BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel : Propolis mixture production and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

Jurnal : Livestock Research for Rural Development, Volume 33, Number 6, June 2021

Penulis : Dr. Ir. Erwan, M.Si.

No.	Perihal	Tanggal
1	Bukti Submit Artikel ke Livestock Research for Rural Development (LRRD) dan Artikel yang di Submit	22 Maret 2021
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4	Bukti Submit Perbaikan/Revisi Artikel dan Artikel Hasil Perbaikan Revisi Pertama	5 April 2021
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BUKTI SUBMIT ARTIKEL KE LIVESTOCK RESEARCH FOR RURAL DEVELOPMENT (LRRD) DAN ARTIKEL YANG DI SUBMIT (22 MARET 2021)

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LRRD

1 pesan

erwan apis <apiserwan@gmail.com> 22 Maret 2021 pukul 11.43
Kepada: Reg Preston <reg.preston@gmail.com>

Dear Professor T R Preston, Ph.D., D.Sc.
as the Senior Editor in LRRD
in Colombia

Good afternoon, we hope Prof. T R Preston, Ph.D., D.Sc. is always healthy, happy in doing the activity every day. I'm Erwan from the Faculty of Animal Science, University of Mataram, Indonesia has been published one paper in LRRD as follows : **Erwan, Astuti M, Syamsuhaidi, Muhsinin M and Agussalim 2020** The effect of different beehives on the activity of foragers, honey pots number and honey production from stingless bee *Tetragonula* sp. *Livestock Research for Rural Development. Volume 32, Article #158.* http://www.lrrd.org/lrrd32/10/apise32158.html

very interesting for our team to send or submit again the second our paper with identity as follows:
Title : Production of propolis and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

Authors: **Erwan**, Suhardin, Syamsuhaidi, Dwi Kusuma Purnamasari, Muhammad Muhsinin and Agussalim

we hope our second paper can be accepted and published in LRRD

Best Regards,

Dr. Erwan
Faculty of Animal Science, University of Mataram, Indonesia

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ARTIKEL YANG DI SUBMIT KE LIVESTOCK RESEARCH FOR RURAL DEVELOPMENT

Production of propolis and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

Erwan, Suhardin, Syamsuhaidi, Dwi Kusuma Purnamasari, Muhammad Muhsinin and Agussalim[1]

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Abstract

The objectives of present study were to determine the foragers exit activity, propolis weight, and propolis production from honey pots and bee bread pots of stingless bee *Tetragonula* sp. from different beehives. In this study was used 30 colonies of *Tetragonula* sp. obtained from bamboo hives and divided into 2 groups (each 15 colonies) consisted of box hives with size 40 x 20 x 15 cm and bamboo hives with diameter was ranged from 7 to 8 cm and length was ranged 40 to 50 cm. The colonies were transferred from natural hives to box and bamboo hives were done at night consisted of a queen bee, workers, drones, and brood cells. The variables were measured consisted of the exit activity of foragers, propolis weight, and propolis production from stingless bee *Tetragonula* sp. Afterwards, all the colonies were meliponiculture for thirty days. The present results showed that the exit activity of foragers, propolis weight, and propolis production from honey pots and bee bread pots (big, medium, small, and total production from stingless bee *Tetragonula* sp. was higher in box hives than in bamboo hives ($p<0.01$).

Keywords: meliponiculture, box hive, nectar, bamboo hive, foragers

Introduction

The stingless bees number in the world that have been identified are 500 species and unidentified are minimum 100 species (Michener 2013). The number of stingless bees species in Indonesia are minimum 46 species from the genus (*tribe*: *Meliponini*) *Austroplebeia* Moure, *Geniotrigona* Moure, *Heterotrigona* Schwarz, *Homotrigona* Moure, *Lepidotrigona* Schwarz, *Lisotrigona* Moure, *Papuatrigona* Michener dan Sakagami, *Pariotrigona* Moure, *Tetragonula* Moure, dan *Wallacetrigona* Engel, and Rasmussen (Kahono et al 2018). Stingless bees consist of three castes are a queen, workers, and drones and each caste have a different work i.e. a queen to produce eggs, drones to mating a young queen, and workers to perform all of the tasks inside the hive (building nest construction, caring brood cells, to produce honey, bee bread, propolis). Furthermore, in the outside of hives the tasks of workers such as collecting nectar, water, pollen, resin, and other materials that are required to build a nest) (Michener 2013).

Indonesia, especially in North Lombok Regency is mostly found in the stingless bees species create a nest in bamboos, sugar palm stalks, and tree or woods (Erwan et al 2020; Agussalim et al 2015). One of the stingless bee species is *Tetragonula* sp. that can produce honey, bee bread, and propolis. Erwan et al (2020) reported production of honey from stingless bee

(*Tetragonula* sp.) in box hives for big, medium, and small pots are 6.68 ml, 7.22 ml, and 4.82 ml, respectively. Furthermore, in bamboo hives is 2.65 ml, 4.07 ml and 2.46 ml for each big, medium, and small pots, respectively after four weeks meliponiculture. In addition, the propolis production from honey pots of stingless bee *Tetragonula* sp. in various beehives ranged from 18.20 to 30.08 g after meliponiculture for two months. Furthermore, production of propolis from stingless bee *Tetragonula laeviceps* is 15.4 to 77.2 g after 4 months meliponiculture (Agussalim et al 2020), but the information of propolis production each pot from honey pots and bee bread pots is lacking. Therefore, the objectives of present study were to determine the foragers exit activity, propolis weight, and propolis production from honey pots and bee bread pots from different beehives.

Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive was used have a length was 40 to 50 cm and diameter was 7 to 8 cm, while the box hive has a size 40 x 20 x 15 cm. The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days.

Foragers daily activity

The daily activity was measured was exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter was ranged from 0.9 to 1.3 cm), medium pot (diameter was ranged from 0.6 to 0.8 cm), and small pot (diameter was ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis production

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested with cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a

digital scale. The plants as the resin source to produce propolis were identified with checking availability of resin from living plants wound.

Statistical analysis

The data production of propolis, propolis weight each pot, the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at $p<0.05$*

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and *Lepidotrigona terminata* (Atmowidi et al 2018), *Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that collected by honeybees or stingless bees from wound of living plants and then mix with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%, essential oils is 10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2). Propolis weight each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives are affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins can be collected by foragers.

Table 2. Propolis weights each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hives (g)	Box hives (g)	SEM	*P*
Honey pots				
Big	0.14^{b}	0.17^{a}	0.006	0.005
Medium	0.08^{b}	0.10^{a}	0.004	0.003
Small	0.05^{b}	0.07^{a}	0.003	0.001
Bee bread pots				
Big	0.13^{b}	0.17^{a}	0.007	0.016
Medium	0.08^{b}	0.10^{a}	0.003	0.017
Small	0.06^{b}	0.08^{a}	0.002	0.001

^{a,b} *Different superscripts within rows indicate differences at $p<0.05$*

Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

Table 3. Propolis production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08[b]	3.08[a]	0.21	0.000
Medium	1.29[b]	2.52[a]	0.16	0.000
Small	0.90[b]	1.80[a]	0.13	0.000
Total	3.28[b]	7.40[a]	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63[b]	0.92[a]	0.07	0.044
Small	0.54[b]	0.97[a]	0.07	0.001
Total	2.04[b]	3.16[a]	0.16	0.000

[a,b] *Different superscripts within rows indicate differences at p<0.05*

The propolis production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). This study indicates that *Tetragonula* sp. foragers collect much more collecting resin to make honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study is not measured. The plant types as the resin sources were mango, cashew, and banana. Propolis production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020). The different propolis production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis production from box hives was higher than bamboo hives in the morning and afternoon.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in box hives was 0.17 g for a big pot, 0.10 g for a medium pot, and ranged from 0.07 to 0.08 g for a small pot.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in bamboo hives ranged from 0.13 to 0.14 g for a big pot, 0.8 g for a medium pot, and 0.5 to 0.6 g for a small pot.
- The total production of propolis from honey pots from stingless bee *Tetragonula* sp. was 7.40 g (box hives), 3.28 g (bamboo hives), 3.16 g for bee bread pots (box hives), and 2.04 g (bamboo hives) after meliponiculture thirty days.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial supporting and for permitting our team to conduct this study.

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On Sun, Mar 21, 2021 at 11:44 PM erwan apis <apiserwan@gmail.com> wrote:

> Dear Professor T R Preston, Ph.D., D.Sc.
> as the Senior Editor in LRRD
> in Colombia
>
> Good afternoon, we hope Prof. T R Preston, Ph.D., D.Sc. is always healthy, happy in doing the activity every day. I'm Erwan from the Faculty of Animal Science, University of Mataram, Indonesia has been published one paper in LRRD as follows : **Erwan, Astuti M, Syamsuhaidi, Muhsinin M and Agussalim 2020** The effect of different beehives on the activity of foragers, honey pots number and honey production from stingless bee *Tetragonula* sp. *Livestock Research for Rural Development. Volume 32, Article #158.* http://www.lrrd.org/lrrd32/10/apise32158.html
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> very interesting for our team to send or submit again the second our paper with identity as follows:
> Title : Production of propolis and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives
>
> Authors: **Erwan**, Suhardin, Syamsuhaidi, Dwi Kusuma Purnamasari, Muhammad Muhsinin and Agussalim
>
> we hope our second paper can be accepted and published in LRRD
>
> Best Regards,
>
> Dr. Erwan
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erwan apis <apiserwan@gmail.com> 23 Maret 2021 pukul 06.45
Kepada: Reg Preston <reg.preston@gmail.com>

Dear Professor T R Preston, Ph.D., D.Sc.
Senior Editor in LRRD

Thanks very much for this information and we wait the revise and comment for our paper

Best Regards,

Dr. Erwan
Faculty of Animal Science, University of Mataram, Indonesia

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Reg Preston <reg.preston@gmail.com> 26 Maret 2021 pukul 22.10
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It is an interesting job. I think they should adjust according to the comments made. I suggest improving the discussion as well. As well as, make inferences about the size of the hive in terms of space (Volume) since there are very large differences between both hives.

