetragonula laeviceps* in Indonesia are known as *Trigona* bee; in the local name especially in Java called *klanceng*. In every region of Indonesia, *Trigona* bee has a different local name. In Indonesia, the *Trigona* sp. has a natural habitat in the trunk of trees or woods, bamboos, sugar palm stalks and in the ground. Making a nest in everything made from bamboo, for instance in bamboo roof of houses and animal cages, and in the living bamboo is the natural habitat of *Tetragonula laeviceps* (Agussalim, 2015). In addition, many stingless bees species created nest in the cavities that could be found in the stem and branches of living trees including bamboo, dead logs either standing or lying on the ground, old and abandoned ant hills, cracks in

walls of houses, and cavities in unused panel doors of buildings (Kwapong *et al*., 2010).

The natural habitat of *Tetragonula laeviceps* could cause some problems such as the difficulty in controlling the health and growth colonies, also the difficulty of harvesting bee-pollen and damage the hive structure in the colony. Thus, one of the strategies is needed to move or transfer the *Tetragonula laeviceps* colonies from their natural habitat into the box that made for example from dried wood boards to ease the harvest (Agussalim, 2015).

All of the honeybees including stingless bees needs the pollen as a source of protein for the colony. The pollen is produced by anthers of plant flowers. Forager bees collect the pollen from the flowers and then was mixed with nectar or with a small dose of salivary glands secretion. The mixture then was placed in a specific basket like, called corbiculae, which located on the tibia of their hind legs, and transported further to the hive (Sihombing, 2005; Komosinska-Vassev *et al*., 2015). Production of bee-pollen or bee-bread by worker bees is depend on the pollen requirement of the colony, the availability of pollen sources like plant flowers, bees activity outside the nest and forager bees experience on collect pollen, and also the temperature and humidity of environment (Sihombing, 2005; Agussalim, 2015). Pollen that has been collected by stingless bees is put and placed in the pot pollen. The aim of this study was to investigate the effect of different beehives size and the daily activities of stingless bee *Tetragonula laeviceps* on bee-pollen production.

Materials and Methods

Research materials

This study was conducted in Ngrandu, Katongan Village, Sub-district of Nglipar Gunungkidul, Yogyakarta. The research materials were stingless bees *Tetragonula laeviceps* (more over similar in diameter and length) as much as forty colonies that were obtained from the bamboos in around the Gunungkidul district area. The collected colonies then transferred into four groups of different beehives size (BS) respectively were BS1 (35 x 17.5 x 13.5 cm), BS2 (35 x 20 x 17.5 cm), BS3 (37.5 x 20 x 20 cm), and BS4 (40 x 20 x 20 cm). Each group of beehives size consisted of ten colonies (or ten beehives) as the replication. The beehives materials were made from dried wood board of Mahogany trees, while the house or cage was made from the woods consist of four stacking racks with the size was of 250 x 250 x 300 cm. Furthermore, the beehives were assisted to the source of food, permitting for the forager bees to collect easer nectar and pollen. The study was using complete randomized design with five different beehives size and ten replications (number of beehives) for each size.

Colony transfer

The colonies obtained from the bamboos were transferred into four different beehives size. Transfer of colonies included of the queen bee, brood cells contain eggs and larvae, worker/forager bees and drones were performed at night to avoid the stress. The hives were then placed on the house or cage for about two months of beekeeping or known as a meliponiculture (Agussalim, 2015). The number of worker bees and drones were not counted because of the difficulty to differentiate between both of them at night. During the beekeeping or meliponiculture period, beehives conditions were controlled once a week from pests especially ants to make sure the properness and ideal conditions of study.

