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Assessment of *Varroa destructor* infestation rate in honey bee colonies from some areas of eastern Libya

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تقييم معدل الإصابة بحلم الفاروا (*Varroa destructor*) في طوائف نحل العسل في بعض مناطق شرق ليبيا

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Abstract:
Varroa destructor is one of the most important ectoparasites affecting honey bee (*Apis mellifera*) colonies worldwide and monitoring its infestation rate is essential for evaluating colony health and improving management practices. This study aimed to assess the infestation rate of V. destructor and investigate its variation among honey bee colonies from some areas of eastern Libya. Adult worker bee samples were collected from 22 honey bee colonies distributed among three study areas: Shahat, Qasr Libya, and South Al-Abyar (Al-Khrouba and Al-Jahishiya), during May and early June 2026. A total of 4,137 adult worker bees were examined using the alcohol wash method, and infestation rates were calculated based on the number of recovered mites relative to the number of examined bees. Data were analyzed using descriptive statistics and the Kruskal–Wallis test to compare infestation rates among the study areas. The overall mean infestation rate was 4.01 ± 5.14%, with mean values of 2.88 ± 3.12% in Qasr Libya, 4.73 ± 1.72% in Shahat, and 4.44 ± 7.23% in South Al-Abyar. The highest individual infestation value was recorded in South Al-Abyar (24.4%). However, no statistically significant differences were detected among the three study areas ($\chi^2 = 2.37$, df = 2, P = 0.305). The observed variation among colonies suggests that infestation levels may be influenced by colony-specific and management-related factors rather than geographical location alone. These findings confirm the presence of *V. destructor* in the studied areas and highlight the importance of regular colony monitoring and appropriate management strategies to maintain honey bee colony health. Further studies incorporating seasonal monitoring and broader geographic coverage are recommended to improve understanding of Varroa population dynamics in Libyan apiaries.

Keywords: *Varroa destructor*, honey bee colonies, infestation rate, *Apis mellifera*, alcohol wash method, eastern Libya.

الملخص:
يُعد حلم الفاروا (*Varroa destructor*) أحد أهم الطفيليات الخارجية التي تؤثر في طوائف نحل العسل (*Apis mellifera*) على مستوى العالم، وتُعد مراقبة معدل الإصابة به أمرًا ضروريًا لتقييم صحة الطوائف وتحسين ممارسات الإدارة. هدفت هذه الدراسة إلى تقييم معدل الإصابة بحلم *V. destructor* ودراسة تباينه بين طوائف نحل العسل في

بعض المناطق من شرق ليبيا. جُمعت عينات من الشغالات البالغة من 22 طائفة نحل عسل موزعة على ثلاث مناطق دراسية: شحات، قصر ليبيا، وجنوب الأبيار (الخروبة والجحيشية)، خلال شهري مايو وبداية يونيو 2026. تم فحص ما مجموعه 4,137 نحلة عاملة باستخدام طريقة الغسل بالكحول، وحُسبت معدلات الإصابة اعتمادًا على نسبة عدد حلم الفاروا المستخرج إلى عدد النحل المفحوص. تم تحليل البيانات باستخدام الإحصاءات الوصفية واختبار كروسكال–واليس لمقارنة معدلات الإصابة بين مناطق الدراسة. بلغ متوسط معدل الإصابة الكلي 4.01 ± 5.14%، حيث سجلت القيم المتوسطة 2.88 ± 3.12% في قصر ليبيا، و4.73 ± 1.72% في شحات، و4.44 ± 7.23% في جنوب الأبيار. وسُجلت أعلى قيمة فردية لمعدل الإصابة في جنوب الأبيار (24.4%). ومع ذلك، لم تظهر فروق ذات دلالة إحصائية بين مناطق الدراسة الثلاث ($\chi^2 = 2.37$، $df = 2$، $P = 0.305$). ويشير التباين الملحوظ بين الطوائف إلى أن مستويات الإصابة قد تتأثر بعوامل خاصة بالطائفة وعوامل مرتبطة بإدارة المنحل أكثر من ارتباطها بالموقع الجغرافي فقط. تؤكد هذه النتائج وجود حلم *V. destructor* في المناطق المدروسة، وتبرز أهمية المراقبة الدورية للطوائف وتطبيق استراتيجيات الإدارة المناسبة للحفاظ على صحة طوائف نحل العسل. كما يُوصى بإجراء دراسات مستقبلية تتضمن المتابعة الموسمية وتغطية جغرافية أوسع لتحسين فهم ديناميكية جماعات الفاروا في المناحل الليبية.