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(26 MARET 2021)

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Commented [U1]: I don't know which is first name and surname, please reference in authors guide

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Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive was used have a length was 40 to 50 cm and diameter was 7 to 8 cm, while the box hive has a size 40 x 20 x 15 cm. The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days.

Commented [U2]: The authors should especifc Authors should specific Length, width and height.

Commented [U3]: As it is verified that the size of the containers are not a product of the size of the hive. The box hive is 30000cc and the bamboo hive is only 2010cc maximum. As we know that the collection processes are not affecting the colony (size) and therefore the production (the harvest of trigones).

Foragers daily activity

The daily activity was measured was exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter was ranged from 0.9 to 1.3 cm), medium pot (diameter was ranged from 0.6 to 0.8 cm), and small pot (diameter was ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis production

Commented [U4]: I am in doubt about the term propolis. Because the authors say that it is the containers of bee bread and honey that are evaluated. I think the containers are made of wax and not propolis.

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested with cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a

digital scale. The plants as the resin source to produce propolis were identified with checking availability of resin from living plants wound.

Statistical analysis

The data production of propolis, propolis weight each pot, the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at p<0.05*

Commented [U5]: The frequency of workers leaving is a consequence of the size of the colony. The size of the colony is influenced by the size of the hive. If the bamboo colonies are smaller, so is their population.

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and *Lepidotrigona terminata* (Atmowidi et al 2018), *Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that collected by honeybees or stingless bees from wound of living plants and then mix with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%, essential oils is 10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2). Propolis weight each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives are affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins can be collected by foragers.

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Table 2. Propolis weights each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hives (g)	Box hives (g)	SEM	*P*
Honey pots				
Big	0.14^{b}	0.17^{a}	0.006	0.005
Medium	0.08^{b}	0.10^{a}	0.004	0.003
Small	0.05^{b}	0.07^{a}	0.003	0.001
Bee bread pots				
Big	0.13^{b}	0.17^{a}	0.007	0.016
Medium	0.08^{b}	0.10^{a}	0.003	0.017
Small	0.06^{b}	0.08^{a}	0.002	0.001

^{a,b} *Different superscripts within rows indicate differences at p<0.05*

Commented [U7]: I think a more equitable analysis should be done. For the reasons stated above that may be influencing the size of the pots and the production of the wax mix. As for example the number of pots and the weight of mixture that they produce per hive.

Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

Table 3. Propolis production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08[b]	3.08[a]	0.21	0.000
Medium	1.29[b]	2.52[a]	0.16	0.000
Small	0.90[b]	1.80[a]	0.13	0.000
Total	3.28[b]	7.40[a]	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63[b]	0.92[a]	0.07	0.044
Small	0.54[b]	0.97[a]	0.07	0.001
Total	2.04[b]	3.16[a]	0.16	0.000

[a,b] *Different superscripts within rows indicate differences at p<0.05*

The propolis production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). This study indicates that *Tetragonula* sp. foragers collect much more collecting resin to make honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study is not measured. The plant types as the resin sources were mango, cashew, and banana. Propolis production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020). The different propolis production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis production from box hives was higher than bamboo hives in the morning and afternoon.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in box hives was 0.17 g for a big pot, 0.10 g for a medium pot, and ranged from 0.07 to 0.08 g for a small pot.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in bamboo hives ranged from 0.13 to 0.14 g for a big pot, 0.8 g for a medium pot, and 0.5 to 0.6 g for a small pot.
- The total production of propolis from honey pots from stingless bee *Tetragonula* sp. was 7.40 g (box hives), 3.28 g (bamboo hives), 3.16 g for bee bread pots (box hives), and 2.04 g (bamboo hives) after meliponiculture thirty days.

Commented [U8]: Unfortunately the conclusions do not consider the effect of the size of the hive and these conclusions can lead to a misinterpretation in the analysis.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial supporting and for permitting our team to conduct this study.

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Kepada: Reg Preston <reg.preston@gmail.com>

Dear Professor T R Preston, Ph.D., D.Sc.
Senior Editor LRRD
in Colombia

Thanks very much for comment our paper and we will revise and improving according to suggestion in the paper file also we improving the inferences or conclusion

Best Regards,

Dr. Erwan
Faculty of Animal Science, University of Mataram
[Kutipan teks disembunyikan]

BUKTI SUBMIT PERBAIKAN/REVISI ARTIKEL DAN ARTIKEL HASIL PERBAIKAN
(5 APRIL 2021)

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erwan apis <apiserwan@gmail.com> 5 April 2021 pukul 20.05
Kepada: Reg Preston <reg.preston@gmail.com>

Dear Professor T R Preston, Ph.D., D.Sc.
Senior Editor in LRRD
in Colombia

We send the revised version our paper and in the text also commented by the reviewer about the propolis. Honey pot and bee bread pot is made from propolis and it is called as the propolis according to references as follows:
1. Ryo Miyata, Muhamad Sahlan, Yoshinobu Ishikawa, Hiroshi Hashimoto, Sari Honda, and Shigenori Kumazawa. 2020. Propolis Components from Stingless Bees Collected on South Sulawesi, Indonesia, and Their Xanthine Oxidase Inhibitory Activity. J. Nat. Prod., 82 (2): 205−210, 10.1021/acs.jnatprod.8b00541. and 2. Abduh M Y, Adam A, Fadhlullah M, Putra R E and Manurung R 2020 Production of propolis and honey from *Tetragonula laeviceps* cultivated in modular *Tetragonula* hives. Heliyon, 6: e05405.

in addition, the methods, discussion, and conclusion have been revised

Best regards,

Dr. Erwan
Faculty of Animal Science, University of Mataram, Indonesia

[Kutipan teks disembunyikan]

LRRD Journal ERWAN March 2021 First Revise.docx
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ARTIKEL HASIL PERBAIKAN PERTAMA
(5 APRIL 2021)

Production of propolis and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

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Abstract

The objectives of present study were to determine the foragers exit activity, propolis weight, and propolis production from honey pots and bee bread pots of stingless bee *Tetragonula* sp. from different beehives. In this study was used 30 colonies of *Tetragonula* sp. obtained from bamboo hives and divided into 2 groups (each 15 colonies) consisted of box hives with size 40 x 20 x 15 cm and bamboo hives with diameter was ranged from 7 to 8 cm and length was ranged 40 to 50 cm. The colonies were transferred from natural hives to box and bamboo hives were done at night consisted of a queen bee, workers, drones, and brood cells. The variables were measured consisted of the exit activity of foragers, propolis weight, and propolis production from stingless bee *Tetragonula* sp. Afterwards, all the colonies were meliponiculture for thirty days. The present results showed that the exit activity of foragers, propolis weight, and propolis production from honey pots and bee bread pots (big, medium, small, and total production from stingless bee *Tetragonula* sp. was higher in box hives than in bamboo hives ($p<0.01$).

Keywords: meliponiculture, box hive, nectar, bamboo hive, foragers

Introduction

The stingless bees number in the world that have been identified are 500 species and unidentified are minimum 100 species (Michener 2013). The number of stingless bees species in Indonesia are minimum 46 species from the genus (*tribe*: *Meliponini*) *Austroplebeia* Moure, *Geniotrigona* Moure, *Heterotrigona* Schwarz, *Homotrigona* Moure, *Lepidotrigona* Schwarz, *Lisotrigona* Moure, *Papuatrigona* Michener dan Sakagami, *Pariotrigona* Moure, *Tetragonula* Moure, dan *Wallacetrigona* Engel, and Rasmussen (Kahono et al 2018). Stingless bees consist of three castes are a queen, workers, and drones and each caste have a different work i.e. a queen to produce eggs, drones to mating a young queen, and workers to perform all of the tasks inside the hive (building nest construction, caring brood cells, to produce honey, bee bread, propolis). Furthermore, in the outside of hives the tasks of workers such as collecting nectar, water, pollen, resin, and other materials that are required to build a nest) (Michener 2013).

