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# PREVALENCE, SEASONALITY, AND ASSOCIATED RISK FACTORS OF VARROA MITE INFESTATION IN THE APIARIES OF TEHSIL-E- MATTA, SWAT, PAKISTAN

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## Abstract
*The ectoparasitic mite Varroa destructor is a primary global threat to apiculture, causing significant colony losses. This study assessed the prevalence of Varroa destructor and identified associated risk factors in the apiaries of Tehsil Matta, District Swat, Pakistan. A total of 251 honey bee hives (Apis mellifera = 80; Apis cerana = 171) were inspected from May to July 2025 using the non-lethal sugar shake method. The overall prevalence was 11.2% (28/251). Statistical analysis revealed significant associations between infestation rates and several management and environmental variables. Infestation was markedly higher in the introduced A. mellifera (26.3%) compared to the native A. cerana (4.1%). Furthermore, modern beekeeping practices were linked to increased risk, with prevalence in wooden hives (26.3%) far exceeding that in traditional earthen hives (3.5%). Urban apiaries showed a 33.3% infestation rate versus 1.0% in rural settings. Infestation escalated with rising seasonal temperatures, from 0% in May to 28.0% in July, correlating with a jump from 4.5% at 32°C to 25.0% at 35°C. Colonies with supplemental feeding and poor hygiene also showed elevated risk. These findings establish a critical baseline for V. Destructor prevalence in the region and demonstrate that the transition to intensive, non-native beekeeping systems significantly elevates parasitic load. The study underscores the urgent need for integrated pest management strategies that favor locally adapted bee subspecies and sustainable husbandry practices to enhance colony resilience.*

## INTRODUCTION

Honey bees (Apis spp.), members of the order Hymenoptera, are eusocial insects of immense global ecological and economic importance (Hickman et al., 2006; Winston, 1987). Beyond their production of honey, a valuable food and medicinal substance for humans, their role as pollinators is indispensable (Klein et al., 2007; Greenleaf & Kremen, 2006). Pollinators, with honey bees as key contributors, are involved in the production of over 70% of the world's leading food crops, directly affecting food security and biodiversity (Dos Santos & Otesbelgue, 2025). In the United States alone, the annual market value of commercial pollination services was over $400 million in 2024, often surpassing honey production revenue for beekeepers (United States Department of Agriculture [USDA ERS], 2025). The genus Apis is diverse, with major species like the Western honey bee (*A. mellifera*), the Asiatic honey

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bee (*A. cerana*), the giant honey bee (*A. dorsata*), and the dwarf honey bee (*A. florea*), each adapted to specific ecological niches across Asia, Europe, and Africa (Ruttner, 1987; Kotthoff et al., 2013).

Despite their significance, honey bee colonies worldwide face severe threats, leading to unsustainable annual losses. These declines are driven by a multifactorial syndrome including habitat loss, pesticide exposure, viral diseases, and, critically, the ectoparasitic mite Varroa destructor (Ellis & Nalen, 2010; Rosenkranz et al., 2010). Varroa destructor, an obligate parasite that feeds on bee hemolymph, is arguably the most devastating single pest of apiculture. Its recent establishment in regions previously free of the mite, such as Australia in 2022, triggers radical changes in beekeeping management and necessitates extensive industry transition plans (National Varroa Mite Management Program, 2024). Management traditionally relies on synthetic chemical treatments (Goodwin & Eaton, 2001; MAF, 2001), but issues of resistance, hive contamination, and food safety residues underscore the urgent need for integrated, sustainable control strategies (Mutinelli & Baggio, 2004; O'Shea-Wheller et al., 2025).

A critical and interconnected stressor is nutritional deficiency. Bees require a balanced diet of pollen and nectar to obtain proteins, lipids, vitamins, and essential sterols. However, climate change and agricultural intensification have degraded floral diversity, depriving colonies of the varied forage they need. Modern landscapes often force beekeepers to rely on commercial pollen substitutes, which are frequently nutritionally incomplete, lacking specific micronutrients vital for bee development (Dos Santos & Otesbelgue, 2025). This malnutrition weakens individual bees and entire colonies, compromising their immune systems and making them more susceptible to parasites like Varroa and other pathogens.

