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Article

The Evaluation Effectiveness of Parasitoids *Phenacoccus manihoti* Matile-Fererro (Hemiptera: Pseudococcidae) on Cassava Plants in Bali Province

I Wayan Supartha ^{1,*}, I Wayan Susila ¹, Aunu Rauf ², Ali Nurmansyah ², Ketut Ayu Yuliadhi ¹, Araz Meilin ³, Dodin Koswanudin ⁴, I Wayan Eka Karya Utama ⁵ and I Kadek Wisma Yudha ⁵

¹ Laboratory of Integrated Pest Management (IPMLab), Faculty of Agriculture, Udayana University, Denpasar City (80231), Bali Province, Indonesia; yansupartha@yahoo.com

² Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor (16680), West Java, Indonesia

³ Research Center for Horticultural and Estate Crops, National Research and Innovation Agency, Jalan Raya Jakarta - Bogor Km. 46, Cibinong 16911, Indonesia

⁴ Research Centre for Applied Zoology, National Research and Innovation Agency, Jalan Raya Jakarta - Bogor Km. 46, Cibinong 16911, Indonesia

⁵ Doctoral Student of Agriculture Science, Faculty of Agriculture, Udayana University, Denpasar City (80231), Bali Province, Indonesia

* Correspondence: yansupartha@yahoo.com

Abstract: *Phenacoccus manihoti* (Hemiptera: Pseudococcidae), is an invasive pest that is very detrimental to cassava cultivation in Indonesia. Control efforts by utilizing natural enemies of the parasitoid have succeeded in overcoming the cassava mealybug in 25 countries such as in Africa and Thailand. The purpose of this study was to specifically evaluate the potential for parasitoid species associated with the mealybug *P. manihoti* by (1) determining the level of parasitization against mealybugs on various varieties of cassava, (2) determining parasitoid preference for *P. manihoti* instars, and (3) determining the type of parasitoid. This study was undertaken in the field to re-evaluate the kinds of parasitoids associated with cassava pests, and in the laboratory to assess the amount of parasitization, predilection, and functional response of parasitoids to *P. manihoti* population density. The findings revealed three of parasitoids linked with *P. manihoti* in the field: *Anagyrus lopezi*, *Acerophagus* sp., and *Blepyrus* sp. The parasitoid *A. lopezi* had the greatest parasitization rate in the field, which was 18.67 %. Furthermore, the parasitoids *A. lopezi* and *Blepyrus* sp. preferred 3rd instar nymphs, but *Acerophagus* sp. preferred 2nd instar nymphs. The parasitoid *A. lopezi* demonstrated a type-III functional response, while *Acerophagus* sp. and *Blepyrus* sp. demonstrated a type-II functional response. The handling capacity (Th) of *A. lopezi* is the shortest at 3.42 minutes. This work is a comprehensive study of the parasitoid *A. lopezi*'s potential as a biological agent, highlighting how it effectively suppresses *P. manihoti* on various cassava varieties in Bali, providing an inexpensive, environmentally friendly, and applicable control in tropical countries.

Keywords: biological control agents; Effectiveness; parasitoid; invasive pest

1. Introduction

Indonesia is the fourth largest cassava-producing country in the world after Brazil, Nigeria, and Thailand [1]. However, from 2010 to 2015, the amount of cassava output in Indonesia varied. The disruption of pests and plant diseases that changed from year to year was one of the elements that produced these swings and reductions. Cassava plant diseases, such as Cassava Bacterial Blight (CBB) caused by *Xanthomonas campestris* pv. *manihoti*, Cassava Mosaic Disease (CMD), and Cassava Brown Streak Disease (CBSD), are an issue in various Asia-Pacific nations [2]. Meanwhile, whiteflies, mealybugs, and red spider mites have emerged as the most serious pests affecting cassava plants in Asia [3]. Mealybugs, among other diseases and pests, are still in the spotlight today due to

the harm they do to cassava fields in multiple nations. According to reports, mealybugs have impacted negatively on cassava crops in Africa and South America [4].