الكلمات المفتاحية: حلم الفاروا(*Varroa destructor*) ، طوائف نحل العسل؛ معدل الإصابة، نحل العسل *Apis mellifera*، طريقة الغسيل بالكحول، شرق ليبيا.

Introduction:

The European honey bee (*Apis mellifera*) is a valuable insect that provides essential ecosystem services and produces honey, wax, propolis, venom, and royal jelly (Hung et al., 2018). Honey bees, together with other insect pollinators, contribute to the pollination of 85–90% of flowering plants, thereby supporting their reproduction (Potts et al., 2010; Pires & Maués, 2020). During foraging, worker bees collect nectar and pollen to meet the colony's nutritional requirements (Nicolson, 2011). Pollen serves as the primary source of protein, whereas nectar provides the carbohydrates required for bee activity and honey production (Ghosh et al., 2020; Hendriksma et al., 2014). However, Goulson et al. (2015) identified several stressors contributing to the global decline of honey bees, including habitat loss, parasites, pesticides, monotonous diets, transportation stress, competition, and climate change. When two or more of these stressors occur simultaneously, their combined effects can significantly increase the risk to honey bee colony health (Neov et al., 2019).

The life cycle of *V. destructor* consists of two main phases: the phoretic phase and the reproductive phase. During the phoretic phase, only the adult female mite uses the adult honey bee as both a means of transport and a source of food. During this period, bees inadvertently contribute to the transmission of Varroa within and between honey bee colonies. The reproductive phase begins when a female mite enters an unsealed brood cell containing a fifth-stage bee larva, where it lays eggs after the cell is sealed. (Häußermann et al.,2020) reported that a virgin mite could initiate the phoretic phase. Under these conditions, the mite enters a brood cell, deposits an unfertilized egg, and subsequently mates with the resulting male offspring. Female *Varroa* reproduce through arrhenotokous parthenogenesis, allowing unfertilized eggs to develop into males. Although Varroa mites were initially believed to feed on honey bee recent studies have shown that they also feed on the honey bee fat body (Ramsey et al., 2019).

Varroa destructor was reportedly introduced into Libya in 1976 or 1977 with honey bee colonies brought from Bulgaria (Crane, 1979). The mite eventually became established and spread throughout the country (Keshlaf & Alfallah, 2019).

Honey bee parasitism by *V. destructor* reduces the body weight and water content of immature emerging bees (Bowen-Walker & Gunn, 2001). The weight loss of future adult bees increases with the number of foundress mites (Duay et al., 2003; Strauss et al., 2016). In honey bees, the amount of spermatozoa is proportional to drone body size (Schlüns et al., 2003). By reducing drone size, Varroa causes a decrease in sperm production and consequently affects reproductive fitness (Duay et al., 2002). Varroa also affects the flying, homing, and orienting abilities of foragers (Kralj & Fuchs, 2006). This may reduce their ability to collect resources required for colony development. Failure to return to the colony can also be viewed as a defensive response adopted by parasitized bees. The effects of *Varroa* on bee behavior may be explained by its ability to alter neural processes, thereby disrupting honey bees' non-associative learning abilities. Indeed, parasitized bees show a reduced sugar response and faster adaptation to olfactory stimuli (Kralj et al., 2007). Additionally, *Varroa* causes proteomic alterations in the honey bee immune response (Erban et al., 2019; Słowińska et al., 2019) and downregulates immune gene expression in emerging infested adults (Nazzi et al., 2012). By interfering with immune response pathways, it disrupts the bee's immune defense (Zaobidna et al., 2017). For example, *Varroa* reduces the expression of prophenol oxidase, which is involved in melanin production, and decreases the number of circulating hemocytes in the hemolymph (Koleoglu et al.,

2018; Wegener et al., 2016). Both melanin and hemocytes contribute to insect immune responses and wound healing by enabling pathogen encapsulation during infection or injury (Kanost & Gorman, 2008). Since fat bodies play an important role in immunity (Arrese & Soulages, 2010), the discovery that mites feed on this tissue may be associated with the decline in honey bee immunity (Annoscia et al., 2019; Yang & Cox-Foster, 2005).