Recent scientific advances are illuminating both the depth of these challenges and novel pathways to resilience. Groundbreaking research by Moore et al. (2025) has identified that a precise mixture of six key pollen sterols is essential for bee larval development and colony reproduction. Using synthetic biology, researchers have engineered yeast (Yarrowia lipolytica) to produce these sterols; feeding trials showed colonies on this enriched diet reared up to 15 times more larvae than those on conventional supplements. Concurrently, there is a push to develop multifunctional feeds that combine nutrition with therapeutic benefits. For example, recent formulations by Muñoz-Fariña et al. (2025) fortified with polyphenol-rich berry extracts and acaricidal essential oils (like oregano oil) have demonstrated dual utility: providing balanced nutrition while achieving over 90% reduction in Varroa mite counts, offering a sustainable "food-as-therapy" alternative to synthetic miticides. This aligns with earlier work suggesting the potential of combined organic treatments, such as oxalic acid with thymol (Mahmood et al., 2012).

Furthermore, the co-evolutionary relationships between bees and their specialized gut microbiota are gaining recognition as a cornerstone of health. These bacterial communities aid in digestion, nutrient absorption, and pathogen defense. A comparative metagenomic study across honey bee species by An et al. (2025) reveals that while core microbial groups exist, their composition and functionality are highly dynamic and host-specific. This suggests that a bee's internal microbial symbionts are a vital component of its overall fitness and adaptation.

Given this context, the pressing need is for holistic management approaches that address the intertwined issues of parasitism and malnutrition. This study, therefore, seeks to contribute to this integrative paradigm. Focusing on a key honey bee species in [Your Region], it will investigate the synergistic relationship between colony nutritional status and *Varroa destructor* infestation levels. By evaluating both the efficacy of novel nutritional supplements and their potential to enhance natural colony defenses against parasites, this research aims to inform sustainable, effective strategies for safeguarding these indispensable pollinators.

## Material and Methods

### 3.1. Study Area and Duration

This study was conducted from May to July 2025 in Tehsil Matta, Swat District, Khyber Pakhtunkhwa, Pakistan. This three-month period spans spring and summer, corresponding with peak honey bee activity and potential ectoparasite development, making it optimal for prevalence surveys.

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### 3.2. Sample Collection

A total of 251 honey bee samples were collected from hives across Tehsil Matta. The sample population comprised 171 *Apis cerana* samples from traditional earthen hives and 80 *Apis mellifera* samples from commercial wooden (forum-type) hives managed by local beekeepers. Live adult honey bees were gently collected from brood frames using a bee brush or sheets of paper and immediately transferred to a shaking jar.

(a) (b) (c)

**Figures** 3.1: **(a), (b), (c) showing the collection of samples**

### 3.3. Materials

The following materials were used for mite detection:

- Shaking jars with mesh lids (3-4 mm pore size)
- Food-grade icing (powdered) sugar
- Tablespoons
- White plastic trays or containers
- Standard beekeeping personal protective equipment (veil, gloves)
- Bee brush
- Stereomicroscope (for post-collection confirmation)
- Sample tubes for mite storage

### 3.4. Detection and Enumeration of Varroa Mites

The non-lethal sugar shake method was employed for the detection and quantification ofvarroa mites (Gregorc, Sampson, et al., 2017; Gregorc, Alburaki, et al., 2017). This method was selected for its efficacy, simplicity, minimal cost, and low impact on bee health, allowing for the safe release of sampled bees.

**3.4.1 Procedure:** Approximately 300 adult bees per sample were collected and placed in a shaking jar. Two tablespoons of icing sugar were added, and the jar was rolled for two minutes to coat the bees. After resting for one minute, the jar was inverted and shaken over a white tray to dislodge mites. If mites were not immediately visible, a small amount of water was added to dissolve the sugar, causing mites to float to the surface. All sampled bees were released at their hive entrance following the procedure. An infestation threshold of ≥3 mites per 300 bees was used to recommend treatment, following established guidelines (Ellis & Nalen, 2010).

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(a) (b)

**Figure 3.2: (a), (b) showing the Varroa mites covered in icing powdered sugar in white tray after shaken off honey bees.**

### 3.5. Mite Identification

Detected mites were collected and preserved.Identification to the species level was performed using established morphological keys (Delfinado & Baker, 1974; De Guzman, Rinderer, & Frake, 2007) under a stereomicroscope.

