

## Performance and Functional Response of Three Parasitoid Species as Potential Biological Control Agents of Three Important Mealybug Pests

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### ABSTRACT

Mealybugs (*Maconellicoccus hirsutus*, *Phenacoccus solenopsis*, *Planococcus citri*), are significant agricultural pests worldwide. This study demonstrated the effectiveness of three parasitoid species (*Leptomastix dactylopii*, *Anagyrus kamali*, *Anagyrus pseudococci*) in controlling these pests. It focused on parasitism rates, host preferences, offspring emergence from parasitized mummies, and parasitoid functional responses. *Leptomastix dactylopii* showed parasitism rates of 51.42% for *P. solenopsis*, 72.85% for *M. hirsutus*, and 77.14% for *P. citri*. In comparison, *Anagyrus kamali* had rates of 75.71%, 92.85%, and 54.28% for the same hosts, while *A. pseudococci* recorded 41.41%, 48.57%, and 78.57%. The highest emergence percentages were 72.57% for *L. dactylopii* from *P. citri*, 82.85% for *A. kamali* from *M. hirsutus*, and 68.57% for *A. pseudococci* from *P. citri*.

*Leptomastix dactylopii* preferred *P. solenopsis* (39.68%) over *M. hirsutus* (36.50%) and *P. citri* (23.81%). *Anagyrus kamali* preferred *M. hirsutus* (41.29%) over *P. solenopsis* (34.09%) and *P. citri* (24.62%). *Anagyrus pseudococci* favored *P. citri* (46.76%) more than *P. solenopsis* (24.2%) and *M. hirsutus* (29.04%). Higher host-parasitoid densities and ratios increased parasitism rates across all three species. All three parasitoids exhibited a type III functional response to the three mealybug species. These findings highlight the potential for developing eco-friendly pest control strategies through mass rearing and release of these parasitoids to manage mealybug populations.

**Keywords:** Biological control, parasitoids, biological parameters, host-parasitoid interaction, mealybugs, functional response

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## INTRODUCTION

Mealybugs are agricultural and horticultural pests that feed on plant sap, causing damage to plants, stunted growth, changes in leaf color, and the transmission of viruses, leading to economic losses. Their honeydew secretions promote sooty mold growth, which further damages plant health. Scale insects in the family Pseudococcidae, including mealybugs, often cluster on plants. They weaken plants and can spread various diseases, worsening the damage they cause (Vercher, González, Sánchez-Domingo, & Sorribas, 2023). Mealybugs are key vectors of viral diseases that threaten plant health, especially among *Planococcus* species, which effectively transmit Ampeloviruses (Sarwar, 2020). This study focuses on important species such as the *Hibiscus* mealybug (*Maconellicoccus hirsutus* Green, 1908), the cotton mealybug (*Phenacoccus solenopsis* Tinsley, 1898), and the citrus mealybug (*Planococcus citri* Risso, 1813), all of which belong to the order Hemiptera and family Pseudococcidae. Virus transmission by mealybugs depends on their life stage, temperature, and available host plants (Tsai, Rowhani, Golino, Daane, & Almeida, 2010). The saliva of some, particularly *M. hirsutus*, can harm plants. *Planococcus* species are especially adept at carrying multiple viruses, including the cocoa swollen shoot virus, with *P. citri* being notable carriers (Dey, Sugikawa, Kerr, & Melzer, 2019). Mealybugs, particularly *M. hirsutus*, *P. solenopsis*, and *P. citri*, are destructive sap-sucking pests that cause significant economic losses worldwide and transmit viral diseases (Ahmed, Apori, & Karim, 2023). Estimating the direct impact of viruses spread by mealybugs is challenging due to various factors, including mechanical damage, combined stressors, mixed infections, environmental and agronomic influences, and vector population dynamics (Franco, Zada, & Mendel, 2009; Ahmed, Apori, & Karim, 2023).

*Maconellicoccus hirsutus* is a highly invasive polyphagous pest worldwide (Culik et al., 2013). In Pakistan, it may spread to other areas through the transport of ornamental plants. Its rapid proliferation and significant impact on crops, particularly *Hibiscus*, emphasize the need for accurate identification and prompt action against infestations (Chong, Aristizábal, & Arthurs, 2015; Bragard et al., 2022).

*Phenacoccus solenopsis*, a notable pest affecting cotton and potatoes, is native to the USA but has spread to over 43 countries, especially in tropical and subtropical areas (Cui et al., 2024). It poses significant risks to agriculture by damaging plants through sap feeding and promoting sooty mold growth, which hinders photosynthesis (Saddiq et al., 2014; Waqas et al., 2021).

*Planococcus citri* is a significant economic pest due to its wide host range and its potential to damage various crops, particularly horticultural plants. This pest is also known for transmitting plant viruses (Ahmed & Abd-Rabou, 2010). In Pakistan, *P. citri* severely impacts citrus production, affecting both the quantity and quality of the fruit (Rehana, Munir, & Asim, 2020). Continuous sap suction by this pest can lead to plant depletion, and infestations during the fruiting period can cause partial drying or complete loss of fruits (Rossati et al., 2025).