Phenacoccus manihoti (Hemiptera: Pseudococcidae) is an invasive pest that has spread and destroyed cassava plants around the country, including Indonesia [4, 5]. *P. manihoti* originated in South America and eventually spread to Africa in 1973, where it caused production losses of up to 82 % [6]. The mealybug *P. manihoti* was discovered in Thailand in 2008 and was related to a 30% yield loss [7]. *P. manihoti* occurred in Indonesia and was found for the first time in Bogor in 2010 [8]. Mealybug attacks on plants are distinguished by the emergence of thick white and/or black substances on the leaf surface, which is a combination of adult insects, ovisacs, and insect nymphs.

However, control measures were implemented against these mealybugs, which are often treated with pesticides. Chemical control is accomplished by soaking cassava cuttings in one of the suggested chemicals [9]. Considering that the usage of pesticides may generate environmental contamination and pest resistance, new control approaches that are more ecologically benign in the long run are required [10]. Biological control strategies have been investigated as alternative control strategies [11, 12]. The use of antagonistic organisms or microorganisms such as parasitoids, predators, and insect pathogens may be used in biological control strategies [13]. This biological control technique is one component of an Integrated Pest Management (IPM) strategy in a tropical country including Indonesia.

Essentially, the employment of potential natural enemies to manage pest populations, particularly cassava mealybugs and other damaging pests, may be attributed to their function in intra and interspecies interactions [14]. In essence, the cassava mealybug must be controlled using the proper manner in order to avoid having a negative influence on the environment. The use of organisms or microbes such as parasitoids, predators, and insect diseases are critical to attempts to attain environmental equilibrium [15]. In 25 countries, including Africa and Thailand, the use of natural enemies in pest management approaches has been found to be effective in eliminating the cassava mealybug. *Plesiochrysa ramburi* and Coccinellidae beetles were utilized as predators, while *Anagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) was used as a parasitoid [16]. In Africa, it has been recorded that 18 natural enemy species attack *P. manihoti*, including solitary parasitoids such as *Epidinocarsis lopezi* (DeSantis) (Hymenoptera: Encyrtidae) and predators such as *Hyperaspis notata* Mulsant, *Diomus* spp. (Coleoptera: Coccinellidae), and *Ocyptamus* spp. (Diptera: Syrphidae) in Africa [17].

Preliminary study has been conducted on the sort of parasitoid that is sensitive to the invasive cassava mealybug in Bali in 2020. The investigation discovered four forms of parasitoids that reacted to the cassava mealybug: *Anagyrus lopezi* (Encyrtidae), *Acerophagus* sp. (Encyrtidae), *Blepyrus* sp. (Encyrtidae), and *Encarsia* sp. (Aphelinidae) [5]. When compared to *Encarsia* sp., *A. lopezi*, *Acerophagus* sp., and *Blepyrus* sp., were shown to be more susceptible to mealybugs, with greater population density, parasitization rate, and distribution rate.

As a consequence, host preference and host specificity seem to be crucial in biological control efforts, as they may impact mass rearing techniques and may accelerate the development of new forms of potential parasitoids [18]. To ensure the strength of the three types of parasitoids' roles, a special assessment of their potential is required, which includes determining parasitoid preference for instar mealybugs *P. manihoti*, and determining the type of functional response of the parasitoid to *P. manihoti* population density. This research re-evaluates the type of parasitoid that responds to *P. manihoti*, and it is intended to give more thorough information and carry out more experiments through preferences and functional response.

2. Materials and Methods

2.1. Description of the Study Area

This research was carried out on a field and laboratory scale from October 2022 – March 2023. The field research was carried out in all districts and cities in the province of Bali. Meanwhile, laboratory research, which includes specimen maintenance, parasitoid identification, preference

testing, and functional response, was carried out at the Integrated Pest and Disease Management Laboratory (IPMLab), Faculty of Agriculture, Udayana University.

2.2. Site Determination and Sampling

Determination of the sampling location in each district/city is done by making five sample points diagonally. The area of each sample point is $\pm 500 \text{ m}^2$. Therefore, $2,500 \text{ m}^2$ is required in each district/city. Leaf sampling was carried out by purposive sampling at each sample point by taking 5 shots of plants attacked by mealybugs. Each leaf sample or shoot of the affected plant is accommodated in a plastic bag with a volume of 2 L. Then it is taken to the laboratory for further counting and recording per leaf. Then, the leaves of plants containing mealybugs were cultured until the time limit for parasitoids to appear.

