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Research Article

The Effectiveness of Stingless Bees on Pollination of Bitter Melon Plants *Momordica charantia* L. (Cucurbitaceae)

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ABSTRACT

This study aimed to measure the effectiveness of stingless bee *Tetragonula* cf. *biroi* pollination on the fruit formation of bitter melon *Momordica charantia* plants. We used hoods on the observed bitter melon plants. In the first hood, stingless bees are inserted to help pollinate 100 bitter melon plants, while in the other hoods, stingless bees are not inserted so that there is no assistance in pollinating the other 100 bitter melon plants. The method used is the focal sampling method for 25 days of observation. Based on the results of the study, stingless bee pollination assistance increased the percentage of the number of flowers that became fruit by 390%, the weight of seeds/fruit by 64%, number of seeds/fruit by 260%, fruit weight by 163%, fruit diameter by 91%, and fruit length by 86%. In addition to the size of the fruit, the shape of the bitter melon pollinated by bees is standard (long and straight). In contrast, the bitter melon that does not get pollination assistance grows with a bent shape resembling the letter "C." Bitter melon is an agricultural commodity that needs pollinating agents such as stingless bees because of its monoecy.

INTRODUCTION

Pollination is an essential factor in forming seeds and fruit of Angiosperms plants through the mechanism of pollen transfer from the anther to the stigma on the flower, which is the initial stage of the reproductive process. Some bees, such as *Amegilla*, *Apis*, stingless bees, and *Xylocopa*, are the primary pollinators of most crops (Delaplane & Mayer 2000). The decline in the population of pollinating bees around agricultural land will decrease the frequency of visits to flowers. Pollination by bees contributed significantly to fruit production, namely 41% on cranberries, 7% on blueberries, 26% on tomatoes, 45% on strawberries, and 22-24% on cotton (Delaplane & Mayer 2000), with estimated economic value amounted to US$ 14,564,000 (FAO 2006). Most horticultural crops require pollinating agents for the successful fertilization process. Bitter melon *Momordica charantia* is one of the crops that require a pollinating agent. Bitter melon is a monoecious plant and has sticky pollen so the presence of pollinating insects can play an essential role in the process of pollen transfer to the stigma. Several studies have reported the effectiveness of insect pollinators on bitter melon plants such as Apidae, Halictidae, Syrpidae, Megachilidae, and Formicidae (Deyto & Cervancia 2009; Subhakar et al. 2011; Balina et al. 2012). Bitter melon is one of vegetables with high economic value in Indonesia. Bitter melon fruit has high nutritional value especially, high ascorbic acid, and iron and as well as medicinal properties against various diseases, including boosting the immune system in HIV-AIDS patients (Behera 2004; Njoroge & van Luijk 2004). The increasing demand for bitter melon is an important reason for optimizing the yield through improved agronomic practices and intensive pollination.

Pollinating bees that intensively visit flowers can speed up the process of pollination and fertilization (Husby et al. 2015). The mutualism symbiosis between pollinating bees and flowering plants provides very good results for the ecosystem, including increasing agricultural crop yields. Diversity and intensive visiting behavior of pollinating insects are positively correlated with fruit yield (Atmowidi et al. 2007). The use of stingless bees in helping pollinate cucurbitaceae plants has been widely reported. The number of seeds produced is better and the size of the fruit is larger (Bisui et al. 2020; Wayo et al. 2020). In this study, we used a stingless bee *T.* cf. *biroi* as a pollinating agent in bitter melon. This study aims to measure the effectiveness of the stingless bee on the success of pollinating bitter melon plants.

MATERIALS AND METHODS

Materials

We conducted this research in March-August 2021 in residential areas outside the agricultural location, Biringere Village, Kec. North Sinjai, Kab. Sinjai, South Sulawesi, Indonesia. In this study, we used one colony of *T.* cf. *biroi* with healthy and active colony group as a pollinating agent against bitter melon plants covered with insect nets.

Methods

Plant setup

A total of 200 bitter melon seeds were sown in polybags that had been given liquid organic fertilizer. After the shoots of bitter melon begin to appear or are about 15-20 days old, the seeds of bitter melon are transferred to land with a spacing of 25 cm x 25 cm. The seeds of bitter melon that have been transferred are watered every day and fertilized once a week using liquid organic fertilizer until the plants flower. The bitter melon plant is covered with insect net if the bitter melon plant has grown and has begun to propagate. Pest control is done manually without using pesticides.