Evaluating the level of *Varroa destructor* infestation in adult honey bees is an essential component of colony health monitoring and supports informed management decisions. The alcohol wash and powdered sugar roll are among the most widely used standardized techniques for assessing mite infestation in adult bees. The efficiency and accuracy of these methods for removing mites from adult bees vary. Because it effectively removes mites from bee bodies and provides a reliable estimate of infestation levels, the alcohol wash is generally considered the most accurate and reliable method. Therefore, it is widely used in standardized monitoring programs and research (Dietemann et al., 2013). In order to determine the distribution and prevalence of *Varroa destructor* in honey bee colonies throughout the United States between 2015 and 2022, Kozak et al. (2024) conducted a comprehensive survey. The study assessed Varroa infestation levels in adult bee samples collected from several geographic locations and revealed significant regional and seasonal variations in infestation levels. The authors emphasized the importance of seasonality and geographic location in determining the population dynamics of *V. destructor* in honey bee colonies.

Giacobino et al. (2017) investigated the factors associated with high *Varroa destructor* infestation levels in honey bee colonies across different ecological regions of Argentina. The findings showed that environmental conditions and beekeeping management practices influenced infestation levels. Additionally, the study identified an association between *Varroa* infestation and other stressors, such as *Nosema* infection, suggesting that environmental factors may contribute to regional differences in infestation intensity.

Smoliński et al. (2021) investigated the long-term effects of climate on *Varroa destructor* population dynamics in Central European honey bee colonies from 1991 to 2020. The study showed that higher autumn *Varroa* infestation levels were associated with increased temperatures during specific periods of the year. Furthermore, the availability of capped brood and colony strength had a considerable influence on mite abundance, highlighting the importance of biological and environmental factors in regulating *Varroa* populations.

Robi et al. (2023) investigated the prevalence and infestation levels of *Varroa destructor* in honey bee colonies from several agroecological zones in Southwest Ethiopia. The study revealed considerable differences in infestation levels among ecological regions, and infestation intensity was associated with several factors, including agroecology, hive type, colony condition, and beekeeping management practices. The authors concluded that environmental and management factors influence Varroa infestation patterns.

Keshlaf et al. (2023) conducted a survey to assess the prevalence of *Varroa destructor* in honey bee colonies from different geographical regions of Libya. The study confirmed the presence of Varroa mites in the investigated regions and showed that infestation levels varied among locations. These findings highlight the influence of local environmental factors and provide important baseline information for future studies on Varroa infestation patterns in Libyan honey bee populations.

To understand the prevalence and severity of this economically important parasite, assessing *Varroa destructor* infestation levels in honey bee colonies is essential. Monitoring *Varroa* infestations provides valuable information for evaluating colony health and developing effective management strategies to reduce colony losses. Although studies conducted worldwide have demonstrated that environmental and management factors influence mite abundance (Kozak et al., 2024; Robi et al., 2023), limited information is available regarding the spatial variation of *Varroa* infestation levels in Libya, particularly in the eastern regions. Therefore, this study aims to provide baseline data on the current status of *V. destructor* infestation in honey bee colonies across different areas of Eastern Libya. The findings may support future research, improve beekeeping practices, and contribute to the development of appropriate *Varroa* control programs under local environmental conditions.

Objectives of the study:

To assess the prevalence and infestation levels of *Varroa destructor* and investigate their variation among selected honey bee colonies from different areas in eastern Libya.

Materials and Methods:

Study area:

This study was conducted in eastern Libya to assess the level of *Varroa destructor* infestation in honey bee colonies (*Apis mellifera L*.). The study included three main regions: Shahat, Qasr Libya, and South Al-Abyar. Within the South Al-Abyar region, sampling was carried out at two sites, Al-Khrouba

and Al-Jahishiya. These sites were selected to compare Varroa destructor infestation levels among honey bee colonies from different regions of eastern Libya.

