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Response of parasitoids to invasive pest *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) on cassava crop in Bali, Indonesia

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Abstract. *Supartha IW, Yudha IKW, Wiradana PA, Susila IW. 2020. Response of parasitoids to invasive pest* Phenacoccus manihoti *Matile-Ferrero* (*Hemiptera:* Pseudococcidae*) on cassava crop in Bali, Indonesia. Biodiversitas 21: 4543-4549. Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) is an invasive pest that entered Indonesia in 2010 and attacks cassava plants, especially in Bali Province. This study aimed to identify species and establish the population distribution mapping, community structures (diversity, abundance, and dominance), and parasitization rate on cassava crops in Bali. This study conducted with a field observation method by taking infested shoots of cassava plants by *P. manihoti* purposively in the field. The cassava mealybug that was present in the leaves or shoots were kept in the laboratory until the parasitoid appeared. The results showed that four species of parasitoids were found to respond to invasive pest, namely *Anagyrus lopezi* (Encyrtidae), *Acerophagus* sp. (Encyrtidae), *Blepyrus* sp. (Encyrtidae) and *Encarsia* sp. (Aphelinidae). Structurally the parasitoid community had a low abundance (R1 = 0.50–0.87) and low diversity index value (H'= 0.27-0.28). While the parasitoid dominance index was in the moderate category with a value D = 0.33–0.59 in Bangli, Klungkung, Badung, Tabanan, and Buleleng Districts. But it had high dominance index values in Gianyar, Denpasar, Jembrana, and Karangasem Districts with a value D = 0.62-0.72. The three species of parasitoid spread evenly in all districts and cities in Bali according to the host distribution map, namely *P. manihoti* on cassava crop. The four parasitoids showed varying parasitic levels in the field with the highest parasitization rate was *A. lopezi* followed by *Acerophagus* sp., *Blepyrus* sp., and *Encarsia* sp.

Keywords: Cassava crop, community structure, invasive pest, parasitoid, *Phenacoccus manihoti*

INTRODUCTION

The cassava mealybug *Phenacoccus manihoti* Matile-Fererro (Hemiptera: Pseudococcidae) is an invasive pest from South America. The pest entered Africa in 1973 and it caused crop losses of up to 82% (Muniappan et al. 2011; Schulthess et al. 1991). In 2008, the pest was reported to have entered Thailand and caused crop losses of around 30% (Parsa et al. 2012; Muhammad et al. 2019). In 2010, *P. manihoti* had entered Indonesia for the first in Bogor (Muniappan et al. 2011). A survey was conducted in the Bogor District showed that the attack cassava crop rate reached 100% with an estimated crop loss of 30-50% (Wardani 2015).

Five important factors affect the life system of cassava mealybug *P. manihoti*, namely: temperature, humidity, plant varieties, and drought stress (Thancharoen et al. 2018; Correa et al. 2012). According to Beltrà (2013) and Charles (2011), natural enemy factors as biological control agents which are the main components in integrated pest control systems are important.

All living things have natural enemies in nature. The role of natural enemies is a key factor in the regulation of populations of pests, especially *P. manihoti* in nature. The utilization and usage of natural enemies to control the population of cassava mealybug need to be sought. The utilization of natural enemy has been successfully carried out to overcome cassava mealybug in 25 countries in Africa and Thailand by using biological control agents such as *Plesiochrysa ramburi* and Coccinellidae beetles as predators of mealybug and *Anagyrus lopezi* (Hymenoptera: Encyrtidae) as cassava mealybug parasitoid (Wyckhuys et al. 2018a; Milonas et al. 2011). Wyckhuys et al. (2018b) also reported that eighteen natural enemy species attacked *P. manihoti*. Among these natural enemies are solitary parasitoids such as *Epidinocarsisn lopezi* (Hymenoptera: Encyrtidae), *Hyperpasic notata*, and *Diomus* spp. (Coleoptera: Coccinellidae), and *Ocyptamus* spp. (Diptera: Syrphidae) that are the most commonly found in Africa.

