Journal of Sustainable Agricultural and Environmental Sciences

Print ISSN : 2735-4377
Online ISSN : 2785-9878
Homepage: https://jsaes.journals.ekb.eg/

Research Article

The Dynamics of Varroa Populations on Honeybee Worker's Brood and Adults in Three Honeybee Hybrids in Egypt

Haitham Mahmoud Ramadan [1,*], Ibrahim Ibrahim Mesbah [1], Ashraf Sherif Fatehe [2], Heba Bumey Abada 1

[1] Plant Protection Department, Faculty of Agriculture, Tanta University, Egypt.
[2] Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
* Correspondence: Haitham Mahmoud Ramadan; hmamramadan@agr.tanta.edu.eg

Article info: -
- **Received:** 13 May 2025
- **Revised:** 3 June 2025
- **Accepted:** 17 June 2025
- **Published:** 1 July 2025

Keywords:
Honeybee hybrids; Varroa; grooming behavior; Varroa destructor; parasitic mites.

Abstract:
This study explores the effects of *Varroa destructor*, a major threat to honeybee populations, on three honeybee hybrids: *Apis mellifera lamarckii*, *A. m. ligustica*, and *A.m. carnica*. It assesses how far the three honeybee hybrids are affected by the infestation of *Varroa destructor* on the brood and adults' levels. Honeybee colonies were observed, and Varroa infestation was measured using the wheat flour shaking method. Also, Statistical analysis helped determine infestation trends. Generally, Results showed that Egyptian bees "*Apis mellifera lamarckii*" experienced significant Varroa peaks during the second season, particularly from August to November. Italian bees "*Apis mellifera ligustica*" had the highest infestation rates in late autumn, whereas Carniolan bees "*Apis mellifera carnica*" had a more stable but notable infestation pattern. Notably, *Apis mellifera lamarckii* and *Apis mellifera carnica* hybrids really had lower Varroa counts in worker brood compared to *Apis mellifera ligustica*, which had higher counts, especially in the later months. Finally, this study suggests that *Apis mellifera lamarckii* and *Apis mellifera Carnica* have better mechanisms for managing Varroa mites than *Apis mellifera ligustica*, indicating that *Apis mellifera ligustica* may require more intensive management strategies.

1. Introduction

The *Varroa destructor* mite is one of the most challenging threats facing honeybee populations today. Originally found in Asia, where it parasitizes the Asian honeybee "*Apis cerana*", this mite has since spread to honeybee colonies worldwide, including those of Egyptian bees "*Apis mellifera lamarckii*", Italian bees "*Apis mellifera ligustica*", and Carniolan bees "*Apis mellifera Carnica*". As a result, this mite is notorious due to its feeding on the body fluids of both adult bees and their brood. Consequently, it leads to weakened colonies, reduced honey yields, and increased susceptibility to other pathogens (Rosenkranz et al., 2010). To control this pervasive threat, it is crucial to understand how *Varroa destructor* affects different honeybee hybrids to develop effective management strategies tailored to their unique traits. Specifically, the Egyptian honeybee "*Apis mellifera lamarckii*" is well-adapted to the harsh environmental conditions of Egypt. This resilience is a result of its evolutionary history in a hot, arid climate. Although Egyptian bees have some natural resistance to Varroa mites, they are not immune to their effects. In particular, these bees have developed certain behavioral and genetic traits that help mitigate the impact of mite. For instance, their grooming behaviors and hygienic practices can reduce mite loads to some extent (de Figueiró et al., 2016; Santos et al., 2015; Al-Kahtani and Taha, 2022). Nevertheless, this inherent tolerance doesn't eliminate the threat, while high levels of Varroa infestation still led to significant declines in colony health and productivity (Lodesani et al., 2014). Additionally, Italian honeybees are highly prized for their gentle nature and strong honey production, but they are particularly susceptible to *Varroa destructor* (Bava et al., 2023). Italian bees face considerable challenges due to their high susceptibility to this mite. Furthermore, the long-term impacts of *Varroa destructor* on Italian honeybee colonies, investigate the effectiveness of different management practices over time (Dainat et al., 2012). Effective management practices are essential to mitigate the effects of *Varroa destructor* and sustain the productivity of Italian bees' colonies (Lodesani et al., 2014). Carniolan bees are native to central and southeastern Europe and known for their adaptability and resistance to various environmental stresses. These bees have developed several mechanisms to cope with *Varroa destructor* (Kovačić et al., 2020). Carniolan bees exhibit a relatively high level of resistance to *Varroa destructor*. Furthermore, it is largely due to their grooming behaviors and varroa-sensitive hygiene (VSH) traits, mite populations within the colony have been efficiently managed. Despite this resistance, *Varroa destructor* still poses a threat to Carniolan bees. While Carniolan bees are better equipped to handle mite infestations, effective management practices are necessary to support their natural defenses (Guichard et al., 2020). Indeed, the resistance mechanisms of Carniolan bees, noting their increased grooming behavior and effective brood removal strategies, while Carniolan bees have a natural edge, continuous management and monitoring are essential to maintaining their health and productivity. Comparative studies provide valuable insights into how different honeybee hybrids respond to *Varroa destructor* (Gregorc et al., 2016). Thus, Carniolan bees demonstrated the highest level of resistance, while Italian bees were the most vulnerable, this variation highlights the need for race-specific management strategies that consider the unique characteristics of each honeybee subspecies. Finally, the successful management of *Varroa destructor* requires regular monitoring, timely intervention, and adaptation of practices to suit the specific needs of different honeybee hybrids (Jack et al., 2021)