Effective management of mealybugs requires a combination of control strategies (Franco, Zada, & Mendel, 2009; Venkatesan, Jalali, Ramya, & Prathibha, 2016). Biological control,

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using natural enemies, is a key environmentally friendly alternative to chemical pesticides (Rasool, 2024a, b; Rasool et al., 2024; Rida et al., 2024; Acevedo-Alcalá et al., 2025). While insecticides are often used in ornamental nurseries, biological control is preferred for urban landscapes, as improper insecticide use can worsen pest issues. Well-established biological control methods primarily involve natural enemies (Franco, Zada, & Mendel, 2009; Beltra, Tena, & Soto, 2013). One effective predator is *Cryptolaemus montrouzieri* Mulsant, 1853 (Coleoptera: Coccinellidae), which works best at high mealybug densities as compared to low densities and may need to be paired with a parasitoid (Gunawardana & Hemachandra, 2020). Various hymenopteran parasitoids are successfully used alongside *C. montrouzieri* in the USA to control *M. hirsutus* and other mealybugs (Lomeli-Flores et al., 2024).

*Leptomastix dactylopii* Howard, 1885 (Hymenoptera: Encyrtidae) is a parasitic wasp that targets mealybugs, common pests of various crops. It lays its eggs inside the mealybug, and the larva consumes it from within, resulting in a mummified host (Cocco et al., 2021; Muştu, Derya, & Tarhanacı, 2022). *Anagyrus kamali* Moursi, 1948, is another important parasitoid wasp of the family Encyrtidae used particularly against *M. hirsutus*. Its effectiveness, host specificity, and ease of mass rearing make it valuable for pest management (de Lopez, Kondo, & Molina-Moreira, 2025).

*Anagyrus pseudococci* Girault, 1915, is also significant for controlling mealybug populations, specifically *P. citri*. As a solitary endoparasitoid, it lays eggs inside a single host, aiding in the management of mealybug infestations in fruit orchards and vineyards (Muştu & Tatar 2024). This study evaluated three parasitoid species as potential agents for controlling *M. hirsutus*, *P. solenopsis*, and *P. citri*. All three species have been extensively researched in other systems (Sagarra, Vincent, & Stewart, 2001; 2002; Chong & Oetting, 2006a; Andreason et al., 2019; Cocco et al., 2021; Muştu, Derya, & Tarhanacı, 2022) but the present research specifically addressed their efficacy and parasitism potential on three mealybug species. It is necessary to determine the effect of high densities typical of mass rearing facilities on parasitism. Mass rearing and augmentative releases are essential for effectively controlling pests in agriculture and other settings (Hassan, 1993; Li et al., 2023).

This research aims to develop biocontrol strategies for managing the economic threat posed by mealybugs. Current effective and environmentally safe control methods are limited, so focus on biological control techniques, particularly the potential of natural enemies like parasitoids. The present study will address the following questions: (i) How do parasitoids of host mealybugs affect parasitism and emergence in all three host species? (ii) How do the host species influence parasitoid performance? (iii) How do different density ratios of parasitoids to hosts impact parasitism potential? (iv) What kind of functional responses do parasitoids show with the three host species?

## MATERIALS AND METHODS

### Rearing of mealybugs

Mealybugs (*M. hirsutus*, *P. solenopsis*, and *P. citri*) were collected from various infested areas in Pakistan and reared on sprouted potatoes in plastic boxes covered with mesh. Each potato sprout was individually infested with 15 adult females of each

mealybug species. Once the colonies of mealybugs were established, third instar and young adults of each species were collected for further experiments (Sagarra, Vincent, & Stewart, 2001). Host insect colonies were maintained in a laboratory at  $26 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and 12:12 h L: D photoperiod.

### **Rearing of parasitoids**

*Anagyrus kamali* from *M. hirsutus*, whereas *L. dactylopii* and *A. pseudococci* from *P. citri* were obtained from field-infected samples collected from different areas of Pakistan and reared on sprouted potatoes as outlined by Fischer (1961). The culture was kept in cages (100 x 100 x 150 cm: L x H x W) covered with muslin cloth away from the pesticide-contaminated laboratory at  $26 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  and the LD 12:12h. Mummies were collected from the laboratory colony and incubated in individual gelatin capsules until adult parasitoids emerged. Within 24 h of emergence, the adults were collected and kept in plastic vials (100 ml) for 48 h, where they were provided with diluted honey as a food source. An equal number of male and female parasitoids was maintained in each vial, reflecting the experimental density. No mealybugs were supplied during this time, so the parasitoids had no ovipositional experience before the experiments.

### **Parasitism and emergence rates of parasitoids on three hosts**

Seventy adult mealybugs of each host were transferred separately onto sprouted potatoes and placed in a transparent plastic cage (100 x 100 x 150 cm: L x H x W) with the top covered in nylon mesh. The mating process was conducted separately in glass tubes (3.5 x 10 cm) for at least 12h. One-day-old mated females were used for experiments. Three adult mated females of each parasitoid were introduced separately into each cage of the host species for 48h. The mealybugs were observed daily to record the emergence of parasitoids. Parasitism was also recorded, and parasitized mealybugs were transferred to another similar cage to complete their developmental time until the emergence of the parasitoid. The experiment was repeated 10 times for each of three hosts and parasitoid separately. Fertilized female wasps used in the experiments were up to 24 h old. Percent parasitism was determined by dividing the parasitized individuals by the total number of individuals and multiplying by 100. Not all parasitized individuals develop as adult parasitoids, and the number of adult parasitoids was recorded to determine how many emerged from the parasitized mealybugs. Laboratory conditions were the same as mentioned for the rearing of parasitoids.