2.3. Mealybug and Parasitoid Maintenance

Maintenance of plant leaves containing mealybugs is carried out until the time limit for parasitoids appears. Observations on the type and number of parasitoids that appeared were carried out every day. To determine the type of parasitoid that appears, identification is carried out under a microscope using an identification method that refers Gordh [19]. Based on the identification results, the number and type of parasitoids were counted and recorded, and each parasitoid was photographed.

2.4. Preparation of Host Plants

The host plant used for the study was the UJ5 variety. The choice of the UJ5 variety as the host plant was made because it was the most widely cultivated by farmers in the field. The size of the cuttings used for host plant seeds was 15 cm, which was bred in a plastic cup pot measuring 5 cm in diameter on the upper surface and 4 cm on the lower surface with a height of 7 cm. Each container is filled with a seedling stem, then poured with water as a planting medium, and then placed in the open. Cassava seeds used as research material were aged for 15-20 days after growing about 5 to 8 leaves.

2.5. Mealybugs Propagation

Nymphs and adult's mealybug were collected from a farmer's cassava plantation in Temesi Village, Gianyar Regency (8028'26"S 115011'225"E). The nymphs and adults were raised in the laboratory by infecting them with pre-prepared cassava plant seeds. on the breeding table, which is located in the propagation room, 40-50 plant pots made of plastic cups containing cassava plant seeds are inserted and arranged in a row. Some propagation outcomes are employed for parasitoid breeding and propagation.

2.6. Parasitoid Breeding

The parasitoids collected in the field were maintained in the lab. In cages with hardwood frames measuring 80 cm long, 45 cm wide, and 65 cm high, parasites were propagated. Gauze is used to cover the cage's top, right, and left walls, as well as the rear wall. Meanwhile, the front wall serves as an access door for entering and removing mealybug and parasitoid-infested plant pots. A total of 10 mealybug-infected cassava plants were included in the study, and the data was kept safe. Parasitoids from the wild were placed in breeding cages and maintained to generate offspring for experimental experiments. A cotton coil with a 10% honey solution was introduced as a source of nutrition to boost the parasitoid's fitness.

2.7. Parasitoid Preference Testing for Nymph Instars and Adults of *P. manihoti*

The assessed host insects are placed in a Petri dish as a container in this test. Each Petri plate included *P. manihoti* instar-2, instar-3, and adult hosts. Following that, previously copulated

parasitoid insects were infected and allowed for 24 hours. The parasitoids were removed after the copulation phase, and the instar- 2, instar-3, and adult host-treated hosts were kept in various containers. Every day, observations and data were recorded until the time limit for mature parasitoids emerged from each host instar. This experiment was conducted 10 times using a totally randomized design. The parasitoids *Acerophagus* sp. and *Blepyrus* sp. were subjected to the same technique.

2.8. Parasitoid preference and parasitization index

The index used to measure preference for *P. manihoti* host instars is the Manly index [20], namely:

$$Bi = \ln (Ri/Ai)/$$

Where B_i = preference index for host instars, A_i = Initial host density: $I = 1;2;3 \dots k$, and R_i = number of unparasitized hosts.

Parasitization rate of parasitoids using the equation:

$$Parasitization = \frac{\Sigma Parasitoid A}{\Sigma Emerging parasitoid + \Sigma Host}$$

Where $\Sigma Parasitoid A$ = the number of one type of parasitoid that appears, $\Sigma Host$ = the total number of hosts.

2.9. Functional responsiveness testing

The functional response is the parasitoid's reaction to the density of the host population. The population density of the examined host was 3; 7; 15; 25; 35; and 50 *P. manihoti* 3rd instar nymphs. Infesting nymph-3 *P. manihoti* on cassava plants housed in plastic glass containers filled with plants provided hosts for each density. The cassava plant is then placed in a cage. After mating the female and adult male parasitoids, the adult female was placed in a cage for 24 hours to lay eggs on the mealybugs. The mealybugs and parasitoid larvae that are present in the host's body are allowed to mature until the adult parasitoids emerge. The test included 6 treatments and was performed 5 times. Every day, observations were made on the number of mature parasitoids that emerged, which were differentiated by sex. The parasitoids that emerged were collected in a 1 mL collection container with 0.5 mL of 70% alcohol.