Utilization of *T.* cf. *biroi* as pollinators of bitter melon plants

A total of 100 bitter melon plants that began to flower were covered with insect net mesh 50, size 3 x 4 x 3 m. After the bitter melon begins to blossom, as many as one colony of stingless bees is put in a hood. We also covered 100 other bitter melon plants, but We did not put stingless bees in the lid to prevent bee pollination from assisting.

Observation of foraging behavior of *T.* cf. *biroi*

The activity of bee visits on bitter melon was observed visually for 25 days using the focal sampling method (Martin & Bateson 1986). The visit activities observed were the number of flowers visited per minute (foraging rate), length of visits per flower (flower handling time), and length of visits to bitter melon plantations (Dafni 1992).

Environmental factor measurement

We measured environmental parameters during the bee diversity observation, including humidity and air temperature, wind speed, and light intensity. Environmental data measurements were carried out at 07.00, 09.00, 11.00, 13.00, and 14.00 WIB. The correlation between physical factors and foraging behavior was analyzed using Principal component analysis (PCA) in PAST software.

Measuring the effectiveness of pollinating bees

The effectiveness of bee pollinators was measured on 100 bitter melon plants caged with gauze and contained stingless bee colonies. Another hundred bitter melon plants were confined with gauze, without stingless bee pollination. The effectiveness of bees in pollinating bitter melon was measured by the percentage of the number of fruits formed, fruit size, number of seeds, and seed weight per fruit.

RESULTS AND DISCUSSION

Results

Foraging behavior of *T.* cf. *biroi* on bitter melon

Observation results show that the foraging *T.* cf. *biroi* activity in the cage occurs when the sun rises at 05.30 hrs until the sun sets at 18.00 hrs. The highest number of flowers visited per minute was in the third week, namely 39.8±0.91 flowers, and the least in the fifth week was 30.23±1.03 flowers (Figure 1a). The highest total number of plants visited per trip was 13.33±4.62 plants (first week), and the least in the third week was 10.39±0.62 plants (Figure 1c).

Correlation between environmental parameters and stingless bee's foraging activity

During the 25-day observation, the air temperature increased at 11.00 and decreased in the afternoon, starting at 16.00. The humidity is highest in the morning and gets lower as the air temperature rises. The highest light intensity is at 11.00, and the lowest is at 16.00 (Table 1). The wind speed is relatively low because this research was carried out in a hood, so the wind does not affect the pollination of bitter melon plants (Table 2).