### **Host preference studies**

In the choice tests, mated females of each parasitoid species were placed individually in a mixed host population within separate cylinders with a volume of 7853.98 cm<sup>3</sup>. Four individuals of parasitoids per species were transferred into the experimental arena. Thirty-six adult mealybugs (12 individuals from each species) were transferred to each cylinder and exposed to the parasitoids for 24 h in the experimental arena. Host females were alternately distributed near the borders of the area, equally distant from each other. The parasitoid female was released in the center of the testing arena, equally

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distant from all the mealybug individuals. After this period, the level of parasitism for each parasitoid was recorded, and the host individuals were transferred into individual Petri dishes (120 × 25 mm) for monitoring their emergence. Each treatment had ten replicates under standardized laboratory conditions, as previously described. The sizes of both hosts and parasitoid individuals were measured using micrometers evo series (Sylvac, Switzerland) and stereoscopic microscopes (Nikon, Japan SMZ-745).

### Host-parasitoid density-dependent effects

The impact of various host and parasitoid densities was assessed to evaluate parasitism potential in controlled conditions, akin to mass rearing. Each mated female parasitoid was provided with honey as food in rearing chambers (50 x 45 x 35 cm: L x H x W), with data collected from eight replicates for each treatment. Four host to parasitoid densities (8:1, 45:5, 100:10, 165:15) were tested with host: parasitoid ratios (8, 9, 10, 11) in four separate rearing chambers ( $RC_1$ - $RC_4$ ) maintained at  $26 \pm 1$  °C with  $65 \pm 5\%$  relative humidity (RH) and 14:10 (L: D) photoperiod. Hosts were exposed to the parasitoids for 24 h after which adults were removed. The process was repeated separately for each host, treatment parasitoid and parasitism data was recorded.

### Statistical analysis

Datasets related to parasitism, emergence, and host-parasitoid density were assessed for normality using the Shapiro-Wilk test. For normally distributed variables, two-way ANOVA followed by Post Hoc Tukey's HSD test was used. Each reported dataset represents the average calculated from various replicates  $\pm$  SE, and additionally it was converted into percent values. Assumptions for each statistical test were verified using the Agricolae package in R (Core Team 2020). The frequency data from choice and non-choice experiments were analyzed using the Chi-square ( $\chi^2$ ) test. Pearson correlation coefficients were calculated to assess relationships between variables.

Polynomial logistic regression analysis was performed to fit data regarding each parasitoid density to a logistic model. This analysis aimed to determine whether the proportion of parasitized mealybugs aligns with the predictions by three functional response models (Juliano, 2001). The logistic regression parameters, linear, quadratic, and cubic, related to the slope of the curve were calculated based on the methods described by Mills & Lacan (2004); Kalinkat, Rall, Uiterwaal, & Uszko (2023). The analysis was conducted using GraphPad Prism 10 software, LLC, <https://www.graphpad.com>. The model parameters, including odds ratios and 95% confidence intervals, Akaike Information Criterion (AIC), maximum likelihood ratios, goodness-of-fit and coefficient of determination, were estimated.

## RESULTS

### Parasitism and emergence from hosts

Differences in parasitism rates were observed among the three parasitoids ( $F_{2,85} = 4.82$ ;  $p = 0.01$ ) and host species ( $F_{2,85} = 12.1$ ;  $p < 0.000024$ ). *Leptomastix dactylopii*

had rates of 51.42% for *P. solenopsis*, 72.85% for *M. hirsutus*, and 77.14% for *P. citri*. *Anagrus kamali* demonstrated rates of 75.71%, 92.85%, and 54.28% for the same hosts, while *A. pseudococci* had 41.41%, 48.57%, and 78.57%, respectively (Fig. 1).

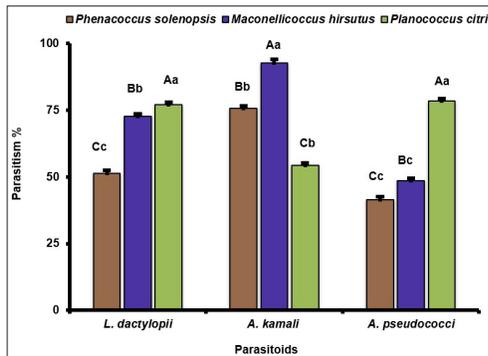


Figure 1. Percentage parasitism rates of *Leptomastix dactylopii*, *Anagrus kamali* and *Anagrus pseudococci* from hosts *P. solenopsis*, *M. hirsutus* & *P. citri*. Differences based on Tukey's test ( $p < 0.05$ ), Capital letters: comparison between hosts within parasitoids, small letters comparison between parasitoids for hosts (No choice experiments).