Honey bee sample collection:

Adult worker honey bee samples were collected from May to early June 2026. The study included 22 honey bee colonies distributed among the study areas, including five colonies from Shahat, seven colonies from Qasr Libya, and ten colonies from South Al-Abyar (Al-Khrouba and Al-Jahishiya).

Adult worker bees were collected from the selected colonies and transported to the laboratory for examination. Due to differences in colony strength, the number of examined worker bees ranged from 100 to 300 workers per colony, with a total of 4,137 examined adult worker bees.

Assessment of *Varroa destructor* infestation:

Varroa destructor infestation was assessed using the alcohol wash method. This method is a standard technique for estimating mite infestation in honey bee colonies and was performed according to the protocols described by Dietemann et al. (2013) and Gregorc and Sampson (2019).

Adult worker bees were placed in containers containing 70% ethyl alcohol and shaken thoroughly to detach *Varroa destructor* mites from their bodies. The bees were then separated from the wash solution using a suitable mesh strainer, after which the wash solution was examined and the detached mites were counted.

For each colony, the number of *Varroa destructor* mites and the number of examined worker bees were recorded. The infestation rate was calculated using the following formula:

Infestation rate (%) = (Number of *Varroa destructor* mites / Number of examined worker bees) × 100

Statistical analysis:

Statistical analyses were performed using jamovi software (version 2.7.37). Descriptive statistics, including the mean, standard deviation, median, minimum, and maximum infestation rates, were calculated for each study area. The normality of the data distribution was assessed using the Shapiro–Wilk test. As the infestation data did not satisfy the assumption of normality, differences in infestation rates among the three study areas were evaluated using the Kruskal–Walli's test. Statistical significance was set at $P \leq 0.05$.

Results:

The infestation rates of *Varroa destructor* were evaluated in honey bee colonies collected from three study areas in eastern Libya: Qasr Libya, Shahat, and South Al-Abyar (Al-Khrouba and Al-Jahishiya). The normality of infestation rate data was assessed using the Shapiro–Wilk test. The results showed that infestation rates in South Al-Abyar deviated significantly from normal distribution ($W = 0.586$, $P < 0.001$), whereas data from Qasr Libya ($W = 0.824$, $P = 0.070$) and Shahat ($W = 0.921$, $P = 0.540$) did not show significant deviations. Since the assumptions of normality were not fully met, the non-parametric Kruskal–Walli's test was used to compare infestation rates among the three study areas.

Descriptive statistics of *Varroa destructor* infestation rates in honey bee colonies from the three study areas are presented in Table 1. The overall mean infestation rate was 4.01 ± 5.14%, with a median value of 3.00%. The mean infestation rate was 2.88 ± 3.12% in Qasr Libya, 4.73 ± 1.72% in Shahat, and 4.44 ± 7.23% in South Al-Abyar. The highest recorded infestation value was observed in South Al-Abyar (24.4%), while the lowest values (0%) were recorded in colonies from Qasr Libya and South Al-Abyar.

Table (1): Descriptive statistics of *Varroa destructor* infestation rates (%) in honey bee colonies from the three study areas.

Study area	N	Mean ± SD (%)	Median
Qasr Libya	7	2.88 ± 3.12	2.00
Shahat	5	4.73 ± 1.72	4.67
South Al-Abyar	10	4.44 ± 7.23	2.19
Total	22	4.01 ± 5.14	3.00

Although infestation rates were numerically higher in Shahat and South Al-Abyar compared with Qasr Libya, the Kruskal–Walli's test indicated no statistically significant differences among the three study areas ($\chi^2 = 2.37$, $df = 2$, $P = 0.305$).

The distribution of infestation rates among honey bee colonies is shown in Figure 1. The box plot indicated variation in infestation levels among colonies, particularly in South Al-Abyar, where greater variability and the highest individual infestation value were observed. However, the distributions of infestation rates showed considerable overlap among the three study areas, indicating similar infestation levels among the studied regions.