Research variables

The variables of the study were the production of bee-pollen *Tetragonula laeviceps* that was measured after two months of beekeeping (meliponiculture). The bee-pollen was harvested by cutting the propolis attached from the beehives and then propolis was separated from bee-pollen. The bee-pollen separated from propolis was weighed by the digital scale (expressed in gram). The daily activity was noted when the worker bees bring in pollen that was characterized by the corbiculae having attached the pollen. Due to the technical problem, the weight of pollen from each corbicula was not measured. The number of worker bees brings in the pollen were counted three times a week (Tuesday, Thursday, and Saturday) by two hand counter manually at a distance of one meter from the entrance. The daily activities of bees was distinguished in two periods of control that were morning (from 07:00 to 11:00 am) and afternoon (from 14:00 to 17:00 pm). The frequency of bees bring pollen into the hives was monitored by counting how many numbers of bees during five minutes per colony enter into the hives. In addition, the types of flowering plants and/or trees as a source of pollen, existed around 100 meters from the hives, were identified and recorded.

Data analysis

The data of production of bee-pollen and daily activity of *Tetragonula laeviceps* at morning and at afternoon were analyzed by one-way variance analysis followed by Duncan's multiple range test and T-test by SPSS for Windows® 23.

Results and Discussion

Results

The forager bees of *Tetragonula laeviceps* started to collect pollen from the plant flowers on the first day after the colonies transferred from bamboos to beehives. The pollen was collected by forager bees in order to produce bee-pollen or bee bread in the colony. The forager bees of *Tetragonula laeviceps* went outside of the hives to collect nectar, pollen and bee glue in the morning started from 05:25 to 05:30 am and bring in to the hives for the first time from 05:40 to 05:45 am.

The pollen collected by forager bees from plant flowers had various colors consisted of light

beige, beige, yellow, orange, and dark depend upon plant types as the source of pollen. The types of the plant as the source of pollen consisted of coconut, spinach, banana, acacia, Mexican creeper, pomelo, rice, maize, and stink beans (Table 1). The result showed that the production of bee-pollen of stingless bee *Tetragonula laeviceps* in four different beehives size (Table 2) did not significantly different (ns).

Based on the trends that production of bee-pollen from the higher to the lower production were respectively BS2 (4.56±3.94 g/colony/2 months or 27.36 g/colony/year), BS1 (2.34±2.48 g/colony/2 months or 14.04 g/colony/year), BS3 (1.30±1.22 g/colony/2 months or 7,80 g/colony/year), and BS4 (1.02±0.63 g/colony/2 months or 6.12 g/colony/year).

Beehive size had significant (P<0.05) offer on daily activity of forager bee bring in pollen at the morning (07:00 to 11:00 am) (Table 2) was significantly different (P<0.05) on BS1 and BS2, but not on BS3 and BS4 (P>0.05). Beehive BS2 was the highest activity (25.36±0.53 times/5 minutes/colony), then followed by BS1 (21.33±0.39 times/5 minutes/colony), BS4 (20.17±0.76 times/5 minutes/colony), and BS3 (20.15±0.82 times/5 minutes/colony). While, the bring in pollen by forager bees at the afternoon (14:00 to 17:00 pm) (Table 2) into beehives were not significantly different among the groups. Daily activity at morning (07:00 to 11:00 am) almost two times higher (P<0.05) compared to the activity at the afternoon (14:00 to 17:00 pm). The results showed activity at the morning vs. the afternoon for BS1: 21.33 vs. 12.19 times/5 minutes/colony, BS2: 25.36 vs. 12.85 times/5 minutes/colony, BS3: 20.15 vs. 12.45 times/5 minutes/colony, and for BS4: 20.17 vs. 12.24 times/5 minutes/colony, respectively (Table 2).

Discussion

Bee-pollen is a natural product collected by the bees from plant flowers. The pollen contains protein, vitamins, and minerals that is required by honeybees or stingless bees to build or made healthy colonies (Sihombing, 2005; Abrol, 2011). Worker bees collect pollen from the flowers by hugging their body repeatedly to the flower so that the pollen attaches to the body hairs, especially on the thorax, jaw, and also tongue. The sticked pollen in the body was collected using a pollen brush on all three feets which are then placed in the corbicula (Sihombing, 2005).