2. Materials and Methods

2.1. Selection of Colonies

We selected honeybee colonies of *Apis mellifera lamarckii, A. m. ligustica*, and *A.m. Carnica.* Hybrids from different apiaries from Gharbia governorate to ensure a representative sample.

2.2. Bee and Brood Collection

For each colony, we collected the following samples: 100 worker bees from the brood nest area using a bee brush and 100 sealed worker brood cells from the brood nest of the three hybrids.

2.3. Mite Detection Techniques

The method for detecting *Varroa* mite infestation in honeybees using wheat flour involves the following steps:

a. Bees collecting: A sample of bees taken from the hive.

b. Adding flour: The bees were put in a container and lightly dusted with wheat flour. The flour stuck to both the bees and the mites.

c. Shaking the Container: The container was shaken vigorously. This helped the mites to fall off the bees and get coated with flour.

2.4. Separate and Check

After shaking, the mixture of bees and flour was poured through a sieve into another container. The flour collected at the bottom contained mites. The mites were counted using a magnifying glass or microscope. This method is popular because it is straight forward and inexpensive. It was proven effective in a study by De Jong et al. (1982) which showed that using flour is a practical way to monitor mite infestations. The total number of bees and brood cells were recorded in each sample. The numbers of mites found in each sample were documented. The infestation rate was calculated by dividing the number of mites by the number of bees or brood cells and multiplying by 100 to obtain a percentage.

2.5. Statistical Analysis

The data were subjected to appropriate statistical analysis, including one-way ANOVA, to determine significant differences among the studied groups. To compare infestation rates between different hybrids, time, and bee types (workers and worker's brood). Software tools SPSS were used for detailed analysis and visualization. Pearson's correlation coefficients were calculated to assess the relationships between Varroa mite infestation levels among the three honeybee hybrids.