Estimation of parameters a and Th for the functional response of *A. lopezi* using the Hassel in Fenlon and Faddy [21] equation:

$$Ne = N0 \{1 - \exp [(d + bN0) (ThNe - T) / (1 + cN0)]\}$$

with $b = 0,209$, $c = 0,027$ and $d = 0$.

Meanwhile, the estimation of a and Th parameters for the functional response of *Acerophagus* sp. and *Blepyrus* sp. using Rogers (1972) [22] equation:

$$Ne = N0 \{1 - \exp [a (ThNe - T)]\}$$

Where Ne is the number of hosts being parasitized, $N0$ the number of hosts provided, the instantaneous search rate, T the length of time the host was exposed to the parasitoid, and Th the length of time the host was handled.

Data analysis for determining the type of functional response (logistic regression) and estimating model parameters (a and Th) with the non-linear regression analysis above was carried out using the R-Studio version 4.1.2 program (RStudio, Inc).

3. Results

3.1. Parasitoid preference for mealybug instar *P. manihoti*

The results showed that the three parasitoid species found were able to parasitize the 2nd instar, the 3rd instar, and the adult *P. manihoti*. However, each parasitoid species has a significantly different level of preference for the host stadia. The parasitoid *A. lopezi* showed a significantly stronger preference for instar-3 than in instar-2 and adults. The strength of this preference was also shown by the higher number of individuals in instar-3 (2.90 individuals) when compared to instar-2 (1.80 individuals) and adults (1.10 individuals).

In this investigation, *Blepyrus* sp. preferred instar-3 but was not significantly different from instar-2 or adults. Although a greater host selection value suggested preference support in instar-3, it was not substantially different when compared to instar-2 and adults. Unlike *Acerophagus* sp., which demonstrated a greater preference for instar-2 than instar-3 and adults, the strength of this preference was further confirmed by a significantly higher value of the instar-2 selection (0.17 individuals) vs instar-3 (0.90 individuals) and adults (0.80 individuals) (Table 1).

Table 1. Test of parasitoid preference on several instars of the mealybug *P. manihoti*.

Treatments	The emergence of parasitoids (ind)			Preference Index (Li)		
	<i>A. lopezi</i>	<i>Acerophagus</i> sp.	<i>Blepyrus</i> sp.	<i>A. lopezi</i>	<i>Acerophagus</i> sp.	<i>Blepyrus</i> sp.
Instar -2	1,80 ± 0,92 ^b	1,70 ± 0,67 ^a	0,40 ± 0,70 ^b	0,18 ± 0,20 ^b	0,19 ± 0,17 ^a	0,03 ± 0,10 ^a
Instar- 3	2,90 ± 0,74 ^a	0,90 ± 0,57 ^b	1,10 ± 0,57 ^a	0,39 ± 0,06 ^a	0,03 ± 0,10 ^b	0,06 ± 0,13 ^a
Adults	1,10 ± 0,74 ^b	0,80 ± 0,63 ^b	0,60 ± 0,52 ^b	0,09 ± 0,15 ^b	0,03 ± 0,10 ^b	0,03 ± 0,10 ^a

Values in the same row followed by the same letter do not show a significant difference (Duncan's test = 5%).

3.2. Effect of host density on parasitization rate of *P. manihoti*

The number of parasitized hosts and the rate of parasitization were both impacted by an increase in host density (Table 2). The number of hosts parasitized by *A. lopezi*, *Acerophagus* sp., and *Blepyrus* sp. was considerably impacted by *P. manihoti* population density. The parasitization rate of *A. lopezi*, on the other hand, exhibited a very sharp increase in the host population density of 3–15 individuals. The rate of increase in the percentage of parasitization peaked at 78 % at a host population density of 15 individuals, following which the rate of increase started to decrease (Table 2). Unlike the parasitization rates of *Acerophagus* sp. and *Blepyrus* sp., which showed the percentage of parasitization that had reached its peak at a population density of 7 and 3 individuals, respectively, 62.86 % at a density of 7 individuals by *Acerophagus* sp., and 60 % at a density of 3 species by *Blepyrus* sp.