Indonesia, especially in North Lombok Regency is mostly found in the stingless bees species create a nest in bamboos, sugar palm stalks, and tree or woods (Erwan et al 2020; Agussalim et al 2015). One of the stingless bee species is *Tetragonula* sp. that can produce honey, bee bread, and propolis. Erwan et al (2020) reported production of honey from stingless bee

(*Tetragonula* sp.) in box hives for big, medium, and small pots are 6.68 ml, 7.22 ml, and 4.82 ml, respectively. Furthermore, in bamboo hives is 2.65 ml, 4.07 ml and 2.46 ml for each big, medium, and small pots, respectively after four weeks meliponiculture. In addition, the propolis production from honey pots of stingless bee *Tetragonula* sp. in various beehives ranged from 18.20 to 30.08 g after meliponiculture for two months. Furthermore, production of propolis from stingless bee *Tetragonula laeviceps* is 15.4 to 77.2 g after 4 months meliponiculture (Agussalim et al 2020), but the information of propolis production each pot from honey pots and bee bread pots is lacking. Therefore, the objectives of present study were to determine the foragers exit activity, propolis weight, and propolis production from honey pots and bee bread pots from different beehives.

Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive was used have a length or height was 50 cm and diameter or width was 8 cm with the volume was 2,514 cm^3, while the box hive has a size 40 x 20 x 15 cm with the volume was 12,000 cm^3. The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days. In addition, the workers number was not counted because very difficult to count them in the night.

Foragers daily activity

The daily activity was measured was exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter was ranged from 0.9 to 1.3 cm), medium pot (diameter was ranged from 0.6 to 0.8 cm), and small pot (diameter was ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis production

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested with cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a

digital scale. The plants as the resin source to produce propolis were identified with checking availability of resin from living plants wound.

Statistical analysis

The data production of propolis, propolis weight each pot, the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The foragers exit was higher in the box hives is affected by the workers number or their population in the hive especially foragers in the box hive might be much more than in the bamboo hive and consequently the exit activity of foragers also higher in box hive than in bamboo hive. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at p<0.05*

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and *Lepidotrigona terminata* (Atmowidi et al 2018), *Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and

light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that collected by honeybees or stingless bees from wound of living plants and then mix with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%, essential oils is 10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2). Propolis weight each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives might be affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins can be collected by foragers is much more.

Table 2. Propolis weight each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hives (g)	Box hives (g)	SEM	*P*
Honey pots				
Big	0.14^{b}	0.17^{a}	0.006	0.005
Medium	0.08^{b}	0.10^{a}	0.004	0.003
Small	0.05^{b}	0.07^{a}	0.003	0.001
Bee bread pots				
Big	0.13^{b}	0.17^{a}	0.007	0.016
Medium	0.08^{b}	0.10^{a}	0.003	0.017
Small	0.06^{b}	0.08^{a}	0.002	0.001

^{a,b} *Different superscripts within rows indicate differences at* $p<0.05$

Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

Table 3. Propolis production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08[b]	3.08[a]	0.21	0.000
Medium	1.29[b]	2.52[a]	0.16	0.000
Small	0.90[b]	1.80[a]	0.13	0.000
Total	3.28[b]	7.40[a]	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63[b]	0.92[a]	0.07	0.044
Small	0.54[b]	0.97[a]	0.07	0.001
Total	2.04[b]	3.16[a]	0.16	0.000

[a,b] *Different superscripts within rows indicate differences at p<0.05*

The propolis production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). Furthermore, affected by number of workers in the box hives might be much more than in bamboo hives, however in our study the workers number not counted. This study indicates that *Tetragonula* sp. foragers collect much more collecting resin to made honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study is not measured. In addition, depend on requirement in the hive, for example if in the hive the honey is fulfilled, afterwards the foragers can be collecting pollen, water, and other materials that required to construct the nest or otherwise. Agussalim et al (2015) reported that various box hives with different volume is not affecting propolis production of stingless bee *Tetragonula* sp., but in our study the different volume of hives (box and bamboo hives) is affecting the propolis production might be the number of workers in both box and bamboo hives is different. The plant types as the resin sources were mango, cashew, and banana. Propolis production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020; Abduh et al 2020). The different propolis production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The volume of hives (box and bamboo hives) is affecting the exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis production in the morning and afternoon from the stingless bee *Tetragonula* sp.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in box hives with volume 12,000 cm^3 is 0.17 g for a big pot, 0.10 g for a medium pot, and ranged from 0.07 to 0.08 g for a small pot.
- Propolis weight from honey pots and bee bread pots of stingless bee (*Tetragonula* sp.) in bamboo hives with volume 2,514 cm^3 is ranged from 0.13 to 0.14 g for a big pot, 0.8 g for a medium pot, and 0.5 to 0.6 g for a small pot.
- The total production of propolis is 7.40 g from honey pots, 3.16 g from bee bread pots for box hives, 3.28 g from honey pots, and 2.04 from bee bread pots for bamboo hives.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial supporting and for permitting our team to conduct this study.

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KOMENTAR KEDUA ARTIKEL DARI REVIEWER
(7 APRIL 2021)

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Reg Preston <reg.preston@gmail.com> 7 April 2021 pukul 23.25
Kepada: erwan apis <apiserwan@gmail.com>

The study is interesting, and that we can publish it, for me it would be good if they improve these two comments
1.Review the term propolis: (when it is really a mixture of resins (propolis), wax and other products). They can change "Propolis production", for propolis mixture production.
2. the production values, it is the unbalanced of the boxes and how these can influence the size of the population, the size of the containers and the production. In their writing they acknowledge it but do not infer about the effects.
• Transfer of colony
The stingless bee (Tetragonula sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive was used have a length or height was 50 cm and diameter or width was 8 cm with the volume was 2,514 cm 3 , while the box hive has a size 40x 20 x 15 cm with the volume was 12,000 cm 3 . The colonies were transferred to bamboo and
box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days. In addition, the workers number was not counted because very difficult to count them in the night.
Taking into account the editorial in the previous issue of the journals , it is suggested to include photos of the boxes or hives and also of the bees, it is already a different species.

Editorial

LRRD encourages the inclusion of photos in articles that contain topics that relate to different local or regional resources (vegetative and animal species, by-products, breeds/varieties), when these are used in the production systems that are the subject of the research. The sources of the photos should be indicated.

Sincerely

Professor T R Preston, PhD, DSc

Investigador Emérito
Centro para la Investigación en Sistemas Sostenibles
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Carrera 25 No 6-62 Cali, Colombia

Senior Editor, Livestock Research for Rural Development
http://www.lrrd.org (The international on-line journal on sustainable livestock-based agriculture)

Tropical Animal Production
http://www.cipav.org.co/TAP/tapindex.htm

Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics
http://www.cipav.org.co/PandL/Preston_Leng.htm

El sitio Web sobre Producción Tropical Sostenible (Universidad de los Llanos, Colombia)
www.produccionтropicalsostenible.info

Web site (old) of MEKARN I

http://hostcambodia.com/mekarn/indexold.htm

---------- Forwarded message ---------
From: **Felix Augusto Moreno Elcure** <famorenoe@ut.edu.co>
Date: Tue, Apr 6, 2021 at 12:01 PM
Subject: Re: 210321apise
To: Reg Preston <reg.preston@gmail.com>

Estimado Dr.
Cordial saludo.
He revisado el archivo y han realizado algunos ajustes al artículo.

Ellos justifican con una referencia el uso inadecuado de un término Propolis (cuando es realmente una mezcla de resinas (propolis), cera y otros productos). Podemos insistir que haga la aclaración o dejar asi. Yo pienso que ellos podrían cambiar el nombre Producción de propóleo "Propolis production", por producción de mezcla de propóleo). Pues se seguirá incurriendo en el error.

propolis [**prop**-*uh*-lis] **SHOW IPA**

---

noun

a reddish resinous cement collected by bees from the buds of trees, used to stop up crevices in the hives, strengthen the cells, etc.

Otra observación, que tiene que ver con los valores de producción, es lo desequilibrado de las cajas y como estas pueden influir en el tamaño de la población, el tamaño de los recipientes y la producción. En su escrito lo reconocen pero no infieren sobre los efectos.

Transfer of colony
The stingless bee (Tetragonula sp.) as much 30 colonies obtained from bamboos were
adapted one week before domesticated and divided into 2 groups each 15 colonies for
bamboo and box hives. The bamboo hive was used have a length or height was 50 cm and
diameter or width was 8 cm with the volume was 2,514 cm 3 , while the box hive has a size 40
x 20 x 15 cm with the volume was 12,000 cm 3 . The colonies were transferred to bamboo and
box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for
thirty days. In addition, the workers number was not counted because very difficult to count
them in the night.

Pero no hacen referencia a este efecto, lo cual es conservador y que debería considerarse para nuevos estudios.

Pienso que ellos quieren publicar sus resultados pero hay problemas de comunicación. Yo creo que el estudio es interesante, y que podemos publicarlo, para mi seria bueno que ellos mejoren estos dos comentarios.