### Results

The present study was conducted on ectoparasites Varroa mites of apiaries of Tehsil Matta District Swat to find out the infestation of Varroa mite. During research work a total 251 colonies of honey bees were visited from different locations of tehsil Matta during the months of May to July 2025. Out of which 171 hives of *Apis cerana* and remaining 80 hives of *Apis mellifera*. Each hives was observed by using standard parasitological procedures like visual inspection and microscopic examination to identify the presence of Ectoparasitic mites in honey bees. The results of the study show that 28 out of 251 hives were found to be infested with Ectoparasite Varroa Mites. So it means that 11% of the honey bee hives were infested with Varroa mites. The infestation of Varroa mites was observed by simply visible signs of mites attached on thorax and abdomen region of adult bees and deformed wings of honey bees and also by using sugar shake method. The infestation of Varroa mites were also strongly associated with different Months of the year, as reflected by the study that highest infestation rate of ectoparasite was detected in July (28%) followed by lower infestation rate in June (9%) and in May (0%).The results of the study also show that Varroa mites infestation also strongly associated with temperature range, no awareness about the control of ectoparasites mites, poor hygienic condition of hives, hives condition, species and absence of treatment strategies regarding the ectoparasites mites. So the overall results of the current study highlight the infestation of ectoparasite Varroa mites in the tehsil Matta.

**Table 4.1: Shows the overall results of the research work.**

| Serial No. | Category | Types | No.of hives observed | Infestation | %age |
|---|---|---|---|---|---|
| 1 | Variety/species | *Apis cerana*(local) | 171 | 7 | 4% |
| | | *Apis mellifera*(Non local) | 80 | 21 | 26% |
| | | Total hives | 251 | 28 | 11% |
| 2 | Construction of hives | Wooden hives | 84 | 22 | 26% |
| | | Earthen hives | 167 | 6 | 3.5% |
| | | Total hives | 251 | 28 | 11% |
| 3 | Hives location | Rural area | 174 | 2 | 1% |

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| | | | | | |
|---|---|---|---|---|---|
| | | Urban area | 77 | 26 | 33% |
| | | Total hives | 251 | 28 | 11% |
| 4 | Treatment of hives | Untreated hives | 45 | 7 | 15% |
| | | Treated hives | 206 | 21 | 10% |
| | | Total hives | 251 | 28 | 11% |
| 5 | Feeding nature of honey bees | Supplemental feeding | 246 | 28 | 11% |
| | | Natural feeding | 5 | 0 | 0% |
| | | Total hives | 251 | 28 | 11% |
| 6 | Condition of hives | Hygienic hives | 233 | 23 | 9% |
| | | Unhygienic hives | 18 | 5 | 27% |
| | | Total hives | 251 | 28 | 11% |
| 7 | Temperature range | 32 | 174 | 8 | 4.5% |
| | | 35 | 77 | 20 | 25% |
| | | Total hives | 251 | 28 | 11% |
| 8 | Months | May | 82 | 0 | 0% |
| | | June | 106 | 10 | 9% |
| | | July | 63 | 18 | 28% |
| | | Total | 251 | 28 | 11% |
| 9 | Experience of beekeeping | 1 year | 54 | 0 | 0% |
| | | More than 1 year | 197 | 28 | 14% |
| | | Total hives | 251 | 28 | 11% |
| 10 | Inspection of hives | Daily | 3 | 0 | 0% |
| | | Weekly | 208 | 21 | 10% |
| | | Monthly | 33 | 6 | 18% |
| | | Rarely | 7 | 1 | 14% |
| | | Total hives | 251 | 28 | 11% |

### 4.2 HIVES LOCATION

The infestation of ectoparasites of honey bees hives in rural and urban area. Out of 251 hives 77 hives are urban while 174 hives are rural. The rate of infestation is high in urban (33%) as compared to rural (1%) as presented in the table 4.2.

**Table 4.2: Shows the infestation of ectoparasites Varroa mites of honey bees in rural and urban area.**

| Hives location | No.of hives | Infestation | %age |
|---|---|---|---|
| Urban hives | 77 | 26 | 33% |
| Rural hives | 174 | 2 | 1% |
| Total | 250 | 28 | 11% |

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### 4.3 CONSTRUCTION OF HIVES

The infestation of ectoparasites of honey bees in wooden hives and earthen hives. Approximately a total 251 hives were visited out of which 167 were earthen hives and 84 were wooden hives. It is reflected from current study that the rate of infestation is high in wooden hives (26%) than earthen hives (3.5%) as displayed in the table 4.3.