Table 2. Effect of host density on parasitization rate of *P. manihoti*.

Host density	Number of parasitized hosts (ind)			Parasitization rate (%)		
	<i>A. lopezi</i>	<i>Acerophagus</i> sp.	<i>Blepyrus</i> sp.	<i>A. lopezi</i>	<i>Acerophagus</i> sp.	<i>Blepyrus</i> sp.
3	2,0 ± 0,71 ^d	1,8 ± 0,84 ^b	1,8 ± 0,84 ^b	66,67 ± 2,35 ^{ab}	60,00 ± 2,78 ^a	60,00 ± 1,27 ^a
7	5,4 ± 0,55 ^c	4,2 ± 0,82 ^a	3,2 ± 0,84 ^a	77,14 ± 1,78 ^a	62,86 ± 1,62 ^a	45,71 ± 1,19 ^a

15	11,8 ± 1,30 ^b	4,8 ± 0,45 ^a	3,2 ± 0,45 ^a	78,67 ± 1,86 ^a	30,67 ± 3,65 ^b	21,33 ± 2,98 ^b
25	14,8 ± 1,64 ^a	5,0 ± 0,00 ^a	3,4 ± 0,55 ^a	59,20 ± 1,65 ^b	20,80 ± 1,79 ^{bc}	13,60 ± 2,19 ^b
35	15,2 ± 1,30 ^a	5,2 ± 0,45 ^a	4,0 ± 0,00 ^a	42,29 ± 3,73 ^c	14,29 ± 2,02 ^{bc}	10,29 ± 1,56 ^b
50	15,6 ± 1,14 ^a	5,6 ± 0,55 ^a	4,0 ± 0,00 ^a	30,80 ± 1,79 ^c	10,00 ± 1,41 ^c	7,20 ± 1,10 ^b

Values in the same row followed by the same letter do not show a significant difference (Duncan's test = 5%).

3.3. Functional Response to Population Density of *P. manihoti*

The logistic regression study of the parasitoid *A. lopezi* revealed that the linear component of the coefficient was positive (0.1252) and the quadratic component was negative (-0.0073) at the 5% level. Both coefficients differed substantially from zero, with a P-value of 0.1699 and 0.0426 (Table 3).

This indicated that the density of *P. manihoti* parasitized by *A. lopezi* showed a type-III functional response model (Figure 1). The parasitoid *Acerophagus* sp. revealed that the linear coefficient component was negative (-0.1691) and the quadratic component was positive (0.0032), with P-values of 0.0754 and 0.4141, respectively. It was indicated that the parasitoid *Acerophagus* sp. exhibited a type-II functional response model. The same thing was also shown by the parasitoid *Blepyrus* sp., which showed a negative linear coefficient value (-0.2523) and a positive quadratic component (0.0068), with P-values of 0.0125 and 0.1067, indicating a functional type model-II. The estimated values of the parasitoid *A. lopezi*'s search capacity (a) and handling time (Th) were 2.54 and 3.42, respectively, based on non-linear regression analysis. Meanwhile, the parasitoids *Acerophagus* sp. were 1.81 and 10.00, and *Blepyrus* sp. was 1.83 and 14.79. (Table 4).

Table 3. Results of logistic regression analysis of functional response curves of parasitoids *A. lopezi*, *Acerophagus* sp., and *Blepyrus* sp.

Coefficient	Estimating	SD ¹	z-count	P-Values
Parasitoid: <i>A. lopezi</i>				
Intercept (P ₀)	0.6093	0.6514	0.94	0.3496
Linear (P ₁)	0.1252	0.0912	1.37	0.1699
Quadratic (P ₂)	-0.0073	0.0036	-2.03	0.0426
Cubic (P ₃)	0.0001	0.0000	2.07	0.0385
Parasitoid: <i>Acerophagus</i> sp.				
Intercept (P ₀)	1.2040	0.6368	1.89	0.0587
Linear (P ₁)	-0.1691	0.0951	-1.78	0.0754
Quadratic (P ₂)	0.0032	0.0039	0.82	0.4141
Cubic (P ₃)	0.0000	0.0000	-0.49	0.6251
Parasitoid: <i>Blepyrus</i> sp.				
Intercept (P ₀)	1.2030	0.6452	1.86	0.0624
Linear (P ₁)	-0.2523	0,1010	-2.50	0.0125
Quadratic (P ₂)	0.0068	0,0042	1.61	0.1067
Cubic (P ₃)	-0.0001	0.0000	-1.29	0.1963