The activity of stingless bee *Tetragonula laeviceps* in Ngrandu, Gunungkidul Indonesia started from 05:25 to 05:30 am to collect pollen from plant flowers and then bring in the pollen into the hives for the first time around 05:40 to 05:45 am. Different result was reported previously by Agussalim (2015) that *Trigona* sp. in North Lombok, West Nusa Tenggara Province, Indonesia, starts collecting nectar, pollen, and resin from plants started at 06:00 am. Different location, geography and time the sun rise may affect the start time of daily bee activity in the morning. Thakur *et al*. (1982) reported that *Apis cerana* foraging on Mustard had considerable high activity in the morning, while *Apis mellifera* start their activity at 10:00 to 10:30 am. Chandel *et al*. (2004) reported that *Apis dorsata* had the maximum foraging period started from 06:30 to 18:55, followed by *Apis cerana* at 06:45 to 18:30 and *Apis mellifera* had the least foraging period at 07:25 to 18:20 on Onion seed crop. *Apis cerana* and *Apis florea* commenced foraging on onion plants between 09:00 and 09:30 and 08:15 and 08:40, respectively (Bhalla *et al*. 1983; Mattu *et al*., 1994). African honey bees *Apis mellifera* collected only pollen in male flowers of maize, with a highest frequency of 9.00 am in year 2009 and between 16.00 and 17.00 pm in year 2010 (Malerbo-Souza, 2011). The different result has been reported by some other researchers. It might be caused by the different origin or geographical, environmental condition (temperature and humidity), standard time zone, species of honeybees or stingless bees.

The higher production of bee-pollen by colony in BS2 (4.56±3.94 g/colony or 27.36 g/colony/year) might be caused by the higher activity of forager bees in bring in bee-pollen at 07:00 to 11:00 am compared to other beehives group. In the morning, the forager bee were more active to collect pollen compared in the afternoon activities. It was shown that the activity in bringing in pollen of the BS2 group at the morning (07:00 to 11:00 am) was significantly higher (25.36 vs. 12.85 times/5 minutes/colony) compared to the afternoon (14:00 to 17:00 pm) (Table 2). This phenomenon might be explained by the fact that in the location of study, majority the flowers had been blooming in the morning, that might increased the activity of forager bees to collect the pollen. The number of worker bees as a forager collect pollen from plants. Brood cells number as the candidate of forager bees may also affected

Table 1. Plant/tree types as source of pollen for stingless bee *Tetragonula laeviceps*

Plant types	Latin name	Indonesian name	Source	Pollen color
Coconut	*Cocos nucifera*	Kelapa	Nectar/Pollen	Beige
Spinach	*Amaranthus* spp.	Bayam	Pollen	Light beige
Banana	*Musa paradisiaca* L.	Pisang	Nectar/Pollen	Light beige
Acacia	*Acacia* spp.	Akasia	Nectar/Pollen	Yellow
Mexican creeper	*Antigonon leptopus*	Air mata pengantin	Nectar/Pollen	Yellow
Pomelo	*Citrus maxima*	Jeruk bali	Nectar/Pollen	Beige
Rice	*Oryza sativa*	Padi	Pollen	Yellow
Stink beans	*Parkia speciosa*	Petai	Pollen	Light beige
Maize	*Zea mays*	Jagung	Pollen	Yellow

Table 2. Production of bee-pollen and in different beehives daily activity of stingless bee *Tetragonula laeviceps*

Variables	Beehives size (BS)			
	35x17.5x13.5 (BS1)	35x20x17.5 (BS2)	37.5x20x20 (BS3)	40x20x20 (BS4)
Production of bee-pollen (g)^{ns}	2.34±2.48	4.56±3.94	1.30±1.22	1.02±0.63
Frequency in the bringing in pollen at the morning (07:00 to 11:00) (times/5 minutes per colony)	21.33±0.39^{bx}	25.36±0.53^{ax}	20.15±0.82^{cx}	20.17±0.76^{cx}
Frequency in the bringing in pollen at the afternoon (14:00 to 17:00) (times/5 minutes per colony)^{ns}	12.19±0.53^{y}	12.85±0.43^{y}	12.45±0.76^{y}	12.24±0.56^{y}