3. Results

3.1. Number of Varroa on adult worker

3.1.1. Number of Varroa on adult workers of the first season

The Varroa mite infestation levels in *A. m. lamarckii* exhibited a distinct seasonal pattern. As shown in Figure (1) starting with a low level of 0.2 mites per bee in August, the infestation gradually increased to 3.6 mites per bee in October and November of the first year. Then, the infestation declined in March and April, reaching 2.6 and 2.2 mites per bee, respectively, and reached its highest level in November of second year, with 18.8 mites per bee. Remarkably, no Varroa mites were detected from May to September. However, the Varroa mite infestation levels in Italian bees, *A. m. ligustica* as shown in Figure (1), indicates that the number of Varroa mites recorded 0.2 mites per bee in August, then in October increased to 4.8 mites per bee in the first year. There was a decrease to 2.4 mites per bee in November, followed by fluctuating counts in the second year ranging from 0.2 mites per bee in May to 3.2 mites per bee in March. The infestation increased notably in October (23.2 mite per bee) and peaked at 43.4 mites per bee in November of the second year. As for *A. m. Carnica,* the initial Varroa count was 0.2 mite per bee in August, rising significantly to 5.0 mites per bee by November of the first year. In the second year, the infestation showed fluctuations, peaking at 6.2 mites per bee in May. Infestation decreased during the summer but increased again in October (4.0 mites per bee) and in November (13.6 mite per bee) of the second year. Overall, the data highlights distinct infestation patterns for each honeybee hybrid, *A. m. lamarckii* experiences a notable peak in November of the second year. *A. m. ligustica* showed the highest infestation levels in October and November of the second year, indicating a rapid increase in Varroa counts towards the end of the season. *A. m. Carnica* exhibited a more consistent pattern, with significant peaks observed in the fall of both years.

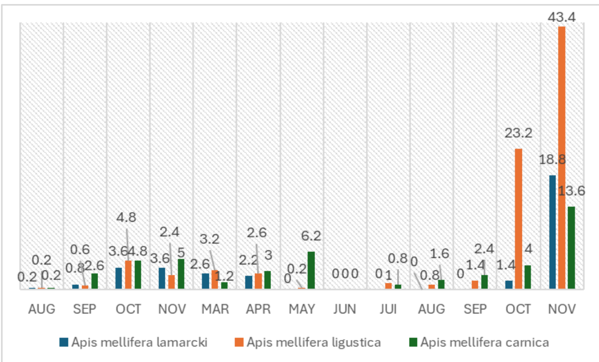

Figure 1. Mean number of Varroa on worker bees of the three hybrids during the first season (from August to November) in the first year and, (from March to November) in the second year.

3.1.2. Number of Varroa on adult workers of the second season

The data presented in Figure (2) showed a notable variation in infestation levels among the three hybrids throughout the second season. Obviously, the Egyptian race had the highest mean of Varroa counts at the beginning of the season, with a significant peak of 52.2 mites per bee in March. Infestation levels drop sharply to 8 in April and further decline to 0 from May to September. This suggests effective management or natural decline in mite populations during the warmer months, followed by a pronounced absence of mites. While in *A. m. ligustica*, the infestation begins at 27.8 mites per bee in March then decreases gradually over the months. The count peaks at 8.6 in April, with subsequent minor fluctuations and lower levels for the rest of the season (0.6 in May, 1.2 in June, 1.8 in July, 2 in August, and 1 in September). This pattern indicates a more stable but still notable infestation trend compared to *A. mellifera lamarckii*. However, the Varroa mite infestation levels in *A. m. Carnica*, at Figure (2) showed that the mean number of Varroa in March was 35.4 mites per bee, which is lower than *A. mellifera lamarckii* but higher than *A. mellifera ligustica*. The count peaked at 23.8 in April, followed by a steady decline over the remaining months. In September, no mites are recorded, reflecting a similar trend of eventual decline in mite populations as seen in the other hybrids.

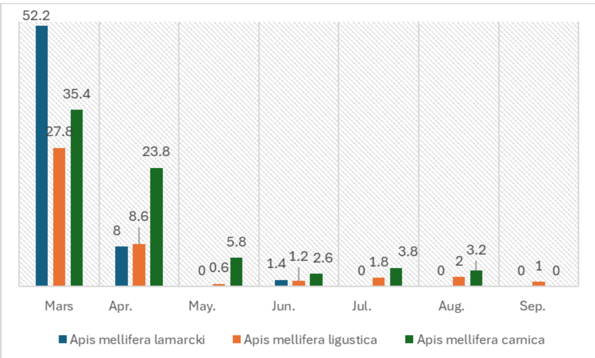

Figure 2. Mean number of Varroa on worker bees of the three hybrids in the second season from May to September.