El mar, 6 abr 2021 a las 10:37, Reg Preston (<reg.preston@gmail.com>) escribió:

> Hola Felix.
> Respondió el autor.
> un abrazo
> Professor T R Preston, PhD, DSc
>
> Investigador Emérito
> Centro para la Investigación en Sistemas Sostenibles
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> Carrera 25 No 6-62 Cali, Colombia
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> Senior Editor, Livestock Research for Rural Development
> http://www.lrrd.org (The international on-line journal on sustainable livestock-based agriculture)
>
> Tropical Animal Production
> http://www.cipav.org.co/TAP/tapindex.htm
>
> Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics
> http://www.cipav.org.co/PandL/Preston_Leng.htm
>
> El sitio Web sobre Producción Tropical Sostenible (Universidad de los Llanos, Colombia)
> www.produccion tropicalsostenible.info
>
> Web site (old) of MEKARN I
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> http://hostcambodia.com/mekarn/indexold.htm
>
> [Kutipan teks disembunyikan]

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BUKTI SUBMIT PERBAIKAN/REVISI ARTIKEL DAN ARTIKEL HASIL PERBAIKAN REVISI KEDUA (8 APRIL 2021)

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erwan apis <apiserwan@gmail.com> 8 April 2021 pukul 14.09
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Dear Professor TR Preston, Ph.D., D.Sc.
Senior Editor LRRD
in Colombia

Thanks very much for the comment our paper and we have been revise the paper and attached the second revised version

Best Regards,

Dr. Erwan
Faculty of Animal Science, University of Mataram, Indonesia

[Kutipan teks disembunyikan]

LRRD Journal ERWAN March 2021 Second Revise.docx
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ARTIKEL HASIL PERBAIKAN REVISI KEDUA
(8 APRIL 2021)

Propolis mixture production and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

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Abstract

The objectives of present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots of stingless bee *Tetragonula* sp. from different beehives. In this study was used 30 colonies of *Tetragonula* sp. obtained from bamboo hives and divided into 2 groups (each 15 colonies) consisted of box hives with size 40 x 20 x 15 cm and bamboo hives with diameter was ranged from 7 to 8 cm and length was ranged 40 to 50 cm. The colonies were transferred from natural hives to box and bamboo hives were done at night consisted of a queen bee, workers, drones, and brood cells. The variables were measured consisted of the exit activity of foragers, propolis weight, and propolis mixture production from stingless bee *Tetragonula* sp. Afterwards, all the colonies were meliponiculture for thirty days. The present results showed that the exit activity of foragers, propolis weight, and propolis mixture production from honey pots and bee bread pots (big, medium, small, and total production from stingless bee *Tetragonula* sp. was higher in box hives than in bamboo hives ($p<0.01$).

Keywords: meliponiculture, box hive, nectar, bamboo hive, foragers

Introduction

The stingless bees number in the world that have been identified are 500 species and unidentified are minimum 100 species (Michener 2013). The number of stingless bees species in Indonesia are minimum 46 species from the genus (*tribe*: *Meliponini*) *Austroplebeia* Moure, *Geniotrigona* Moure, *Heterotrigona* Schwarz, *Homotrigona* Moure, *Lepidotrigona* Schwarz, *Lisotrigona* Moure, *Papuatrigona* Michener dan Sakagami, *Pariotrigona* Moure, *Tetragonula* Moure, dan *Wallacetrigona* Engel, and Rasmussen (Kahono et al 2018). Stingless bees consist of three castes are a queen, workers, and drones and each caste have a different work i.e. a queen to produce eggs, drones to mating a young queen, and workers to perform all of the tasks inside the hive (building nest construction, caring brood cells, to produce honey, bee bread, propolis). Furthermore, in the outside of hives the tasks of workers such as collecting nectar, water, pollen, resin, and other materials that are required to build a nest) (Michener 2013).

Indonesia, especially in North Lombok Regency is mostly found in the stingless bees species create a nest in bamboos, sugar palm stalks, and tree or woods (Erwan et al 2020; Agussalim et al 2015). One of the stingless bee species is *Tetragonula* sp. that can produce honey, bee bread, and propolis. Erwan et al (2020) reported production of honey from stingless bee

(*Tetragonula* sp.) in box hives for big, medium, and small pots are 6.68 ml, 7.22 ml, and 4.82 ml, respectively. Furthermore, in bamboo hives is 2.65 ml, 4.07 ml and 2.46 ml for each big, medium, and small pots, respectively after four weeks meliponiculture. In addition, the propolis mixture production from honey pots of stingless bee *Tetragonula* sp. in various beehives ranged from 18.20 to 30.08 g after meliponiculture for two months. Furthermore, production of propolis from stingless bee *Tetragonula laeviceps* is 15.4 to 77.2 g after 4 months meliponiculture (Agussalim et al 2020), but the information of propolis mixture production each pot from honey pots and bee bread pots is lacking. Therefore, the objectives of present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots from different beehives.

Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive volume was 2,514 cm^3 (length was 50 cm and diameter was 8 cm), while the box hive volume was 12,000 cm^3 (length was 40, width was 20, and height was 15 cm). The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days. In addition, the workers number was not counted because very difficult to count them in the night.

Foragers daily activity

The daily activity was measured was exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter was ranged from 0.9 to 1.3 cm), medium pot (diameter was ranged from 0.6 to 0.8 cm), and small pot (diameter was ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis mixture production

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested with cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a

digital scale. The plants as the resin source to produce propolis were identified with checking availability of resin from living plants wound. The describe of colony of stingless bee *Tetragonula* sp. was shown in Figure 1 and 2.

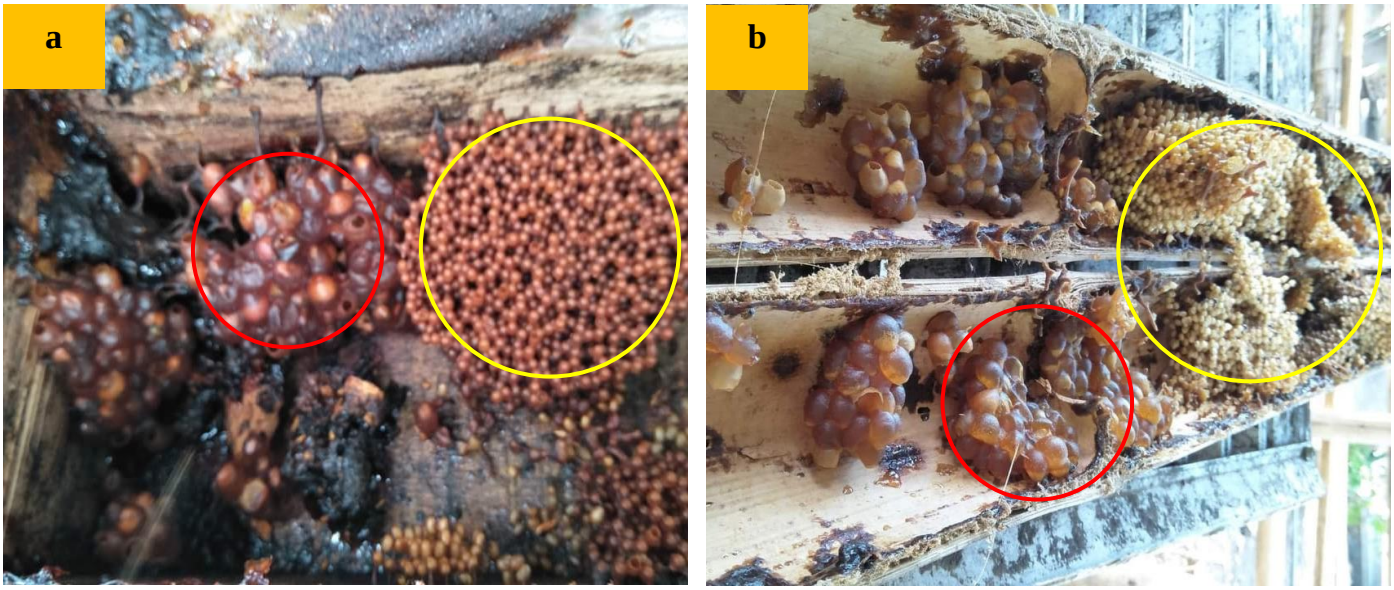

Figure 1. The describe of colony from stingless bee *Tetragonula* sp. in each hive (a. box hive, b. bamboo hive, red circle was honey pots, and yellow circle was brood cells).

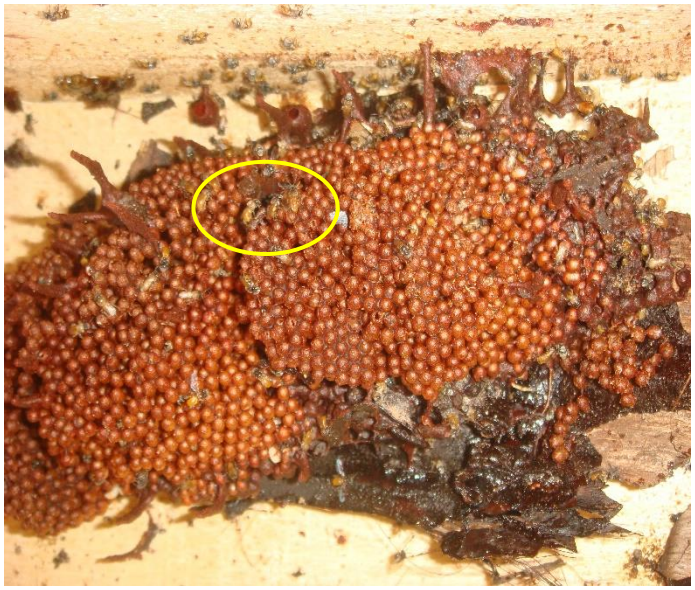

Figure 2. The stingless bee *Tetragonula* sp. was characterized by the abdomen is yellow color (inside yellow circle)

Statistical analysis

The data production of propolis, propolis weight each pot, the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The foragers exit was higher in the box hives is affected by the workers number or their population in the hive especially foragers in the box hive might be much more than in the bamboo hive and consequently the exit activity of foragers also higher in box hive than in bamboo hive. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at $p<0.05$*

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and *Lepidotrigona terminata* (Atmowidi et al 2018), *Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that collected by honeybees or stingless bees from wound of living plants and then mix with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%, essential oils is

10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2).