^{abc}The different superscripts in the same row indicate significantly different (P<0.05)
^{xy}The different superscripts in the same column indicate significantly different (P<0.05)
ns: not significant.

the production of bee-pollen. In this present study, the number of workers bee were not counted due to the technical constraints. The similar result was reported previously by Agussalim (2015) that *Trigona* sp. in Papak, North Lombok, West Nusa Tenggara Province had produced bee-pollen of 4.76±3.94 g/colony/2 months beekeeping period with relatively similar in variety of plants with this present study such as of coconut, banana, and aratiles as the source of pollen.

Type of plants or trees, and its flowering potential may provide relatively the same potential of pollen. The species of the plant as the source of pollen in the Ngrandu, Gunungkidul Yogyakarta consisted of coconut, spinach, banana, acacia, mexican creeper, pomelo, rice, stink beans, and maize plants that have different period of blooming and duration of flowering. Agussalim *et al*. (2018) reported that coconut, banana, acacia, rice, and maize are the source of pollen that highly potential and available in Gunungkidul, Yogyakarta, Indonesia. Martínez-Hernández *et al*. (1994) reported that plants as the source of nectar and pollen for *Nannotrigona testaceicornis, Trigona (Tetragonisca) angustula, Scaptotrigona mexicana and Plebeia sp.* in the Tacaná region, Chiapas, Mexico consisted of *Ageratum houstonianum, Alchornea latifolia, Trema micrantha, Coffea arabica,* and *Citrus limon*. Malerbo-Souza (2011) stated that corn was an important source of pollen for African honey bees *Apis mellifera*.

Sihombing (2005) explained that the amount of pollen collected by forager bees depended on several factors i.e. honeybees or stingless bees and environmental factors (temperature, humidity, flowering plants and trees availability etc.). The honeybees or stingless bees might be influenced by bee species and size of colony that will then determine the size of corbicula, the experience of forager bees to find and collecting pollen. The environmental factors such as temperature, humidity, light intensity, and season or climate may also the important role in the honeybee products. Indeed, the most probable important factor which determines the collectable pollen is the presence of the phytosterol compound. The production of bee-pollen in the colonies dependent on the requirement of honeybees of stingless bees especially queen bee.

The lower production of bee-pollen from BS1, BS3, and BS4 might be caused by several factors for example the poor of colonies development such as the number of brood cells, number of worker bees (forager bee), the low activity for exit and entrance of the hives which impact on the low activity in the bringing in pollen by forager bees (Table 2). In addition, the low production of bee-pollen might be also caused by the low productivity of queen bee in each beehive, that may impact on the low number of forager bees. The queen bee requirement for bee-pollen as the main source of protein that required in the process for the eggs production is also one of the important factors (Sihombing, 2005).

Conclusions

Production of bee-pollen from colonies of stingless bee *Tetragonula laeviceps* was influenced by the daily activity of forager bees to collect pollen from the plant flowers and the availability of plants or trees in surrounding areas. The production of bee-pollen in the beehive size of 35 x 20 x 17.5 cm (BS2 group) was higher (4.56±3.94 g/colony or 27.36 g/colony/year) than other groups. Daily activity in the bringing in pollen at the morning (07:00 to 11:00) was also higher (25.36 times/5 minutes/colony) compared to the other beehives. For all beehives size, the daily activities at the morning is also higher than at the afternoon. It is much of interest in the future that the study on the chemical composition and biological properties of bee-pollen from Indonesia that is produced by stingless bees. The bee-pollen might be usefull as the functional food, therapetic agency, and for traditional or modern medicine to improve human health. Further study need to be done.

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