3.2. Number of varroa on workers' brood

3.2.1. Number of Varroa on workers' brood during the first season

The data revealed in Figure (3) showed a significant variation in infestation levels among these hybrids throughout the first season, data of *A. m. lamarckii*, started with no detectable Varroa mites in August. Infestation levels gradually increased over the subsequent months, peaking at 12.6 mites per bee in October. This number of Varroa mites then decreases to 5.2 mites per bee in November. In March of the following year, the number dropped low to 1.2 mites per bee, suggesting either effective control measures or natural decline in mite populations. Notably, no Varroa mites were observed from April to June, indicating either successful management or environmental factors contributing to the decline.

Apis m. ligustica was similar to *A. m. lamarckii,* whereas it started with no Varroa mites in August. The infestation peaks at 11.8 mites per bee in October and declines to 5.8 mites per bee in November. In the subsequent season, the infestation level rose to 1 mite per bee in March and then increased significantly to 26.8 mites per bee in October, before decreasing slightly to 19.8 mites per bee in November. Also, *A. m. Carnica* started with no Varroa mites in August. Infestation levels increased over the months, reaching a peak at 7.2 mites per bee in October. The mite's number are relatively lower than those for *A. mellifera ligustica*, with a high of 20.4 mites per bee in November of the following year. Although there is some fluctuation, *A. mellifera Carnica* maintained lower mean infestation levels compared to *A. mellifera ligustica*, indicating a potentially better overall resistance to Varroa mites.

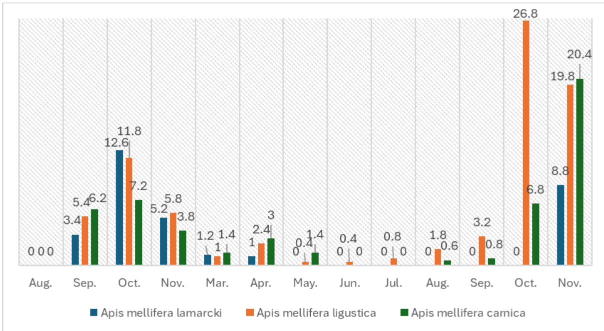

Figure 3. Mean Number of Varroa on workers' brood of the first season (from August to November in the first year and from March to November in the second year).

3.2.2. Number of Varroa on workers' brood during the second season

The data revealed in Figure (4) showed that the levels of infestation vary significantly among the different hybrids and indicates that some hybrids were more susceptible to mite infestation than others

In March, the infestation in *A. m. lamarckii* started high at 24 mites. In April, the number dropped to 6.2, and by August and September, it went to zero. This suggests that effective control measures were applied, leading to a significant recovery in the bees' health by the end of the season.

The infestation in *A. m. ligustica* started at 20.4 mites in March, which is moderate. The number decreased to 9.8 in April and remained relatively steady, fluctuating slightly over the subsequent months (from 1.6 in May to 3 in August). In September, the number decreased to 0.5. Although this type of bee can manage infestations, this race appears to manage mite infestation more consistently than *A. m. lamarckii.*

The race of *A. m. Carnica* has the highest infestation in March with 32 mites. The numbers dropped significantly over the next few months (27.4 in April, 19.4 in May, and 7.2 in June) and stabilized around 2-3 mites in July and August. In September, the mite count reaches zero, indicating that, despite the initial high infestation, the bees respond well to management practices.

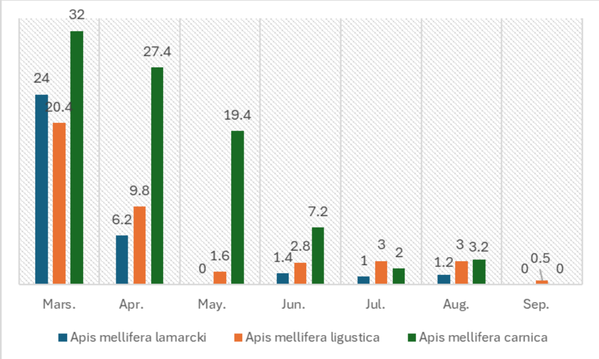

Figure 4. Mean Number of Varroa on workers' brood during the second season (from March to September).