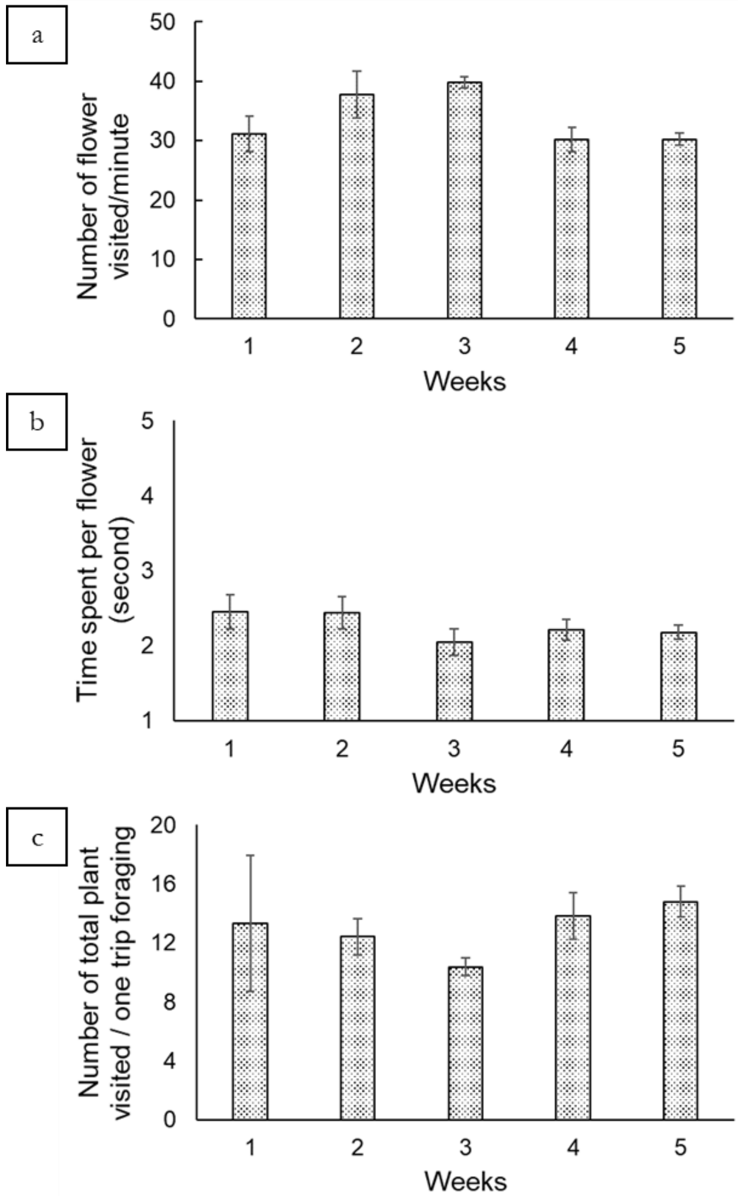

Figure 1. The behavior of *T.* cf. *biroi* visits on caged bitter melon. a) foraging rate, b) flower handling time, c) plant handling time. The first to third week is in May 2021, and the fourth to fifth week is the beginning of June 2021. Bars = standard deviation.

Table 1. Conditions of abiotic factors in the hood for 25 days of observation.

Times	**Abiotic factors**											
	Temperature			Humidity			Light intensity			Wind speed		
	Max	Min	Avrg	Max	Min	Avrg	Max	Min	Avrg	Max	Min	Avrg
07.00	32	17	21,98	73	23	59	1548	176	662,09	1,5	0	0,13
09.00	37	22	27,56	67	23	46,65	1304	99	793,68	10,6	0	0,70
11.00	36	24	29,28	64	19	41,28	1861	210	886,40	20,1	0	1,33
13.00	38	22	27,15	63	27	40,34	1502	113	743	10,5	0	0,79
16.00	30	19	24,8	72	30	36,09	897	53	540,51	0,2	0	0,72

Max = maximum; Min = Minimum; Avrg = Average

Table 2. Pearson correlation between the number of individuals and environmental parameters.

	Number of individuals	Temperature	Humidity	Light intensity	Wind speed
Number of individuals	1				
Temperature (°C)	0,092724996	1			
Humidity (%)	0,32651301	-0,69726	1		
Light intensity (Lux)	0,1070385	0,54137	-0,50304	1	
Wind speed (m/s)	-0,089986299	0,228328	-0,3444	0,159456	1

The effectiveness of *T.* cf. *biroi* on pollinating bitter melon plants

Significant differences were seen in bee-pollinated plants with non-pollinated plants (Table 3). In plants pollinated by *T.* cf. *biroi*, bitter melon fruit is longer and wider in diameter than plants without the help of bee pollination. The number of seeds per fruit in bee-pollinated plants is also more, and the weight of the seeds is heavier than plants that are not pollinated. The percentage of flowers that become fruit on plants pollinated by bees is 98%, while plants that are not pollinated by bees are only 20%, meaning that most (80%) flowers fail to become fruit. The shape of the bee-pollinated bitter melon also looks better, with a straight shape, long and 30.18±1.56 cm larger in size. Meanwhile, plants that were not pollinated by bees had an abnormal shape, bent, with a shorter size of 16.14±2.03 cm (Figure 2).

Figure 2. Bitter melon fruit pollinated by bees (top), and unpollinated bitter melon fruit (bottom).

Table 3. Comparison of fruit pollinated by a stingless bee with fruit without pollination assistance.

Component	n	Plantation		Enhancement (%)
		Pollinated by bees	Bees do not pollinate them	
Fruit length (cm)	25	$30{,}18^{b} \pm 1{,}56$	$16{,}14^{a} \pm 2{,}03$	86
Fruit diameter (cm)	25	$7{,}72^{b} \pm 0{,}51$	$4{,}04^{a} \pm 0{,}92$	91
Fruit weight (g)	25	$415{,}46^{b} \pm 35{,}37$	$157{,}69^{a} \pm 26{,}5$	163
Number of seeds/fruit	25	$31{,}75^{b} \pm 4{,}94$	$8{,}8^{a} \pm 3{,}1$	260
Seed weight/fruit (g)	25	$2{,}34^{b} \pm 0{,}36$	$1{,}42^{a} \pm 0{,}32$	64
Flower to fruit (%)	25	98	20	390

*Different letters in the same row indicate a significant difference with the 95% level T-test.