The entry of new pest species into an area is usually responded to by natural enemies in the area, although their role is sometimes not optimal. Similar cases had been reported in invasive potato leafminer *Liriomyza huidobrensis* and *L. sativae* that entered Indonesia around the 1990s, which were responded by natural enemies from the parasitoids in Indonesia. There were 17 types identified of parasitoid Indonesia associated with *Liriomyza* spp. (Supartha 2003). Supartha (2003) and Wahyuni et al. (2017) also reported the same evidence of the response of parasitoid to the entry of invasive pest *L. huidobrensis* and *L. sativae* to Bali and Lesser Sunda.

Preliminary results showed that the cassava mealybug *P. manihoti* that entered and attacked cassava crops in Bali were also responded to by parasitoids. There were four types of parasitoids associated with cassava mealybug *P. manihoti* in the field, namely *Anagyrus lopezi* (Encyrtidae), *Acerophagus* sp. (Encyrtidae), *Blepyrus* sp. (Encyrtidae), and *Encarsia* sp. (Aphelinidae). Based on the results of the exploration, it is necessary to understand community structure (diversity, abundance, and dominance), map the population distribution and the level of parasitization to ensure the natural enemies' potential as biological control agents of cassava mealybug *P. manihoti* on cassava crop in Bali.

MATERIALS AND METHODS

Study area

Phenacoccus manihoti and their parasitoids were sampled simultaneously on cassava crops carried out in eight districts and one city in Bali Province, Indonesia namely in Badung, Bangli, Buleleng, Gianyar, Jembrana, Karangasem, Klungkung, Tabanan Districts, and Denpasar City from March to May 2019.

Procedures

Sampling was done by diagonal method with an area of 500 m^2 per sample unit. The leaf was taken purposively sampling of 3 varieties of cassava crops which were indicated with *P. manihoti* attack symptoms. The sample was put in a clear plastic bag measuring 2 liters in size separately for each sample which were then stored temporarily in a cool box to be brought to the Integrated Pest Management Laboratory (IPMLaB) of the Faculty of Agriculture, Udayana University, Bali, Indonesia. The leaves or shoots of plants containing mealybugs were sorted and put into a maintenance hood made of mylar plastic measuring 90 cm high with a diameter of 18 cm. All samples were then transferred into a growth chamber and maintained at room temperature (28^{o}C) for further development of their parasitoids. The emerged adults of parasitoids were carefully separately, and each was kept in a small vial volume of 10 mL containing 80% alcohol for further identification. The samples parasitoid were identified by morphological using the key provided by Noyes and Hayat (1986) Noyes (2000).

Data analysis

To determine the community structure of the parasitoid, measurements were made on the index of species diversity, abundance index, and dominance index of the parasitoid.

Measurement of diversity index used an index developed by Shannon and Wiener (H`) (Magurran 2005) through the following equation:

$$H' = -\Sigma pi \ln pi$$
$$pi = \sum ni/N$$

Where:
H' = Shannon Wiener Index
ni= Number of individuals for the species observed
N= Total number of individuals

The diversity index was grouped into three categories, namely: if H'<1.5. diversity is low. Furthermore, if H'= 1.5-3.5 the diversity is moderate, and if the value of H > 3.5, the diversity is high.

Measurement of abundance index used the Margalef index (Magurran 2005).

$$R1 = \frac{S-1}{\ln N}$$

Where:
R1 = Abundance index
S = Number of species found
ln = Nature logarithm
N = Total number of individuals
Values:
R1 < 3.5 = low
R1 >3.5-<5.0 = moderate
R1 > 5.0 = high

Measurement of dominance index used the Menheinick index (Magurran 2005).

$$D = \sum \left(\frac{ni\,(ni-1)}{N\,(N-1)} \right)$$

Where:
D = Dominance index
N = Total number of individuals
Ni = Number of i-species individuals
Values:
D = ≤ 0.00 – 0.30: low
D = > 0.30 – 0.60: moderate
D = ≥ 0.60 – 1.00: high

Parasitization level of parasitoid

$$Parasitization = \frac{\sum Parasitoid\ A}{\sum \text{Appeared parasitoid} + \sum Host}$$

Where:
∑ Parasitoid A = The number of one type of appeared parasitoid
∑ Host = Total number of the host

The results of data analysis were presented in tables and map images.