3.3. Effect of Hybrids

3.3.1. Effect of Hybrids on the Dependent Variable: Worker's Varroa Infestation

The data revealed in Table (1), showed the significance level was 0.041, which was below the commonly accepted threshold of 0.05, indicating a statistically significant difference between the Egyptian and Italian bees. The 95% confidence interval for the difference in means ranges from -0.90 to -0.02, suggesting that Egyptian bees have a significantly lower mean value compared to Italian bees. Also, the significance level was 0.114, which is above the 0.05 limit, indicating that the difference between the Egyptian and Carniolan bees was not statistically significant. The 95% confidence interval ranges from -0.79 to 0.09, which included zero, further supporting the lack of significant difference.

Furthermore, the significance level was 0.639, well above 0.05, indicating no significant difference between Italian and Craniolan bees. The 95% confidence interval ranged from -0.54 to 0.33, which also included zero, further supporting the conclusion of no significant difference.

3.3.2. Effect of Hybrids on the Dependent Variable: Worker Brood Varroa Infestation

Also, Table (1) illustrated that the significance level between the Egyptian and Italian bees was 0.000, which is much lower than 0.05, indicating a highly statistically significant difference. The 95% confidence interval ranged from -1.24 to -0.54, showing that Egyptian bees have a significantly lower mean compared to Italian. At the same pattern, the significance level between Egyptian and Carniolan bees were 0.000 indicating a highly statistically significant difference. The 95% confidence interval ranged from -1.40 to -0.70, suggesting that Egyptian race has a significantly lower mean compared to Carniolan. The significance level was 0.355, which was above 0.05, indicating no significant difference between Italian and Carniolan bees. The 95% confidence interval ranged from -0.52 to 0.19, which included zero, supporting the absence of a significant difference. Generally, for the bee workers infestation with Varroa, only the comparison between Egyptian and Italian bees was significant, the Egyptian bees has a lower mean value. For the bee brood infestation with Varroa, Both the Egyptian and Italian bees and the Egyptian and Carniolan bees' comparisons showed significant differences, with the Egyptian bees consistently having lower mean values. The comparison between Italian and Carniolan bees brood was not significant.

Table 1. The statistical results included comparisons between different races (Egyptian, Italian, and Carniolan) for two dependent variables, worker varroa and worker's Brood Varroa.

Dependent Variable	(I) Race	(J) Race	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Worker's Infestation	Egyptian	Italian	.041*	-.90-	-.02-
		Carniolan	.114	-.79-	.09
	Italian	Egyptian	.041*	.02	.90
		Carniolan	.639	-.33-	.54
	Carniolan	Egyptian	.114	-.09-	.79
		Italian	.639	-.54-	.33
work's Brood Varroa	Egyptian	Italian	.000*	-1.24-	-.54-
		Carniolan	.000*	-1.40-	-.70-
	Italian	Egyptian	.000*	.54	1.24
		Carniolan	.355	-.52-	.19
	Carniolan	Egyptian	.000*	.70	1.40
		Italian	.355	-.19-	.52

*The *mean difference is significant at the .05 level*

3.4. Correlations

3.4.1. Correlations with Hybrids

a. Worker's Infestation with Varroa Mites

Basically, the number of hybrids didn't seem to affect the number of Varroa workers. So, the correlation with Varroa workers is negligible (r = 0.019), indicating that hybrids do not significantly influence the number of Varroa workers.

b. Brood Worker's Infestation with Varroa Mites

More hybrids are associated with more workers' brood. Then, the positive correlation (r = 0.168**) indicates that as the number of hybrids increases, the worker**'s** brood also increased moderately. This suggested that hybrids may enhance certain aspects of brood development

3.4.2. Correlations with Varroa Mites: Worker's Brood.

A strong positive correlation (r = 0.603) was found. Higher Varroa worker counts were linked with more workers' brood.