Discussion

Visiting activities can be a measure of the effectiveness of pollinating insects. A species has the potential as a pollinator if its visitation activity is intensive on one type of plant and is supported by distinctive morphological characters, such as the number of hairs on its body, and has a pollen basket as a pollen storage place (Dafni 1992).

The foraging activity of bees is influenced by the availability of feed and environmental conditions (Sadeh et al. 2007). The flying activity of bees is reduced at low temperatures and high humidity because the bees require a large amount of energy to heat the thoracic temperature to 35 °C (Yao et al. 2006). Visiting activities and the suitability of pollinating bees' body characters with flowers affect the success of pollination. Pollinating bees that visit flowers intensively can speed up the process of pollination and fertilization (Husby et al. 2015). The length of the bee's visit to the flower is influenced by the behavior of taking pollen (Mensah & Kudom 2011; Depra et al. 2014). Bitter melon plants are monoecious, where on one tree, male and female flowers are separated, with the ratio of male and female flowers being 15:1 (Kishan et al. 2017). In general, the Cucurbitaceae have large, sticky pollen. The character of the flower causes the bitter melon plant to need a pollinating agent to transfer pollen to the stigma. Dorjay et al. (2017) reported that the percentage of bitter melon fertilization increased by 87.14%, while in plants that carried out self-pollination, they formed only 2.17% of the fruit. *Cucumis melo* pollinated by a stingless bee produces more fruits and seeds, larger fruit size, and sweeter taste (Azmi et al. 2018). Increases in fruit length and weight and the number of fruits per plant also increased in cucumbers pollinated by *Tetragonula irridipennis* in India (Kishan et al. 2017). In this study, bitter melon fruit from stingless bee pollination had larger fruit, straight fruit shape, and more seeds.

Meanwhile, bitter melon plants that are not pollinated have smaller fruit, fewer seeds, and a curved fruit shape resembling the letter "C". In plants that are not pollinated by bees, there is a decrease in pollen viability after a few hours or a few days (Kahriman et al. 2015). This condition can cause fertilization failure, or fruit malformation occurs when fertilization occurs when pollen viability has decreased.

The number of individuals and the highest number of pollinating bee species occurred in the morning and the least in the afternoon. It was also reported by and Rianti (2010) that the highest abundance of pollinating insects occurred in the morning. This can be affected by the time the bitter melon bloom in the morning. Perfectly blooming flowers with yellow inflorescences are more attractive to pollinating bees (Faheem et al. 2004; Atmowidi et al. 2007).

Environmental factors influence the local distribution of bees in foraging, reproducing and molting (Gottlieb et al. 2005). Light intensity was positively correlated with the number of individuals and species of pollinating bees. Bee fly activity is reduced at low temperatures and high humidity, because it requires a large amount of energy to heat the chest temperature to 35°C (Yao et al. 2006). At low temperatures, the number of individual bees looking for food is also reduced, because the calories needed are also higher (Gerling et al. 1989). Klein et al. (2002) also reported an increase in the number of solitary bees in coffee plantations, along with an increase in light intensity. However, the number of species and the number of individuals were negatively correlated with wind speed.

CONCLUSION

The stingless bee, *T.* cf. *biroi* is a potential pollinator for monoecious bitter melon plants. Pollination by stingless bees can increase the quality and quantity of bitter melon fruit set. The match between the morphology of the bitter melon flower and the body size of the stingless bee causes pollination to occur optimally.

AUTHORS CONTRIBUTION

In this research, A.G.M. was tasked sampling and observing the behavior of bees in the field. RC.H.S. supervised all data analysis. R.E.P. designed the research and supervised all the process. R.R. controlled the sampling activity of bee behavior data and its effectiveness. H.P. supervised the results of data analysis and the manuscript. A.A. supervised data analysis and manuscript writing. S.K. controlled sampling activities in the field, and manuscript writing.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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