Differences were observed among the three parasitoids ( $F_{2,85} = 6.88$ ;  $p = 0.0017$ ) and the three hosts ( $F_{2,85} = 9.47$ ;  $p < 0.00019$ ) in terms of emergence rates. The highest emergence percentages were recorded as follows: *L. dactylopii* for *P. citri* (72.57%), *A. kamali* for *M. hirsutus* (82.85%), and *A. pseudococci* for *P. citri* (68.57%). Additionally, *L. dactylopii* also exhibited emergence of (70% and 41.43%) from the other two hosts, *M. hirsutus* and *P. solenopsis*. In contrast, *A. kamali* showed emergence rates of 67.14% and 42.85% for *P. solenopsis* and *P. citri*, respectively. Similarly, *A. pseudococci* demonstrated emergence rates of 35.71% and 42.85% for *P. solenopsis* and *M. hirsutus* (Fig. 2).

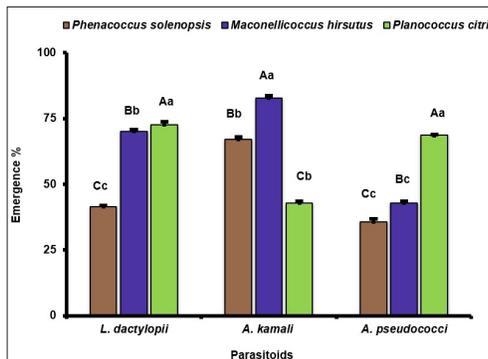


Figure 2. Percentage emergence rates of *Leptomastix dactylopii*, *Anagrus kamali* and *Anagrus pseudococci* from hosts *P. solenopsis*, *M. hirsutus* & *P. citri*. Differences based on Tukey's test ( $p < 0.05$ ), Capital letters: comparison between hosts within parasitoids, small letters, comparison between parasitoids for hosts. (No choice experiments).

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### Host preference of parasitoids (Choice experiments)

Among the parasitoids, *A. kamali* exhibited higher parasitism rates compared to the other two species ( $F_{2,85} = 6.7204$ ,  $p < 0.001952$ ) (Fig. 3). *Leptomastix dactylopii* preferred to parasitize *P. citri* over *M. hirsutus* and *P. solenopsis*: 39.68% vs 36.50% and 23.81%. In contrast, *A. kamali* preferred to parasitize *M. hirsutus* over *P. solenopsis* and *P. citri* 41.29% vs 34.09% and 24.62%. Whereas *A. pseudococci* preferred to parasitize *P. citri* over *P. solenopsis* and *M. hirsutus*: 46.76% vs 24.2% and 29.04% (Fig. 3). The sizes of the mealybugs were approximately The sizes of the immature mealybugs were approximately (1.97 to 2.25 mm; *P. solenopsis*), (1.94 - 2.54 mm; *M. hirsutus*) and *P. citri* (2.4 to 3.0 mm).

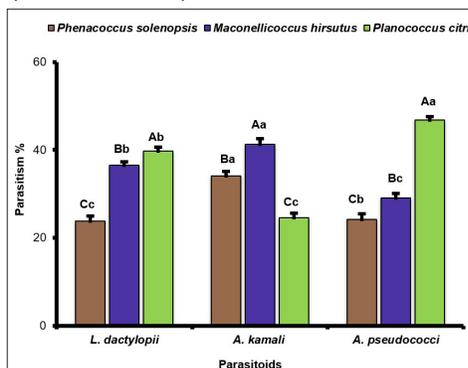


Figure 3. Host preference of *Leptomastix dactylopii*, *Anagyrus kamali* & *Anagyrus pseudococci* given a choice of *P. solenopsis*, *M. hirsutus* & *P. citri*. Capital letters: comparison between hosts within parasitoids, small letters comparison between parasitoids for hosts. The treatments differed significantly based on Tukey's test,  $p \leq 0.05$ .

### Effect of host and parasitoid densities on Parasitism

The parasitism average of *L. dactylopii*, *A. kamali*, and *A. pseudococci* differed significantly for *P. solenopsis* ( $F_{2,90} = 62.28$ ,  $p < 0.0001$ ), *M. hirsutus* ( $F_{2,90} = 123.35$ ,  $p < 0.0001$ ) and *P. citri* ( $F_{2,90} = 20.30$ ,  $p < 0.0001$ ). Host-parasitoid density significantly affected parasitism rates in both *P. solenopsis* ( $F_{3,90} = 2223.22$ ,  $p < 0.0001$ ), *M. hirsutus* ( $F_{3,90} = 2552.19$ ,  $p < 0.0001$ ) and *P. citri* ( $F_{2,90} = 4398.27$ ,  $p < 0.0001$ ) according to the relative ratios in different rearing chambers (RC<sub>1</sub> to RC<sub>4</sub>).