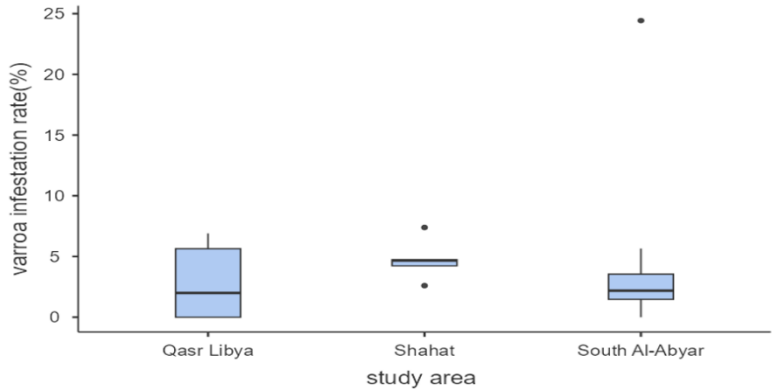

Figure (1): Box plot showing the *distribution of Varroa* destructor infestation rates (%) among honey bee colonies in the three study areas.

Discussion:

The present study recorded an overall *Varroa destructor* infestation rate of 4.01% in honey bee colonies from some areas of eastern Libya. This value is close to the overall infestation rate (3.5%) reported by Keshlaf et al. (2023) in Libyan honey bee colonies, suggesting that the infestation level observed in the selected areas is consistent with the pattern previously reported for Libyan apiaries. Nevertheless, the detection of *V. destructor* in all investigated areas confirms the presence of this parasite in all studied locations. Although the overall infestation level was relatively moderate, its presence highlights the need for regular monitoring because unmanaged mite populations can increase rapidly and negatively affect colony health and productivity (Keshlaf et al., 2023; Rosenkranz et al., 2010; Warner et al., 2024).

Despite the numerical differences in mean infestation rates among the three study areas, the Kruskal–Walli's test detected no statistically significant differences. Although Shahat showed the highest mean infestation rate (4.73%), this difference was not statistically significant among the study areas. Therefore, the observed variation should be interpreted as a numerical difference rather than a geographical effect. This finding indicates that geographical location alone did not have a measurable influence on infestation levels during the study period. Instead, the observed variation is more likely associated with colony-level factors, including brood availability, colony strength, queen performance, hygienic behavior, and apiary management practices, which are recognized as important factors affecting *Varroa* population development .

Considerable variation in infestation rates was observed among individual colonies, particularly in South Al-Abyar, where one colony reached an infestation rate of 24.4%, whereas others showed little or no detectable infestation. Such variation is characteristic of *Varroa* populations, which are rarely distributed uniformly among colonies. Differences in brood production, hygienic behavior, colony demography, and management conditions can result in substantial differences in mite abundance, even among colonies within the same apiary (Dietemann et al., 2013; Rosenkranz et al., 2010). This finding highlights the importance of evaluating infestation at the colony level, as relying only on mean infestation rates for each study area may overlook highly infested colonies that could contribute to mite transmission.

The present study provides additional information on *Varroa destructor* infestation in selected areas of eastern Libya. Although infestation levels were generally comparable among the investigated areas, the detection of the mite across all locations indicates its continuous occurrence within the studied areas. These findings emphasize the importance of regular surveillance and integrated mite management strategies to reduce the risk of colony losses and support sustainable beekeeping (Ceccotti et al., 2025). Future studies involving larger sample sizes, repeated seasonal sampling, and evaluation of environmental and management-related factors would provide a more comprehensive understanding of Varroa dynamics in Libyan honey bee populations.

Conclusion:

The present study provides further evidence on the occurrence of *Varroa destructor* in honey bee colonies from selected areas of eastern Libya. The findings indicate that infestation levels were generally moderate during the study period, while differences among colonies highlight the complex nature of Varroa population dynamics. These results emphasize the importance of regular colony assessment and appropriate management practices to support honey bee health. Future studies incorporating broader geographic coverage, seasonal monitoring, and additional ecological factors are needed to improve understanding of *Varroa* dynamics in Libyan apiaries.

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