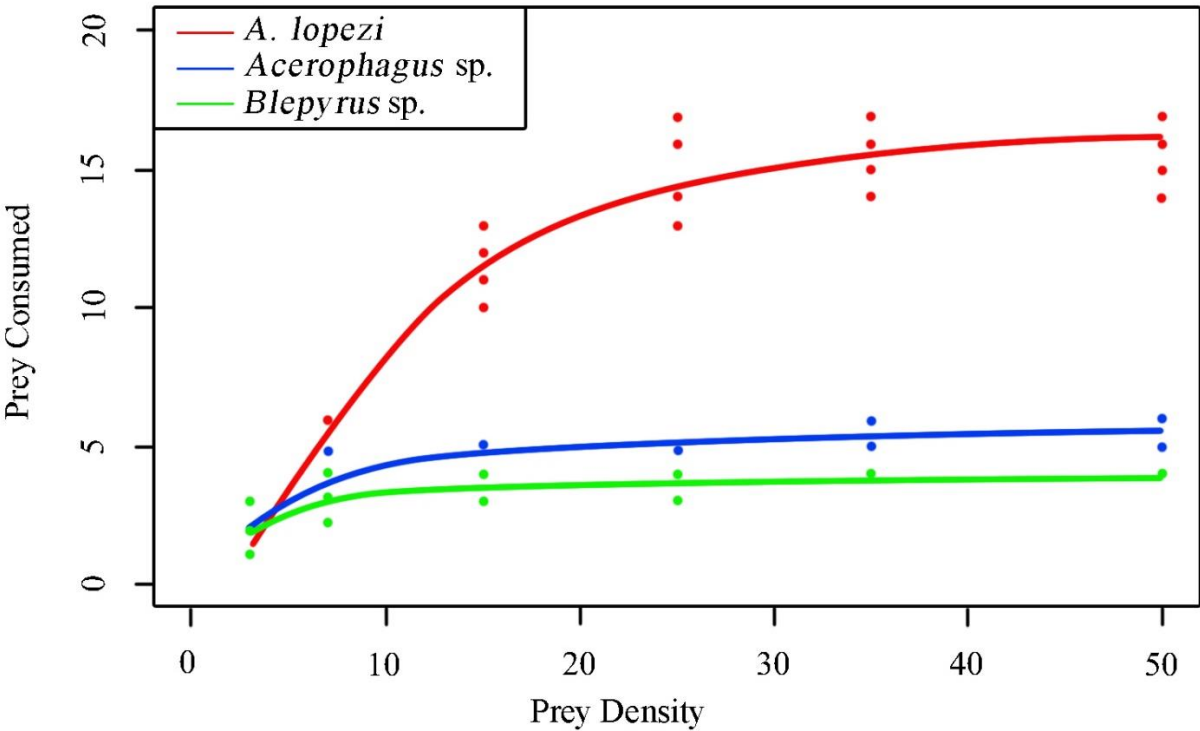


Figure 1. Functional response curves for the three parasitoids.

Table 4. The results of the estimation of model parameters (a and Th) using non-linear regression analysis for the three parasitoids.

Parasitoid	a (mealybugs/hour)	Th (minutes)
<i>A. lopezi</i>	2,54 ± 1,47	3,42 ± 0,52
<i>Acerophagus</i> sp.	1,81 ± 0,62	10,00 ± 1,18
<i>Blepyrus</i> sp.	1,83 ± 0,90	14,79 ± 2,00

4. Discussion

The potential of *A. lopezi* to parasitize *P. manihoti* instars 2–3 and adults may be related to *A. lopezi*'s properties as a particular parasitoid on the host [17]. Similarly, *Acerophagus papayae*. selection of instar-2 This is due to physical considerations such as having a lower body size range of 0.55–0.55 mm, therefore they prefer the 2nd instar host [23]. In addition, there is a possibility of host response to parasitoids, such as encapsulation of parasitoid eggs, which causes the development of parasitoids in the host's body to be less optimal in early instars [24]. Encapsulation is a common form of host insect defense in response to invasion by endoparasitoids or other foreign organisms [13]. *Blepyrus* sp. chose instar-3 due to its larger size, which is about 1.0 mm–1.2 mm, thus preferring instar-3 with a body length range of 1.1 mm–1.5 mm [25]. Therefore, *Blepyrus* sp. prefers instar-3 and adults as hosts in several families of Pseudococcidae [26].