*Anagyrus kamali* exhibited higher parasitism rates in *P. solenopsis* than *L. dactylopii*, while *A. pseudococci* had lower rates across all four tested relative densities (Fig. 4a). *Anagyrus kamali*, in turn, showed higher rates in *M. hirsutus* compared to *L. dactylopii* and *A. pseudococci* (Fig. 4b). However, *A. pseudococci* had higher parasitism rates in *P. citri* than both *L. dactylopii* and *A. kamali* (Fig. 4c). The RC<sub>4</sub> ratio (165:15) exhibited higher parasitism, revealing a strong positive correlation between densities and parasitism. Mean parasitism rates for *L. dactylopii*, *A. kamali*, and *A. pseudococci* were (72%, 82.50%, and 57.25%) in *P. solenopsis*; (82%, 85%, and 54%) in *M. hirsutus*; and (72.50%, 71%, and 80.25%) in *P. citri* (Figs. 4a to 4c).

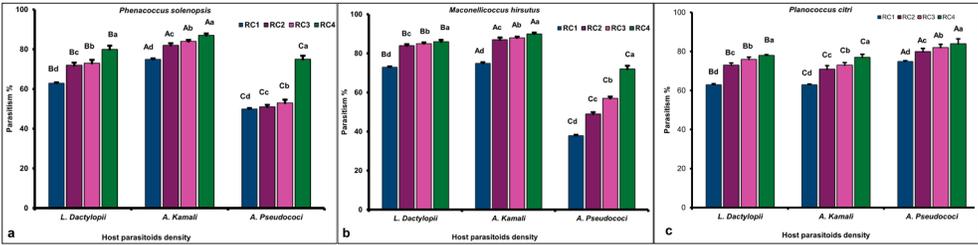


Figure 4. Percent parasitism of (*Leptomastix dactylopii*, *Anagyrus kamali* and *Anagyrus pseudococci*) parasitoids at different densities in the hosts a) *P. solenopsis*, b) *M. hirsutus*, c) *P. citri*. Tukey’s test,  $p \leq 0.05$ . (Capital letters: comparison between hosts within parasitoids, small letters comparison between parasitoids for hosts).

### Functional response of three parasitoids and host mealybugs

At each parasitoid-host ratio, the number and proportion of mealybugs parasitized increased with the density of the parasitoids (Fig. 4). Parasitism rates exhibited an exponential rise as the host density ratio increased (Fig. 4). All three parasitoids (*L. dactylopii*, *A. kamali*, and *A. pseudococci*) showed a type III functional response to the mealybug species (*P. solenopsis*, *M. hirsutus*, and *P. citri*). Maximum likelihood analysis revealed a positive linear relationship, indicating that the proportion of parasitized mealybugs rose with mealybug density across all parasitoid densities (Table 1). The Pearson correlation values for the three parasitoids (*L. dactylopii*, *A. kamali*, and *A. pseudococci*) in host *P. solenopsis* were (0.989, 0.992, 0.957), for *M. hirsutus* (0.992, 0.992, 0.970), and for *P. citri* (0.990, 0.989, 0.991), respectively (Table 1).

Table 1. Comparison between host-parasitoid densities and parasitism through Pearson correlation coefficients and maximum likelihood ratio.

Host parasitoid (RC <sub>1</sub> -RC <sub>4</sub> )	<i>Leptomastix dactylopii</i>			<i>Anagyrus kamali</i>			<i>Anagyrus pseudococci</i>		
	r	Maximum likelihood ratio	p	r	Maximum likelihood ratio	p	r	Maximum likelihood ratio	p
<i>Phenacoccus solenopsis</i>	0.989	10.560	0.01436	0.992	10.626	0.01393	0.957	10.008	0.01850
<i>Macronelliccoccus hirsutus</i>	0.992	10.635	0.01387	0.992	10.622	0.01395	0.970	10.149	0.01734
<i>Planococcus citri</i>	0.990	10.573	0.01427	0.989	10.561	0.01436	0.991	10.606	0.01406

The polynomial logistic regression model analyzed three parasitoids (*L. dactylopii*, *A. kamali*, and *A. pseudococci*) and three host species (*P. solenopsis*, *M. hirsutus*, and *P. citri*), indicating a Type III functional response (Tables 2-4). The results showed positive estimates for the linear component ( $P_1 > 0$ ) and negative estimates for the quadratic component ( $P_2 < 0$ ). This model better fits the data, revealing that parasitism efficiency was lower at low host densities and increased at intermediate densities. Significant differences in parasitism rates among the parasitoids were observed, with lower rates at a host-to-parasitoid density of (RC<sub>1</sub>) and higher rates at (RC<sub>2</sub> to RC<sub>4</sub>). All three parasitoids exhibited a Type III functional response with a stabilizing pattern (Tables 2-4).

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The data indicated that the consumption rate began low, then increased rapidly, and ultimately leveled off as host density rose. The logistic growth curve revealed a Type III functional response with a stabilizing pattern. This suggests that parasitoids consume few hosts at low densities, increase their consumption as host numbers rise, and eventually reach a saturation point. Unlike Type II responses, which can lead to destabilization, a Type III functional response, often driven by factors such as prey switching, learning, or refuges, facilitates the stabilization of host-parasitoid population dynamics (Figs. 5a-5c). This interaction is characterized as density-dependent, illustrating a stabilizing relationship between the parasitoid and its host.

Table 2. Parameter estimates from polynomial logistic regression analysis of the proportion of *Phenacoccus solenopsis* parasitized by *Leptomastix dactylopii*, *Anagyrus kamali* and *Anagyrus pseudococci*.