Table 2. Propolis weight each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hives (g)	Box hives (g)	SEM	*P*
Honey pots				
Big	0.14[b]	0.17[a]	0.006	0.005
Medium	0.08[b]	0.10[a]	0.004	0.003
Small	0.05[b]	0.07[a]	0.003	0.001
Bee bread pots				
Big	0.13[b]	0.17[a]	0.007	0.016
Medium	0.08[b]	0.10[a]	0.003	0.017
Small	0.06[b]	0.08[a]	0.002	0.001

[a,b] *Different superscripts within rows indicate differences at p<0.05*

Propolis weight each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives might be affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins can be collected by foragers is much more. Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis mixture production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis mixture production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis mixture production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis mixture production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

The propolis mixture production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). Furthermore, affected by number of workers and the availability of bee bread in the box hives might be much more than in bamboo hives, however in our study not measured. The bee bread is the main source of protein in the hive that required by the workers to produce royal jelly as the queen bee feed and consequently the productivity of queen bee is increases to producing eggs as the workers and propolis mixture production in box hives.

Table 3. Propolis mixture production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis mixture production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08[b]	3.08[a]	0.21	0.000
Medium	1.29[b]	2.52[a]	0.16	0.000
Small	0.90[b]	1.80[a]	0.13	0.000
Total	3.28[b]	7.40[a]	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63[b]	0.92[a]	0.07	0.044
Small	0.54[b]	0.97[a]	0.07	0.001
Total	2.04[b]	3.16[a]	0.16	0.000

[a,b] *Different superscripts within rows indicate differences at p<0.05*

This study indicates that *Tetragonula* sp. foragers collect much more collecting resin to made honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study is not measured. In addition, depend on requirement in the hive, for example if in the hive the honey is fulfilled, afterwards the foragers can be collecting pollen, water, and other materials that required to construct the nest or otherwise. Agussalim et al (2015) reported that various box hives with different volume is not affecting propolis mixture production of stingless bee *Tetragonula* sp., but in our study the different volume of hives (box and bamboo hives) is affecting the propolis mixture production might be the number of workers in both box and bamboo hives is different. The plant types as the resin sources were mango, cashew, and banana. Propolis mixture production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020; Abduh et al 2020). The different propolis mixture production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The volume of hives (box and bamboo hives) is affecting the exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis mixture production in the morning and afternoon from the stingless bee *Tetragonula* sp.
- Propolis weight, propolis mixture production, and daily activities in the box hives is higher than in bamboo hives from honey pots and bee bread pots.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial supporting and for permitting our team to conduct this study.

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Propolis mixture production and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

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Abstract

The objectives of the present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots of stingless bee *Tetragonula* sp. from different beehives. In this study was used 30 colonies of *Tetragonula* sp. obtained from bamboo hives and divided into 2 groups (each 15 colonies) consisted of box hives with size 40 x 20 x 15 cm and bamboo hives with diameter was 8 cm and length was 50 cm. The colonies were transferred from natural hives to box and bamboo hives were done at night consisted of a queen bee, workers, drones, and brood cells. The variables measured consisted of the exit activity of foragers, propolis weight, and propolis mixture production from stingless bee *Tetragonula* sp. Afterwards, all the colonies were meliponiculture for thirty days. The present results showed that the exit activity of foragers, propolis weight, and propolis mixture production from honey pots and bee bread pots (big, medium, small, and total production from stingless bee *Tetragonula* sp. was higher in box hives than in bamboo hives ($p<0.01$).

Keywords: meliponiculture, box hive, nectar, bamboo hive, foragers

Introduction

The stingless bees number in the world that have been identified are 500 species and unidentified are minimum 100 species (Michener 2013). The number of stingless bees species in Indonesia are minimum 46 species from the genus (*tribe*: *Meliponini*) *Austroplebeia* Moure, *Geniotrigona* Moure, *Heterotrigona* Schwarz, *Homotrigona* Moure, *Lepidotrigona* Schwarz, *Lisotrigona* Moure, *Papuatrigona* Michener dan Sakagami, *Pariotrigona* Moure, *Tetragonula* Moure, dan *Wallacetrigona* Engel, and Rasmussen (Kahono et al 2018). Stingless bees consist of three castes are a queen, workers, and drones and each caste have a different work i.e. a queen to produce eggs, drones to mating a young queen, and workers to perform all of the tasks inside the hive (building nest construction, caring brood cells, to produce honey, bee bread, propolis). Furthermore, in the outside of hives the tasks of workers such as collecting nectar, water, pollen, resin, and other materials that are required to build a nest) (Michener 2013).

Indonesia, especially in North Lombok Regency is mostly found in the stingless bees species that create a nest in bamboos, sugar palm stalks, and tree or woods (Erwan et al 2020; Agussalim et al 2015). One of the stingless bee species is *Tetragonula* sp. that can produce honey, bee bread, and propolis. Erwan et al (2020) reported production of honey from stingless bee (*Tetragonula* sp.) in box hives for big, medium, and small pots are 6.68 ml, 7.22 ml, and

4.82 ml, respectively. Furthermore, in bamboo hives is 2.65 ml, 4.07 ml and 2.46 ml for each big, medium, and small pots, respectively after four weeks of meliponiculture. In addition, the propolis mixture production from honey pots of stingless bee *Tetragonula* sp. in various beehives ranged from 18.20 to 30.08 g after meliponiculture for two months. Furthermore, production of propolis from stingless bee *Tetragonula laeviceps* is 15.4 to 77.2 g after 4 months of meliponiculture (Agussalim et al 2020), but the information of propolis mixture production each pot from honey pots and bee bread pots is lacking. Therefore, the objectives of the present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots from different beehives.

Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as many as 30 colonies obtained from bamboos were adapted one week before domestication and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive volume was 2,514 cm^3 (length was 50 cm and diameter was 8 cm), while the box hive volume was 12,000 cm^3 (length was 40, width was 20, and height was 15 cm). The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days. In addition, the workers' numbers were not counted because it was very difficult to count them at night.

Foragers daily activity

The daily activity was measured as exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at a distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants and was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter ranged from 0.9 to 1.3 cm), medium pot (diameter ranged from 0.6 to 0.8 cm), and small pot (diameter ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis mixture production

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested by cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a digital scale. The plants as the resin source to produce propolis were identified with checking

availability of resin from living plants wound. The description of the colony of stingless bee *Tetragonula* sp. was shown in Figure 1 and 2.

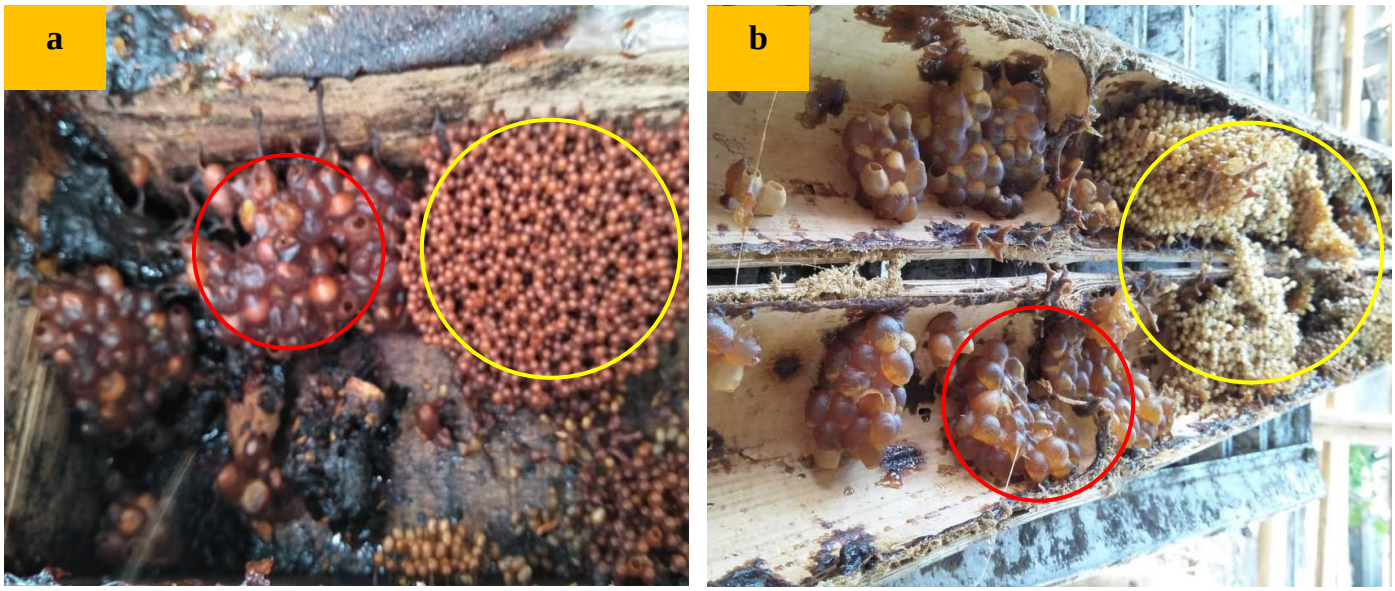

Figure 1. The description of colony from stingless bee *Tetragonula* sp. in each hive (a. box hive, b. bamboo hive, red circle was honey pots, and yellow circle was brood cells).