The selection of instars 2 and 3 by parasitoid species in mealybugs as hosts is associated with the fulfillment of more nutrients and is beneficial to parasitoid progeny growth. Furthermore, the requirement for quantity and quality nutrients is strongly associated with offspring that mature into adult females in order to be more active [27, 28, 29, 30]. The cuticle of the 2nd and 3rd instar hosts was softer, and the kairomone generated by the young instar host was more specialized for triggering parasitoid activity. Similar studies show that parasitoid species grow 9.5 days quicker in nymph-3 mealybugs than in nymph-1 [31].

The amount of parasitization of the three examined parasitoids revealed considerable variations in the density of *P. manihoti*'s host population. The relationship between parasitization level and host density is important in the dynamics of parasitoid and host populations. Previous studies revealed that a host density of 2–100 had a substantial influence on the quantity of *P. manihoti* parasitized by *A. lopezi* [32,33]. The parasitoid *Anagyrus kamali*, which parasitized *Maconellicoccus hirsutus*, was likewise impacted by host density in terms of parasitization, progeny, and sex ratio [34,35,36]. Interestingly, in large numbers, parasitoids lay just a few eggs on a single host. As a result, the fewer individuals that grow in the host egg, the greater the body size [37], which may be associated to the number of offspring (adult eggs) [38]. This has also been seen in wasps associated with *Anagyrus* spp., where the quantity of developed eggs increased as host population density increased [39].

The parasitoid *A. lopezi* showed a type-II functional response to *P. manihoti* in a study by Maharani et al. [40] at a host density of 2–100 individuals. Type-II is the functional reaction of the parasitoid *Acerophagus papayae* to the nymph *Paracoccus marginatus* [41]. The parameters in the laboratory, however, are not identical to those in the field. Temperature and competition with other insects are two elements that influence functional responsiveness in the field [42]. In a recent investigation, we found the parasitoid *Blepyrus* sp. parasitizing *P. manihoti* on cassava plants in the environment. *Gronotoma micromorpha* parasitoid we utilized against *Liriomyza huidobrensis*, on the other hand, demonstrated a type-2 functional response in which the amount of parasitization increased as the host density of *L. huidobrensis* increased [43].

The search efficiency of parasitoids is an important concept in understanding parasitoid host population dynamics, parasitoid evolution, and host behavior, as well as the use of parasitoids as natural enemies of insect pests in biological management [44,45]. Because the host search pattern is carried out systematically and parasitoids are no longer visited by parasitoids, different estimate values of a and T_h might be considered [22].

According to these findings, *A. lopezi* is the most promising parasitoid as a biological control agent for *P. manihoti*. The use of these three parasitoids, on the other hand, has the potential to raise their function as biological control agents for *P. manihoti* pests in the field.

5. Conclusions

In conclusion, our findings indicate that the three species of parasitoids, namely *A. lopezi*, *Acerophagus* sp., and *Blepyrus* sp., are associated with *P. manihoti* in the field. *A. lopezi* had the highest level of parasitization, whereas *Blepyrus* sp. had the smallest percentage of parasitization. Our results emphasize *A. lopezi*'s capacity to parasitize all three *P. manihoti* instars, particularly the third instar, in contrast to *Acerophagus* sp. and *Blepyrus* sp., which prefer the 2nd and 3rd instars, respectively. The parasitoid *A. lopezi* demonstrated a type-III functional response, while the parasitoids *Acerophagus* sp. and *Blepyrus* sp. had a type-II functional response.

As a result of our findings, we recommend further research on the potential for biological control by utilizing *A. lopezi* on a field scale, so that the information obtained can be used to design integrated pest management strategies for *A. lopezi* conservation in the future, as well as support organic farming of cassava commodities in Bali Province, Indonesia.

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