Strain	Coefficients	Estimates	SE	$\chi^2$ (p-value)	AIC	coefficient of determination R <sup>2</sup>	Response Type
<i>L. dactylopii</i>	intercept (P <sub>0</sub> )	59.44	2.039	10.270 (<0.016)	488	0.9771	Type III
	Linear (P <sub>1</sub> )	0.4880	0.1319				
	Quadratic (P <sub>2</sub> )	-0.005557	0.001940				
	Cubic (P <sub>3</sub> )	0.00002033	0.000007459				
<i>A. kamali</i>	intercept (P <sub>0</sub> )	72.49	1.174	11.323 (<0.010)	355	0.9935	Type III
	Linear (P <sub>1</sub> )	0.3401	0.07592				
	Quadratic (P <sub>2</sub> )	-0.003359	0.001117				
	Cubic (P <sub>3</sub> )	0.00001110	0.000004295				
<i>A. pseudococi</i>	Intercept (P <sub>0</sub> )	49.27	1.938	8.163 (<0.043)	475	0.9773	Type III
	Linear (P <sub>1</sub> )	0.1087	0.1253				
	Quadratic (P <sub>2</sub> )	-0.002253	0.001843				
	Cubic (P <sub>3</sub> )	0.00001539	0.000007088				

Table 3. Parameter estimates from polynomial logistic regression analysis of the proportion of *Maconelliococcus hirsutus* parasitized by *Leptomastix dactylopii*, *Anagyrus kamali* and *Anagyrus pseudococci*.

Strain	Coefficients	Estimates	SE	$\chi^2$ (p-value)	AIC	coefficient of determination R <sup>2</sup>	Response Type
<i>L. dactylopii</i>	intercept (P <sub>0</sub> )	67.82	1.092	11.381 (<0.010)	308	0.9955	Type III
	Linear (P <sub>1</sub> )	5.765	0.7178				
	Quadratic (P <sub>2</sub> )	-0.6071	0.1106				
	Cubic (P <sub>3</sub> )	0.02024	0.004572				
<i>A. kamali</i>	intercept (P <sub>0</sub> )	69.26	1.229	11.203 (<0.011)	336	0.9948	Type III
	Linear (P <sub>1</sub> )	6.404	0.8082				
	Quadratic (P <sub>2</sub> )	-0.6895	0.1245				
	Cubic (P <sub>3</sub> )	0.02365	0.005148				
<i>A. pseudococi</i>	Intercept (P <sub>0</sub> )	33.65	1.735	8.165 (<0.014)	419	0.9908	Type III
	Linear (P <sub>1</sub> )	4.760	1.141				
	Quadratic (P <sub>2</sub> )	-0.4338	0.1757				
	Cubic (P <sub>3</sub> )	0.01913	0.007265				

Table 4. Parameter estimates from polynomial logistic regression analysis of the proportion of *P. citri* parasitized by *Leptomastix dactylopii*, *Anagyrus kamali* and *Anagyrus pseudococci*.

Strain	Coefficients	Estimates	SE	Pearson Chi statistic (p-value)	AIC	Goodness of fit/ coefficient of determination R <sup>2</sup>	Response Type
<i>L. dactylopii</i>	intercept (P <sub>0</sub> )	58.76	1.375	10.597 (<0.014)	363	0.9914	Type III
	Linear (P <sub>1</sub> )	4.654	0.9036				
	Quadratic (P <sub>2</sub> )	-0.4295	0.1392				
	Cubic (P <sub>3</sub> )	0.01365	0.005756				
<i>A. kamali</i>	intercept (P <sub>0</sub> )	59.33	2.254	10.642 (<0.014)	482	0.9765	Type III
	Linear (P <sub>1</sub> )	4.078	1.482				
	Quadratic (P <sub>2</sub> )	-0.4267	0.2283				
	Cubic (P <sub>3</sub> )	0.01556	0.009439				
<i>A. pseudococci</i>	Intercept (P <sub>0</sub> )	72.94	2.876	11.665 (<0.009)	540	0.9682	Type III
	Linear (P <sub>1</sub> )	2.255	1.890				
	Quadratic (P <sub>2</sub> )	-0.2024	0.2913				
	Cubic (P <sub>3</sub> )	0.006746	0.01204				

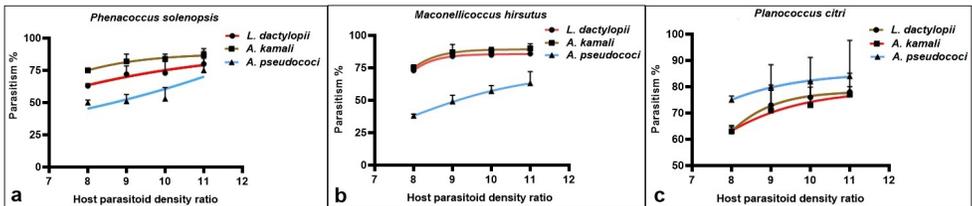


Figure 5. Percentage of parasitism of (*Leptomastix dactylopii*; *Anagyrus kamali* and *Anagyrus pseudococci*) parasitoids at different host-parasitoid density ratios in the hosts a) *Phenacoccus solenopsis*, b) *Maconellicoccus hirsutus*, c) *Planococcus citri*. The curves in the panel predicted type III functional response models for three host species. The error bars, accompanied by respective colored logistic growth trend lines in the panel, depict the % parasitism of three host species parasitized by three parasitoids.