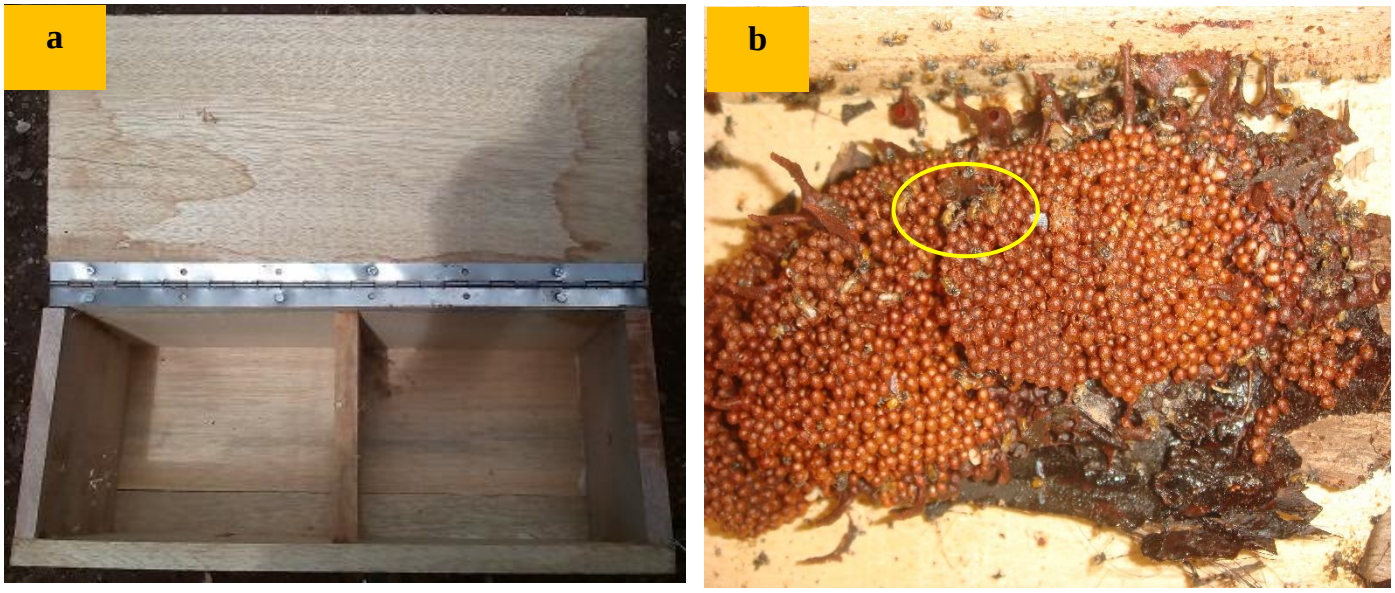

Figure 2. The box hive was used in the study (a) and stingless bee *Tetragonula* sp. was characterized by the abdomen is yellow color (inside yellow circle) (b)

Statistical analysis

The data production of propolis, propolis weight each pot, and the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The foragers exit was higher in the box hives is affected by the workers number or their population in the hive especially foragers in the box hive might be much more than in the bamboo hive and consequently the exit activity of foragers also higher in box hive than in bamboo hive. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at p<0.05*

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and *Lepidotrigona terminata* (Atmowidi et al 2018), *Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that is collected by honeybees or stingless bees from the wound of living plants and then mixed with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%,

essential oils is 10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2).

Table 2. Propolis weight each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hives (g)	Box hives (g)	SEM	*P*
Honey pots				
Big	0.14[b]	0.17[a]	0.006	0.005
Medium	0.08[b]	0.10[a]	0.004	0.003
Small	0.05[b]	0.07[a]	0.003	0.001
Bee bread pots				
Big	0.13[b]	0.17[a]	0.007	0.016
Medium	0.08[b]	0.10[a]	0.003	0.017
Small	0.06[b]	0.08[a]	0.002	0.001

[a,b] *Different superscripts within rows indicate differences at p<0.05*

Propolis weight of each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives might be affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins that can be collected by foragers is much more. Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis mixture production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis mixture production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis mixture production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis mixture production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

The propolis mixture production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). Furthermore, affected by the number of workers and the availability of bee bread in the box hives might be much more than in bamboo hives, however in our study they were not measured. The bee bread is the main source of protein in the hive that is required by the workers to produce royal jelly as the queen bee feed and consequently the productivity of queen bee is increased to produce eggs as the workers and propolis mixture production in box hives.

Table 3. Propolis mixture production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis mixture production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08[b]	3.08[a]	0.21	0.000
Medium	1.29[b]	2.52[a]	0.16	0.000
Small	0.90[b]	1.80[a]	0.13	0.000
Total	3.28[b]	7.40[a]	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63[b]	0.92[a]	0.07	0.044
Small	0.54[b]	0.97[a]	0.07	0.001
Total	2.04[b]	3.16[a]	0.16	0.000

[a,b] *Different superscripts within rows indicate differences at p<0.05*

This study indicates that *Tetragonula* sp. foragers collect much more resin to make honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study it is not measured. In addition, depending on requirements in the hive, for example if in the hive the honey is fulfilled, afterwards the foragers can be collecting pollen, water, and other materials that are required to construct the nest or otherwise. Agussalim et al (2015) reported that various box hives with different volume is not affecting propolis mixture production of stingless bee *Tetragonula* sp., but in our study the different volume of hives (box and bamboo hives) is affecting the propolis mixture production might be the number of workers in both box and bamboo hives is different. The plant types as the resin sources were mango, cashew, and banana. Propolis mixture production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020; Abduh et al 2020). The different propolis mixture production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The volume of hives (box and bamboo hives) is affecting the exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis mixture production in the morning and afternoon from the stingless bee *Tetragonula* sp.
- Propolis weight, propolis mixture production, and daily activities in the box hives is higher than in bamboo hives from honey pots and bee bread pots.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial support and for permitting our team to conduct this study.

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Propolis mixture production and foragers daily activity of stingless bee *Tetragonula* sp. in bamboo and box hives

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Abstract

The objectives of present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots of stingless bee *Tetragonula* sp. from different beehives. In this study was used 30 colonies of *Tetragonula* sp. obtained from bamboo hives and divided into 2 groups (each 15 colonies) consisted of box hives with size 40 x 20 x 15 cm and bamboo hives with diameter was ranged from 7 to 8 cm and length was ranged 40 to 50 cm. The colonies were transferred from natural hives to box and bamboo hives were done at night consisted of a queen bee, workers, drones, and brood cells. The variables were measured consisted of the exit activity of foragers, propolis weight, and propolis mixture production from stingless bee *Tetragonula* sp. Afterwards, all the colonies were meliponiculture for thirty days. The present results showed that the exit activity of foragers, propolis weight, and propolis mixture production from honey pots and bee bread pots (big, medium, small, and total production from stingless bee *Tetragonula* sp. was higher in box hives than in bamboo hives ($p<0.01$).

Keywords: *bamboo hive, box hive, foragers meliponiculture, nectar*

Introduction

The stingless bees number in the world that have been identified are 500 species and unidentified are minimum 100 species (Michener 2013). The number of stingless bees species in Indonesia are minimum 46 species from the genus (*tribe*: *Meliponini*) *Austroplebeia* Moure, *Geniotrigona* Moure, *Heterotrigona* Schwarz,*Homotrigona* Moure, *Lepidotrigona* Schwarz,*Lisotrigona* Moure, *Papuatrigona* Michener dan Sakagami,*Pariotrigona* Moure, *Tetragonula* Moure, dan *Wallacetrigona* Engel, and Rasmussen (Kahono et al 2018). Stingless bees consist of three castes are a queen, workers, and drones and each caste have a different work i.e. a queen to produce eggs, drones to mating a young queen, and workers to perform all of the tasks inside the hive (building nest construction, caring brood cells, to produce honey, bee bread, propolis). Furthermore, in the outside of hives the tasks of workers such as collecting nectar, water, pollen, resin, and other materials that are required to build a nest) (Michener 2013).

Indonesia, especially in North Lombok Regency is mostly found in the stingless bees species create a nest in bamboos, sugar palm stalks, and tree or woods (Erwan et al 2020; Agussalim et al 2015). One of the stingless bee species is *Tetragonula* sp. that can produce honey, bee bread, and propolis. Erwan et al (2020) reported production of honey from stingless bee (*Tetragonula* sp.) in box hives for big, medium, and small pots are 6.68 ml, 7.22 ml, and 4.82 ml, respectively. Furthermore, in bamboo hives is 2.65 ml, 4.07 ml and 2.46 ml for each big, medium, and small pots, respectively after four weeks meliponiculture. In addition, the propolis mixture production from honey pots of stingless bee *Tetragonula* sp. in various beehives ranged from 18.20 to 30.08 g after meliponiculture for two months. Furthermore, production of propolis from stingless bee *Tetragonula laeviceps* is 15.4 to 77.2 g after 4 months meliponiculture (Agussalim et al 2020), but the information of propolis mixture production each pot from honey pots and bee bread pots is lacking. Therefore, the objectives of present study were to determine the foragers exit activity, propolis weight, and propolis mixture production from honey pots and bee bread pots from different beehives.