RESULTS AND DISCUSSION

The results showed that four species of parasitoids were responsive to the invasive of cassava mealybug *P. manihoti* namely *A. lopezi*, *Acerophagus* sp., *Blepyrus* sp., and *Encarsia* sp. (Figure 1.). Except for *Encarsia* sp., all of the species were found in Bangli, Gianyar, Klungkung, Karangasem, Badung, Denpasar, Tabanan, and Jembrana Districts, while *Encarsia* sp. (Hymenoptera: Aphelinidae) was only found in Buleleng District.

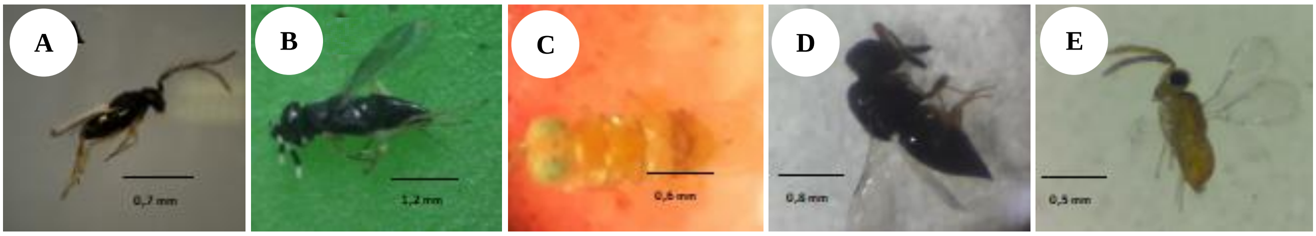

Figure 1. A. Male *Anagyrus lopezi* parasitoid, B. Female *A. lopezi*, C. *Acerophagus* sp., D. *Blepyrus* sp., and E. *Encarcia* sp.

Community structure of parasitoid

The community structure of parasitoids varies in each location as indicated by variations in the diversity index value, abundance index, and species dominance index in each location or district (Table 1). In absolute terms, the abundance of parasitoid populations that showed a response to cassava mealybug in each district classified as low, were 30 adults (Bangli), 54 adults (Gianyar), 29 adults (Klungkung), 44 adults (Denpasar), 35 adults (Badung), 21 adults (Tabanan), 37 adults (Jembrana), 32 adults (Buleleng) and 26 adults (Karangasem). Community structure (abundance, diversity, and dominance) of the four types of parasitoids associated with cassava mealybug *P. manihoti* in cassava crop in the field had varying values (Table 1.). The abundance of the parasitoid population in Bali was relatively low (<3.5) with an abundance of parasitoid index (R1) around = 0.50-0.87 while the diversity index values (H') were also low (<1.5) in all districts, between 0.27-0.28. Unlike the parasitoid dominance index values were in the moderate categories in Bangli, Klungkung, Badung, Tabanan, and Buleleng Districts (≥0.30-0.60) with a range of D = 0.33-0.59, while the dominance index values were high in Gianyar, Denpasar, Jembrana and Karangasem Districts (≥ 0.60-1.00) with a range of D= 0.62-0.72.

According to Supartha (2003), the abundance and diversity of insect population in the field are influenced by intrinsic and extrinsic factors. Extrinsic factors include the number or host density of parasitoid (Wyckhuys et al. 2014; Attia 2012; Bugila et al. 2014a), host resistance such as the power of egg parasitoid encapsulation so that the development of the parasitoid eggs did not become optimal on the initial instar (Suma et al. 2011; Suma et al. 2012b; Bugila et al. 2014b). Host defense behaviors such as rapid movements avoiding parasitoid or bending body up and down can inhibit the parasitoid from penetrating the host (Tschopp et al. 2013; Firlej et al. 2010). Other factors such as the adequacy of food, climate, space, and competition also influence these events (Moursi et al. 2012). The stability of the environment in each cassava crop area also affects diversity, abundance, and dominance of natural enemies (Pekas et al. 2011; Essien et al. 2013). Intercropping patterns carried out on cultivated plants also affect the increasing role of parasitoid and parasitoid reliability (Wahyuni et al. 2017). Mixed cropping systems also directly influence the stability of the relationship between plants, pests, and parasitoid, by suppressing the development of pest populations through increasing the role of natural enemies caused by the availability of nutritional sources such as nectar for parasitoid (Divya et al. 2011; Dhami et al. 2011; Zhou et al. 2012). Host plants are needed as an energy source for parasitoids to be able to carry out living activities such as population, growth, and reproduction (Franco et al. 2011). The existence of human intervention in modifying plant ecosystems also affects the existence of natural enemies. Cropping pattern systems carried out by farmers in increasing plant diversity such as mixed cropping systems (intercropping) have an impact and response to host plants, pests, and natural enemies for ecosystem stability. So that pests will find it difficult to find the main host (Altieri 2012; Shah et al. 2015).