## DISCUSSION

Current research focused on developing cost-effective methods for the biological control and mass production of parasitoids essential for managing economically important crop pests (Heimpel & Mills, 2017; Romero, Benito, & Soto, 2025). This study assessed the parasitism performance of three parasitoid species on three mealybug species. While statistical significance in high-density lab conditions may show differences, it does not guarantee biological relevance. Key factors such as parasitoid species, host type, and release environment are crucial for effective pest control. The research evaluated parasitism rates, emergence rates, host preferences, and the interactions between host-parasitoid density ratios and parasitoid responses. The findings provide valuable insights for managing significant pests in Pakistan, including *P. solenopsis*, *M. hirsutus*, and *P. citri*.

The parasitism rates of *L. dactylopii* and *A. pseudococci* were higher for *P. citri* than for *A. kamali*. Conversely, *A. kamali* and *L. dactylopii* performed better with *M. hirsutus* than with *A. pseudococci*. Earlier Studies indicated that *L. dactylopii* and *A.*

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*pseudococci* were more effective with *P. citri* (Serguei, González, Vickerman, Noyes, & White, 2007; Attia & Awadallah, 2016; Cocco et al., 2021; Al-Shami & Qureshi 2024; Muştu & Tatar 2024). Meanwhile, *A. kamali* and *L. dactylopii* have shown effectiveness against *M. hirsutus* (Sagarra, Vincent, & Stewart, 2000b; Abd-Rabou, 2005; Montes-Rodríguez, Kondo, & Gaimari, 2024), and *A. kamali* is also recognized as a parasitoid of *P. solenopsis* (Hao-jie, Yan, Zihao, Ying, & Ming-xing, 2019). Additionally, *L. dactylopii* has been documented to affect various *Phenacoccus* species (Chong & Oetting, 2007; Al-Shami & Qureshi 2024). Releasing a combination of parasitoid species is suggested for effectively targeting pests (Chen, Li, Pang, Zhu, & Zhang, 2021). Different parasitoids vary in their effectiveness against mealybug species like *M. hirsutus*, *P. solenopsis*, and *P. citri*. An applied ecologist should consider co-evolved parasitoids, as their impact can differ by habitat (Blumberg, 1997; Charles, 2011). Therefore, selecting parasitoid species should be specific to the pest in question. Scale insects utilize physical and structural traits for defense, impacting how effectively parasitoids can locate and attack them. These traits can block host location cues and help resist internal attacks (Apostolos & Alejandro, 2015). The effectiveness of biological control agents depends on factors such as their specificity (generalist or specialist), type, timing, release method, synchrony with the host, field conditions, and release rate (Beirne, 1975; Collier & van Steenwyk 2004; Stiling & Cornelissen 2005). For mass-producing agents for scale insects, evaluating parasitism and emergence rates is crucial for determining host suitability and production efficiency (Herren, Hesketh, Meyling, & Dunn, 2023). High emergence and parasitism rates are vital for ensuring a reliable supply of effective parasitoids for pest control (Collier & van Steenwyk, 2004; Crowder, 2007; Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). The percent emergence was different between the hosts and among parasitoids tested which suggests that all three species affected the development of the parasitoid (Ricciardi et al., 2021; Romero, Benito, & Soto, 2025). Host preferences are critical to parasitoid performance (Cherif, Mansour, & Grissa-Lebdi, 2021). The choice of specific mealybug species and their life stages affects parasitism rates and the success of biological control programs. These preferences are influenced by factors such as host size, nutritional quality, and defensive mechanisms (Wang & Yang, 2010; Rezaei, Asghar, & Zahra, 2019).

The study indicates that *L. dactylopii* effectively controls *M. hirsutus* and *P. citri*, while *A. kamali* targets *M. hirsutus* and *P. solenopsis*. Additionally, *A. pseudococci* is effective for managing *P. citri*. Future tests will evaluate the release of these species individually and in combination under field and greenhouse conditions. Variation in host and parasitoid densities significantly affects parasitism rates, with higher densities leading to better quality parasitoids (Barnay et al., 1999; Lin, Shaukat, & Jianhui 2018; Rasool, 2024a; Rasool et al., 2024; Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). Maintaining optimal host-parasitoid densities is crucial for effective performance in mass-rearing facilities (Taylor, 1988; Barnay et al., 1999; Paranhos, Sivinski, Stuhl, Holler, & Aluja, 2013). To enhance parasitoid parasitism and achieve successful biological pest control, it is vital to reduce pesticide use, match natural enemies to the host and