Materials and methods

Transfer of colony

The stingless bee (*Tetragonula* sp.) as much 30 colonies obtained from bamboos were adapted one week before domesticated and divided into 2 groups each 15 colonies for bamboo and box hives. The bamboo hive volume was 2,514 cm^3 (length was 50 cm and diameter was 8 cm), while the box hive volume was 12,000 cm^3 (length was 40, width was 20, and height was 15 cm). The colonies were transferred to bamboo and box hives were a queen bee, workers, drones, and brood cells and then meliponiculture for thirty days. In addition, the workers number was not counted because very difficult to count them in the night.

Figure 1. The box hive was used in the study

Foragers daily activity

The daily activity was measured was exit activity of foragers from the hive was counted using hand counter check every day for thirty days. The exit activity of foragers was counted at distance 1.5 meter from the hive entrance (5 minutes/hive) was done in the morning at 08:00 am and in the afternoon at 04:00 pm.

Plant types for resin sources

The plant types as the resin sources as the raw material to produce propolis by workers *Tetragonula* sp. was identified with checking the availability of resin from living plants was characterized by sticky material from wound plants.

Propolis weight

Propolis weight consists of propolis from the honey pot and bee bread pot. The propolis from each pot was divided in three categories were big pot (diameter was ranged from 0.9 to 1.3 cm), medium pot (diameter was ranged from 0.6 to 0.8 cm), and small pot (diameter was ranged from 0.3 to 0.5 cm). Propolis from each honey pot and bee bread pots were taken one pot sample and then weighed using a digital scale.

Propolis mixture production

Production of propolis from the stingless bee *Tetragonula* sp. were propolis from the honey pot and bee bread pot after meliponiculture for thirty days. In brief, propolis from each pot was harvested with cutting the propolis in the base as the nest construction and cleared from honey and bee bread. Afterwards, the clean propolis was placed in plastic and then weighed using a digital scale. The plants as the resin source to produce propolis were identified with checking availability of resin from living plants wound. The describe of colony of stingless bee *Tetragonula* sp. was shown in Figure 1 and 2.

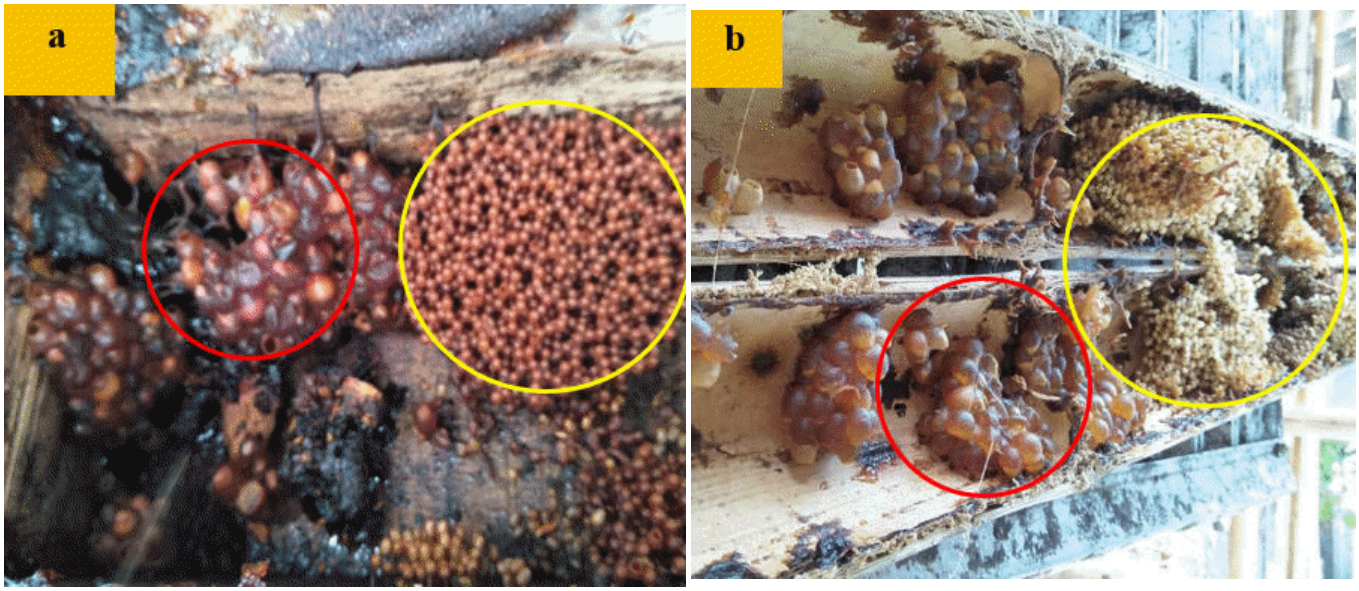

Figure 2. The describe of colony from stingless bee *Tetragonula* sp. in each hive (a. box hive, b. bamboo hive, red circle was honey pots, and yellow circle was brood cells).

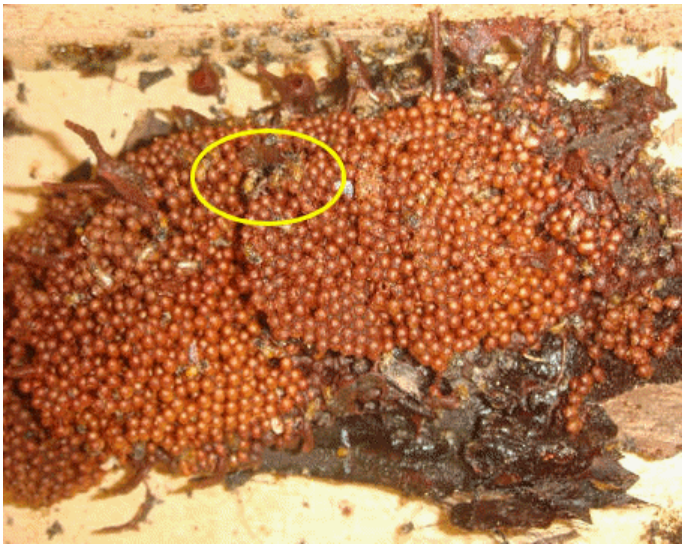

Figure 3. The stingless bee *Tetragonula* sp. was characterized by the abdomen is yellow color (inside yellow circle)

Statistical analysis

The data production of propolis, propolis weight each pot, the foragers exit activity of stingless bee (*Tetragonula* sp.) were analyzed by independent-samples T-test using SPSS statistics version 23.

Results and discussion

Foragers daily activity

The present results showed that the foragers exit activity of stingless bee (*Tetragonula* sp.) in box hives every week was higher than in bamboo hives in the morning and afternoon. The foragers exit activity from box hives in the morning ranged from 49.2 to 51.3 heads in 5 minutes, while in the afternoon it ranged from 29.0 to 29.6 heads in 5 minutes. Furthermore, bamboo hives ranged from 34.7 to 37.5 heads in 5 minutes in the morning and ranged from 24.9 to 25.5 heads in 5 minutes in the afternoon. The foragers exit was higher in the box hives is affected by the workers number or their population in the hive especially foragers in the box hive might be much more than in the bamboo hive and consequently the exit activity of foragers also higher in box hive than in bamboo hive. The higher foragers exit activity in the morning related to the availability of resin from living plants might be much in the morning than in afternoon. In addition, Bankova et al (2000) explained that plants can be secreted substances actively and exuded wounds from plants like materials of lipophilic on leaves, mucilages, gums, resins, and lattices that were collected by foragers to produce propolis.

Table 1. Foragers exit activity from the hive of stingless bee (*Tetragonula* sp.) in bamboo and box hives

Time of observation (weeks)	Exit activity of foragers		SEM	*P*
	Bamboo hives (heads in 5 minutes)	Box hives (heads in 5 minutes)		
Morning at 08:00 am				
First	34.7^{bx}	49.2^{ax}	0.94	0.000
Second	37.3^{bx}	50.2^{ax}	0.91	0.000
Third	36.8^{bx}	51.3^{ax}	0.98	0.000
Fourth	37.5^{bx}	49.8^{ax}	0.93	0.000
Afternoon at 04:00 pm				
First	24.9^{by}	29.6^{ay}	0.46	0.000
Second	25.4^{by}	29.2^{ay}	0.44	0.000
Third	25.5^{by}	29.4^{ay}	0.45	0.000
Fourth	25.2^{by}	29.0^{ay}	0.44	0.000

^{a,b,x,y} *Different superscripts within rows and columns indicate differences at p<0.05*

The foragers exit activity of stingless bee (*Tetragonula* sp.) (Table 1) was differ with previous study for the species of stingless bees were *Tetragonula laeviceps* (Agus et al 2019; Gadhiya and Pastagia 2019; Atmowidi et al 2018), *Heterotrigona itama*, and*Lepidotrigona terminata* (Atmowidi et al 2018),*Tetragonula* sp. (Erwan et al 2020; Agussalim et al 2015), and *Trigona carbonaria* (Heard and Hendrikz 1993). The different exit activity from the hive is affected by the different species of stingless bees, environment conditions (temperature, humidity, and light intensity). Furthermore, affected by the number of workers especially the foragers number.