Table 1. The community structure of parasitoid associated with cassava mealybug *Phenacoccus manihoti* on cassava crop in all districts of Bali Province, Indonesia.

Parasitoid species / indications	**Bangli**	**Gianyar**	**Klungkung**	**Denpasar**	**Badung**	**Tabanan**	**Negara**	**Buleleng**	**Karangasem**
	Total individuals (adult)								
Anagyrus lopezi	21	42	23	36	17	16	30	20	22
Acerophagus sp.	6	8	5	5	13	3	4	6	2
Blepyrus sp.	3	2	1	3	5	2	3	3	2
Encarcia sp.	0	0	0	0	0	0	0	3	0
N Parasitoid	30	54	29	44	35	21	37	32	26
N *P. manihoti*	220	230	130	210	210	100	155	205	170
S Parasitoid	3	3	3	3	3	3	3	4	3
R1	0.59	0.50	0,59	0.53	0.56	0.66	0.55	0.87	0.61
H'	0.50	0.36	0.27	0.42	0.59	0.27	0.28	0.76	0.34
D	0.52	0.62	0.59	0.68	0.38	0.33	0.66	0.43	0.72

Notes: N: Individual abundance, S: Species abundance, R1: Abundance Index, H': Diversity index, D: Dominance Index

Parasitoid distribution mapping associated with cassava mealybug *Phenacoccus manihoti* on cassava crop in Bali

The results showed that the distribution of parasitoid responsive to *P. manihoti* mealybug distribution evenly in cassava crop in all districts in Bali (Figure 2). The four types of parasitoid (Figure 1) that were responsive to the presence of invasive pests spread evenly across all districts and city in Bali, except *Encarsia* sp which was only found in Buleleng District.

The intrinsic and extrinsic factors such as parasitoid behavior, climate differences, host availability, and cropping patterns in each region can strongly influence the spread of parasitoid (Westwood et al. 2010; Thomson 2010; Adly et al. 2016), biotic factor such as host plants spread in the field greatly affect the distribution of parasitoid to find cassava mealybug *P. Manihoti*. *A. lopezi* can be actively dispersed through the wind, or carried by mummies attached to seedling cuttings (Mware et al. 2010; Fallahzadeh et al. 2011). In Africa, the *A. lopezi* parasitoid was reported to be able to scatter to a radius of 50-100 km (Herren et al., 1987).

Level of parasitoid parasitization of cassava mealybug *Phenacoccus manihoti* on cassava crop in Bali

The response of parasitoids to cassava mealybug *P. manihoti* can also be seen from the ability of parasitoid to parasitism *P. manihoti* which was spread throughout the districts in Bali (Table 2.). The value of parasitization rate of the parasitoid *A. lopezi* (Encyrtidae), *Acerophagus* sp. (Encyrtidae), *Blepyrus* sp. (Encyrtidae), and *Encarsia* sp (Aphelinidae) were different. The highest parasitization rate was shown by *A. Lopezi* (8.71%-16.22%), followed by *Acerophagus* sp. (1.16%-3.70%), *Blepyrus* sp. (0.76%-2.33%), and *Encarcia* sp. (1.44%). The highest parasitization rate of parasitoid *A. lopezi* in the suspected field due to the smell of cassava plants infested by *P. manihoti* was used as a guiding signal by female *A. lopezi* parasitoid to find the host. Volatile chemical compounds in cassava, wax secretion, and honeydew, can be a guiding compound for *A. lopezi* to find hosts (Bugila et al. 2014c). Host plants attacked by herbivorous insects in the form of damage to parts of plants during the feeding process can be synomon for parasitoid in host finding (Bugila et al. 2014a; Bodlah et al. 2010). On the contrary, the low level of parasitization indicated by the *Acerophagus* sp. parasitoid is strongly suspected because of its preference difference to *P. manihoti* because the parasitoid is the main parasitoid for other types of mealybug, *Paracoccus marginatus* (Zuparko 2015), while *Blepyrus* sp. parasitoid is also the main parasitoid species for another mealybug, *Ferisia virgata* (Attia 2012).