4. Discussion

Varroa mite infestation patterns varied significantly among the three honeybee hybrids studied. *A. m. lamarckii* exhibited a notable peak in November of the second year, while *A. m. ligustica* experienced the highest infestation levels in October and November, indicating a sharp increase toward the end of the season. In contrast, *A. m. carnica* displayed a more consistent infestation pattern, with significant peaks in the fall of both years. These variations highlight the differing susceptibilities of each race to *Varroa destructor*, emphasizing the need for race-specific management strategies.

The results indicate that *A. m. lamarckii* was the most affected by Varroa mites at the start of the season but showed a sharp decline in mite numbers over time. This decline may be attributed to strong grooming behaviors, a trait previously documented in Egyptian bees that helps reduce mite loads (El-Seedi et al., 2022). Conversely, *A. m. ligustica* exhibited the lowest infestation levels at the beginning of the season but showed a significant increase in mite numbers later in the year. The late-season peaks suggest a prolonged brood cycle, providing mites with extended opportunities for reproduction (Spivak and Reuter, 2001). Moderate infestation levels were maintained *in A. m. carnica* throughout the study, with peaks in the fall, this aligned with previous findings suggesting that the hygienic behaviors in Carniolan honeybees can mitigate but not eliminate Varroa mite populations (Büchler et al., 2010; Rosenkranz et al., 2010).

Race-Specific Management Strategies given the observed differences; each race required a tailored approach to Varroa mite management. *A. m. lamarckii,* is well adapted to hot, arid environments, a factor that contributes to its natural defense mechanisms, particularly grooming behaviors that help dislodge mites. Although initial infestation levels may be high, they tend to decline as the season progresses, suggesting that mechanical control methods (e.g., powdered sugar dusting or screened bottom boards) may be particularly effective (El-Seedi et al., 2022). Despite *A. m. carnica* has moderate infestation levels, this race demonstrated a relatively stable Varroa population, due to its hygienic behavior and efficient detection of infested brood (Büchler et al., 2014). Management strategies should focus on integrated pest management (IPM) approaches that combine selective breeding for hygienic traits with biological controls like predatory mites. In spite of *A. m. ligustica* has prolonged brood cycle and high late-season infestation levels, this race requires intensive management strategies, including chemical treatments such as oxalic acid or formic acid applications. Additionally, brood interruption techniques may be beneficial to disrupt the reproductive cycle of Varroa mites (Spivak and Reuter, 2001).

At the start of August, all three hybrids exhibited negligible mite infestations. However, *A. m. ligustica* demonstrated the highest increase in Varroa counts in the following months, reinforcing its susceptibility. In contrast, *A. m. carnica* showed a more stable infestation pattern, suggesting a degree of resistance or more effective natural control mechanisms. The sharp decline in *A. m. lamarckii* infestations after March aligned with previous research indicating that environmental factors, such as high temperatures, may limit mite (Büchler et al., 2014).

A comparison of worker bee infestations revealed that the difference between Egyptian and Italian hybrids was significant, with *A. m. lamarckii* showing lower mean infestation levels. Similarly, for brood infestations, significant differences were observed between the Egyptian and Italian, as well as the Egyptian and Carniolan hybrids, whereas the Egyptian race consistently exhibiting lower mite loads. However, the comparison between Italian and Carniolan hybrids, did not reveal any significant differences, suggesting a similar level of susceptibility to *Varroa* infestation in both hybrids

Correlation analysis revealed that bee workers infestation with Varroa mites showed a negligible relationship with hybrids ($r = 0.019$), indicating that mite presence in bee workers was not significantly influenced by the hybrids. However, brood infestation rates showed positive correlation with hybrids number ($r = 0.168^{**}$), suggesting that race type may have a moderate influence on mite loads within the brood (Büchler et al., 2014; Spivak and Reuter, 2001).

5. Conclusions

These findings highlight the variability in Varroa mite infestations among different honeybee hybrids, underscoring the necessity of hybrid-specific monitoring and management strategies. The unique strengths and vulnerabilities of each race require targeted approaches based on their genetic traits, brood cycles, and environmental interactions. By integrating selective breeding, biotechnical methods, and chemical control strategies, beekeepers can mitigate Varroa mite impacts and enhance colony health and productivity.

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