environment, time their release with the pest's life cycle, and monitor environmental conditions. Additionally, selecting the right parasitoid species and employing effective rearing and shipping techniques are essential for maximizing their efficacy (Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). Earlier research indicated that mealybug populations were influenced by landscape composition, strain variability, and host compatibility (Smith, 1996; Plata, Tena, Beitia, Sousa, & Paredes, 2024). Assessing environmental risks and interactions with non-target species is crucial for sustainable control measures (Catania et al., 2024; Sarwar, Rasool, Shah, & Ahmad, 2023). Understanding host preferences for different mealybug species helps optimize management while reducing unintended effects (Si et al., 2025). Utilizing parasitoids for pest control can be challenging due to biological factors and environmental variability (Abdalbaset, Bugila, da Silva, & Franco, 2014; Ricciardi et al., 2021; Romero, Benito, & Soto, 2025). These strategies support sustainable agriculture by focusing on pest life stages while safeguarding non-target organisms (Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). A multispecies approach in field trials can improve pest management and promotes sustainability (Rasool, 2024a; Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). All three parasitoids parasitized the pests, but *L. dactylopii* was less effective on *P. solenopsis*, and *A. kamali* performed poorly on *P. citri*. *A. pseudococci* also showed reduced effectiveness against *M. hirsutus* and *P. solenopsis*. These parasitoids can be mass-reared with their host pests, and host or parasitoid density did not negatively affect returns. It's important to explore the use of factitious hosts for cost-effectiveness. Additionally, assessing the ability of these parasitoids to parasitize hosts in field conditions is crucial for effective biological control (Smith, 1996; Villavicencio-Vasquez, Espinoza-Lozano, Espinoza-Lozano, & Coronel-Leon, 2025). Researchers use functional responses to describe interactions between consumers and their resources, such as predator-prey and parasitoid-host relationships (DeLong, 2021; Gobin et al., 2022) in three types of functional responses: Type I (linear), Type II (hyperbolic), and Type III (sigmoid). All three parasitoid species (*L. dactylopii*, *A. kamali*, and *A. pseudococci*) displayed a type III functional response when foraging among host species (*M. hirsutus*, *P. solenopsis*, and *P. citri*). Type I functional responses are rare among parasitoids of homopteran insects; an example is the whitefly parasitoid *Eretmocerus mundus* Mercet (Jones, Greenberg, & Legaspi, 1999). Most studies show either a type II (Gonzalez-Hernandez, Pandey, & Johnson, 2005) or type III response (Sagarra, Vincent, & Stewart, 2000a; Jones et al., 2003; Chong & Oetting, 2006b), with type III responses potentially more common than previously recognized (Kalinkat, Rall, Uiterwaal, & Uszko, 2023; DeLong, Coblenz, & Uiterwaal, 2025). *Anagyrus kamali* exhibited a type III response in arenas where it could choose residence time (Sagarra, Vincent, & Stewart, 2000a), whereas those confined to arenas displayed a type II response. Similar limitations may have impacted reports of type II responses in *Anagyrus* sp. nov. nr. *sinope* (Chong & Oetting, 2006b) and *Anagyrus ananatis* Gahan (Gonzalez-Hernandez, Pandey, & Johnson, 2005). The Type III functional response is considered as a more stable ecological interaction than the Type II response, as it helps regulate host populations. This response is crucial for ecosystem health, allowing prey populations to recover at low densities, thus preventing local extinction and promoting stability. It enables coexistence among various prey types,

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enhancing species richness. Furthermore, Type III responses can prevent the paradox of enrichment, which may lead to unstable population dynamics in productive environments. Predators with Type III responses are often effective biocontrol agents, as they manage pest populations without driving them to extinction (Cicero et al., 2024). Researcher Real (1977) introduced the generalized functional response, a flexible sigmoidal model for representing predator-prey interactions. This approach better accommodates the diverse functional response patterns observed in these systems compared to types I, II, or III (Kalinkat et al., 2013; Rosenbaum & Rall, 2018). This study observed a type III functional response, characterized by a slow initial rate of parasitism that increased as the host-parasitoid density rose. Low parasitism rates at low host densities suggest a learning phase in locating hosts. The experiments indicate that parasitoids learn cues from specific host species, aligning with previous findings (Donnelly & Phillips, 2001).

Furthermore, the study revealed that parasitoid responses depend on the densities and ratios of hosts and parasitoids, enhancing their parasitism efficiency. This is significant for utilizing parasitoids as biological control agents against mealybugs. The type III response allows parasitoids to maintain stable dynamics post-release, as their efficiency increases at low host densities and stabilizes at high densities. In contrast, type II responses often lead to chaotic dynamics. However, our experimental design may have limited our observations. Future studies with free-moving parasitoids in field conditions could improve predictions regarding their responses. Additionally, releasing a large number of parasitoids in greenhouses may effectively manage mealybug populations. The present study determined that the optimal host-to-parasitoid ratio for maximizing parasitism efficiency is 165:15.

## CONCLUSIONS

This study found that *L. dactylopii* effectively controls *M. hirsutus* and *P. citri*, while *A. kamali* targets *M. hirsutus* and *P. solenopsis*. Nevertheless, *A. pseudococci* is suitable for managing *P. citri*. Additionally, functional responses of three parasitoids to three host mealybug species were assessed. The finding demonstrates the potential for ecosystem-friendly pest control through parasitoids to manage mealybugs affecting key crops. This research highlights the benefits of using multiple parasitoid species to improve pest management and reduce pesticide residues in sustainable agriculture.

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