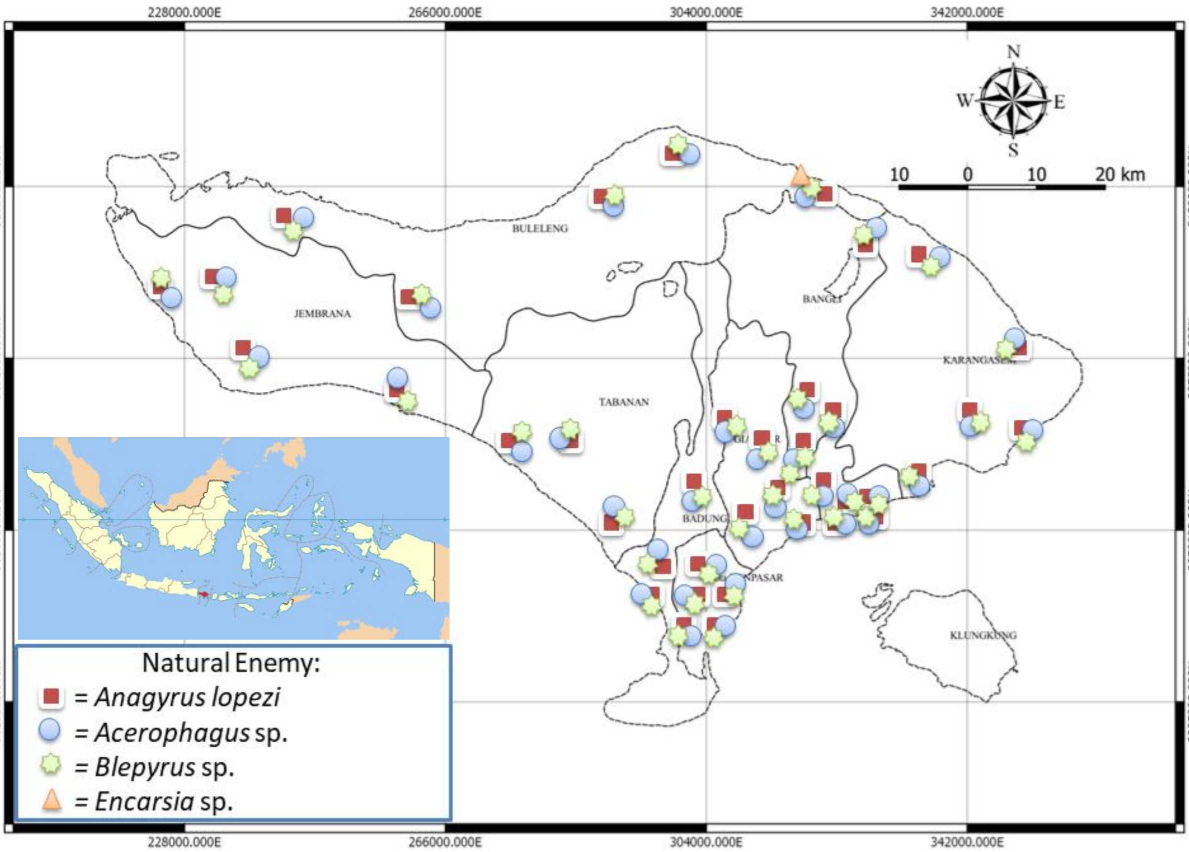

Figure 2. Map of parasitoid distribution associated with cassava mealybug *Phenacoccus manihoti* on cassava crop in Bali Province, Indonesia