Propolis weight

Propolis is a sticky dark material that collected by honeybees or stingless bees from wound of living plants and then mix with wax to construct their nest (Bankova et al 2000). Propolis is made from resins or balsams is 45 to 55%, waxes and fatty acids is 25 to 35%, essential oils is 10%, pollen is 5%, and other organics and minerals is 5% (Cherbuliez 2013). The present results showed that propolis weight for one-pot from honey pots and bee bread pots in box hives were higher than propolis weight from bamboo hives (Table 2).

Table 2. Propolis weight each pot for one-pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee *Tetragonula* sp.

Propolis weight	Bamboo hive s (g)	Box hive s (g)	SEM	*P*
Honey pots				
Big	0.14^{b}	0.17^{a}	0.006	0.005
Medium	0.08^{b}	0.10^{a}	0.004	0.003
Small	0.05^{b}	0.07^{a}	0.003	0.001
Bee bread pots				
Big	0.13^{b}	0.17^{a}	0.007	0.016
Medium	0.08^{b}	0.10^{a}	0.003	0.017
Small	0.06^{b}	0.08^{a}	0.002	0.001

^{a,b} Different superscripts within rows indicate differences at $p<0.05$

Propolis weight each pot from honey pots in box hives compared with bamboo hives were 0.17 g versus 0.14 g (big pot), 0.10 g versus 0.08 g (medium pot), and 0.07 g versus 0.05 g (small pot). Furthermore, 0.17 g versus 0.13 g (big pot), 0.10 g versus 0.08 g (medium pot), 0.08 g versus 0.06 g (small pot) for propolis weight from bee bread pots. The higher propolis weight in box hives than bamboo hives might be affected by the workers number in box hives much more than workers number in bamboo hives that impact the number of resins can be collected by foragers is much more. Propolis weight for each pot (big, medium, and small) from honey pots and bee bread pots in the same hives were similar, it might be the same species did not affect the pot size and propolis weight. The size and weight of propolis are affected by the different species of stingless bees, the exit activity from the hives that are involved in collecting resin, and the availability of resin from living plants (Agussalim et al 2015).

Propolis mixture production

The present results showed that production of propolis from honey pots and bee bread pots was higher in the box hives than in bamboo hives. Propolis mixture production from honey pots in box hives compared with bamboo hives was 3.08 g versus 1.08 g of a big pot, 2.52 g versus 1.29 g for a medium pot, 1.80 g versus 0.90 g for a small pot, and total production was 7.40 g versus 3.28 g. In addition, propolis mixture production from bee bread pots in box hives compared with bamboo hives was 1.28 g versus 0.87 g for a big pot (did not differ), 0.92 g versus 0.63 g for a medium pot, 0.97 g versus 0.54 g for a small pot, and total production was 3.16 g versus 2.04 g (Table 3). In addition, our study showed propolis mixture production from stingless bee *Tetragonula* sp. from honey pots much more or higher than in bee bread pots for each pot size.

The propolis mixture production from honey pots and bee bread pots in box hives was higher than bamboo hives because supported by the exit activity of foragers was higher to collect resin from plants living (especially from wound plant) in the morning and afternoon (Table 1). Furthermore, affected by number of workers and the availability of bee bread in the box hives might be much more than in bamboo hives, however in our study not measured. The bee bread is the main source of protein in the hive that required by the workers to produce royal jelly as the queen bee feed and consequently the productivity of queen bee is increases to producing eggs as the workers and propolis mixture production in box hives.

Table 3. Propolis mixture production each pot (big, medium, and small pots) from honey pots and bee bread pots of stingless bee Tetragonula sp.

Propolis mixture production	Bamboos hive (g)	Boxes hive (g)	SEM	*P*
Honey pots				
Big	1.08^{b}	3.08^{a}	0.21	0.000
Medium	1.29^{b}	2.52^{a}	0.16	0.000
Small	0.90^{b}	1.80^{a}	0.13	0.000
Total	3.28^{b}	7.40^{a}	0.45	0.000
Bee bread pots				
Big	0.87	1.28	0.12	0.082
Medium	0.63^{b}	0.92^{a}	0.07	0.044
Small	0.54^{b}	0.97^{a}	0.07	0.001
Total	2.04^{b}	3.16^{a}	0.16	0.000

a,b Different superscripts within rows indicate differences at $p<0.05$

This study indicates that *Tetragonula* sp. foragers collect much more collecting resin to made honey pots than bee bread pots and will have an impact on the honey production is higher than bee bread production, however in our study is not measured. In addition, depend on requirement in the hive, for example if in the hive the honey is fulfilled, afterwards the foragers can be collecting pollen, water, and other materials that required to construct the nest or otherwise. Agussalim et al (2015) reported that various box hives with different volume is not affecting propolis mixture production of stingless bee *Tetragonula* sp., but in our study the different volume of hives (box and bamboo hives) is affecting the propolis mixture production might be the number of workers in both box and bamboo hives is different. The plant types as the resin sources were mango, cashew, and banana. Propolis mixture production of stingless bee (*Tetragonula* sp.) from honey pots and bee bread pots (Table 3) was differ from the previous study for stingless bee *Tetragonula* sp. (Agussalim et al 2015) and *Tetragonula laeviceps* (Agussalim et al 2020; Abduh et al 2020). The different propolis mixture production is affected by the different foragers exit activity, resin source from plants wound, the number of workers or foragers in the colony, and environment conditions (temperature, humidity, and light intensity) (Agussalim et al 2020).

Conclusions

- The volume of hives (box and bamboo hives) is affecting the exit activity of *Tetragonula* sp. foragers, propolis weight, and propolis mixture production in the morning and afternoon from the stingless bee *Tetragonula* sp.
- Propolis weight, propolis mixture production, and daily activities in the box hives is higher than in bamboo hives from honey pots and bee bread pots.

Acknowledgments

The authors thank the Regional Development Planning Agency, Regency of North Lombok, Province of West Nusa Tenggara, Indonesia for financial supporting and for permitting our team to conduct this study.

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Received 21 March 2021; Accepted 17 May 2021; Published 2 June 2021

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Schwarz,*Lisotrigona* -- Schwarz, *Lisotrigona*

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In Materials and methods

Transfer of colony

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In Propolis mixture production

The last sentence

The describe -- The description

Figure 1 and 2. -- Figure 2 and 3.

Figure 2. The describe -- Figure 2. The description

In Results and Discussion

Foragers daily activity

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In Propolis mixture production

In Table 3.

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more collecting resin -- more resin

(*Tetragonula* sp.) -- (*Tetragonula* sp.)

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The describe -- The description

Figure 1 and 2. -- Figure 2 and 3.

Figure 2. The describe -- Figure 2. The description

In Results and Discussion

Foragers daily activity

In Second Paragraph

and*Lepidotrigona* – and *Lepidotrigona*

2018),*Tetragonula* -- 2018), *Tetragonula*

In Propolis mixture production

In Table 3.

Tetragonula sp. -- *Tetragonula* sp.

In third paragraph

First sentence

more collecting resin -- more resin

(*Tetragonula* sp.) -- (*Tetragonula* sp.)

If difficult to delete box label in box hives, we has attach the box hive photo without label in word file

Thanks very much,

--
Best Regards,

Dr. Ir. Erwan, M.Si.
Faculty of Animal Science, University of Mataram, Indonesia

Box hive photo Erwan Journal.docx
150K

Reg Preston <reg.preston@gmail.com> 27 Mei 2021 pukul 07.38
Kepada: erwan apis <apiserwan@gmail.com>

Dear author
The changes have already been made, surely the new version has not been uploaded to the URL.

Professor T R Preston, PhD, DSc

Investigador Emérito
Centro para la Investigación en Sistemas Sostenibles
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Senior Editor, Livestock Research for Rural Development
http://www.lrrd.org (The international on-line journal on sustainable livestock-based agriculture)

Tropical Animal Production
http://www.cipav.org.co/TAP/tapindex.htm

Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics
http://www.cipav.org.co/PandL/Preston_Leng.htm

El sitio Web sobre Producción Tropical Sostenible (Universidad de los Llanos, Colombia)
www.produccion tropicalsostenible.info

Web site (old) of MEKARN I

http://hostcambodia.com/mekarn/indexold.htm

[Kutipan teks disembunyikan]

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erwan apis <apiserwan@gmail.com> 27 Mei 2021 pukul 09.43
Kepada: Reg Preston <reg.preston@gmail.com>

Dear Professor TR Preston, Ph.D., DSc.
Senior Editor LRRD
in Colombia

Thanks very much for this information and we have been check in the website for proofread, but we found some word not yet changed as follows :

in Abstract

was from 8 cm -- was 8 cm

in Introduction

In first paragraph

Michener dan -- Michener and

dan *Wallacetrigona* -- and *Wallacetrigona*

last sentence

nest) -- nest

Materials and methods

in Figure 1. can be replaced with a new photo without label a ? (if can we attach the photo)

Figure 2. The describtion -- **Figure 2.** The description

Thanks very much

[Kutipan teks disembunyikan]

Box hive photo Erwan Journal.docx
150K