There were three crop varieties found in the field, namely UJ-5, Adira-1, and Malang-6. All of them are classified as varieties that contain high cyanide compounds (Balitkabi 2005). Host plants that contain high cyanide compounds are preferred by cassava mealybug *P. manihoti* (Dhami et al. 2011). Parasitoid uses the cyanide as a cue compound to come to the cassava which is attacked by cassava mealybug *P. manihoti*. The sex ratios of the four types of parasitoids were different (Table 2). The sex ratio of *A. lopezi* parasitoid found from the appearance of an adult in various districts in Bali had a very high ratio of the number of males and females which was around 10.78: 14.44, while the parasitoid *Acerophagus* sp. was around 3.33: 2.67; *Blepyrus* sp. around 2.11: 0.56 and *Encarisa* sp. around 3: 0. The high sex ratio of *A. lopezi* parasitoid was due to the host *A. lopezi* parasitoid specification in which only develops well into the imago of *P. manihoti* host. *A. lopezi* is widely reported as a solitary endoparasite that naturally can control the nymph and imago *P. manihoti* (Karamaouna et al. 2011; Rameshkumar et al. 2013; Suma et al. 2012a). The high level of fitness of the parasitoid in the host was able to increase the length of life so that it had more time to parasitize more hosts. The ability of the parasitoid will be higher if the availability of hosts and plants that produce nutrients such as honey around cassava crop (Thancharoen et al. 2018). The existence of other supporting resources such as alternative hosts, shelter, refugia area, microenvironment has a positive effect on the life span of the parasitoid. Chemical compounds released by plants (*kairomones)* and material sourced from host feces (*honeydew)*, body parts (*body and body filaments)*, and secretions (*silk*, *salivary glands, pheromone markers)* produced by mealybug can affect the ability of parasitoid to search for hosts and handle their hosts in nature (Hilker and Fatouros 2015). The difference in host instar also has a significant effect on the average number offspring of parasitoid that appears and its sex ratio (Bugila et al. 2014a).

Table 2. Parasitization rate of parasitoid *Phenacoccus manihoti* on cassava crop in Bali Province, Indonesia

Districts	**Cassava varieties**	**Natural enemy**	**Σ Inang (adult)**	**Sex ratio**		**Σ number of natural enemies (adult)**	**Parasitization rate (%)**
				Σ Male (adult)	**Σ Female (adult)**		
Bangli	UJ 5	*Anagyrus lopezi*		10	11	21	8.71
	Adira 1	*Acerophagus* sp.	220	3	3	6	2.65
	Malang 6	*Blepyrus* sp.		3	0	3	1.35
Gianyar	UJ 5	*A. lopezi*		13	29	42	15.44
	Adira 1	*Acerophagus* sp.	230	8	2	10	3.36
		Blepyrus sp.		2	0	2	0.86
Klungkung	UJ 5	*A. lopezi*		12	11	23	14.47
	Adira 1	*Acerophagus* sp.	130	3	2	5	3.70
		Blepyrus sp.		1	0	1	0.76
Denpasar	UJ 5	*A. lopezi*		18	18	36	14.63
		Acerophagus sp.	210	4	1	5	2.33
		Blepyrus sp.		2	1	3	1.41
Badung	UJ 5	*A. lopezi*		13	4	17	7.49
	Adira 1	*Acerophagus* sp.	210	9	4	13	5.83
	Malang 6	*Blepyrus* sp.		3	2	5	2.33
Tabanan	UJ 5	*A. lopezi*		6	10	16	10.71
	Adira 1	*Acerophagus* sp.	100	0	3	3	2.91
		Blepyrus sp.		2	0	2	1.96
Jembrana	UJ 5	*A. lopezi*		12	18	30	16.22
	Adira 1	*Acerophagus* sp.	155	0	4	4	2.52
		Blepyrus sp.		2	1	3	1.90
Buleleng	UJ 5	*A. lopezi*		6	14	20	8.89
	Adira 1	*Acerophagus* sp.	205	1	5	6	2.84
	Malang 6	*Blepyrus* sp.		2	1	3	1.44
		Encarsia sp.		3	0	3	1.44
Karangasem	UJ 5	*A. lopezi*		7	15	22	11.46
	Adira 1	*Acerophagus* sp.	170	2	0	2	1.16
	Malang 6	*Blepyrus* sp.		2	0	2	1.16

In conclusion, this study concluded that the parasitoid was very responsive to the invasive pest *Phenacoccus manihoti* which attacks cassava crops in Bali. This response was indicated by the values of diversity, abundance, and dominance index of these parasitoid species against *P. manihoti* pest on cassava crop. Apart from that, the parasitoid also showed a strong response to the spread of *P. manihoti* in all districts and city in Bali with varying levels of parasitization between locations. Overall the parasitization rate of *A. lopezi* was very dominant in all locations with values range from 8.71-16.22%

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