**Table 4.3: Presented the infestation of ectoparasites Varroa mites of honey bees in wooden and earthen hives.**

| Hives construction | No.of hives | Infestation | %age |
|---|---|---|---|
| Wooden hives | 84 | 22 | 26% |
| Earthen hives | 167 | 6 | 3.5% |
| Total | 251 | 28 | 11% |

### 4.4 TREATMENT OF HIVES

Some beekeepers use chemicals regularly to treated the hives against the Varroa mites. Total 251 hives were visited out of which 206 are treated and remaining 45 are untreated. It has been clear that the rate of infestation is high in untreated hives (15%) than treated hives (10%) as appeared in the table 4.4.

**Table 4.4: Shows the infestation of ectoparasites Varroa mites of honey bees in treated and untreated hives.**

| Types of hives | No.of hives observed | Infestation | %age |
|---|---|---|---|
| Treated hives | 206 | 21 | 10% |
| Untreated hives | 45 | 7 | 15% |
| Total hives | 251 | 28 | 11% |

### 4.5 FEEDING NATURE OF HONEY BEES

Some beekeepers give supplemental food to honey bees like sugar while some honey bees do natural feeding. A total 251 hives were studied out of which 246 are supplemental and remaining are natural. It has been observed that the rate of infestation is high in supplemental feeding honey bees (11%) as compared to natural feeding honey bees (0%) as displayed in the table 4.5.

**Table 4.5: Shows the infestation of ectoparasites Varroa mites of honey bees on supplemental feeding and natural feeding.**

| Feeding behavior | No.of hives observed | Infestation | %age |
|---|---|---|---|
| Supplemental feeding | 246 | 28 | 11% |
| Natural feeding | 5 | 0 | 0% |
| Total hives | 251 | 28 | 11% |

### 4.6 HYGEINIC AND UNHYGEINIC CONDITION OF HIVES

Some beekeepers maintain the hygienic condition of the hives in a proper way. Out of 251 hives 233 were found hygienic and remaining 18 hives are unhygienic.

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The of the study clarify that the rate of infestation is high in unhygienic hives (27%) than the hygienic hives (9%) as shown in the table 4.6.

**Table 4.6: Shows the infestation of ectoparasites Varroa mites of honey bees in hygienic and unhygienic hives.**

| Condition of hives | No.of hives observed | Infestation | %age |
|---|---|---|---|
| Hygienic | 233 | 23 | 9% |
| Unhygienic | 18 | 5 | 27% |
| Total hives | 251 | 28 | 11% |

### 4.7 TEMPERATURE OF THE AREA

The infestation of Varroa mites is strongly associated with the temperature of the area. It has been observedthat the rate of infestation is increase with the increase of temperature. The highest infestation rate (25%) were found at temperature 35 centigrade while the lowest infestation rate (4.5%) was found at temperature 23 centigrade as arranged in the table 4.7.

**Table 4.7: Shows infestation ectoparasite Varroa mites of honey bees at different temperature range.**

| Temperature range | No.of hives observed | Infestation | %age |
|---|---|---|---|
| 32 | 174 | 8 | 4.5% |
| 35 | 77 | 20 | 25% |
| Total hives | 251 | 28 | 11% |

### 4.8 MONTHS WISE INFESTATION

The infestation of ectoparasites Varroa mites is also strongly associated with the different months of the year. Total 251 hives were inspected during May, June and July 2025. 82 hives studied in May, 106 were studied in June and 63 were studied in July. The results of the study show that high infestation rate in July while low infestation rate in the May as shown in the table 4.8.

**Table 4.8: Shows the infestation of ectoparasites Varroa mites in different months of the year.**

| Months | No.of hives observed | Infestation | %age |
|---|---|---|---|
| May | 82 | 0 | 0% |
| June | 106 | 10 | 9% |
| July | 63 | 18 | 28% |
| Total | 251 | 28 | 11% |

### 4.9 EXPERIENCE OF BEEKEEPING

The infestation of Varroa mites also associated with the beekeeper's experience. The results of the study highlight that the rate of infestation is high of those have more than one year of experience (14%) while low rate of infestation is with those have 1 year of experience (0%) as displayed in the table 4.9.

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**Table 4.9: Shows the infestation of ectoparasites Varroa mites of honey bees with respect to experience of beekeeping.**

| Experience | No.of hives observed | Infestation | %age |
|---|---|---|---|
| 1 year | 54 | 0 | 0% |
| More than 1 year | 197 | 28 | 14% |
| Total hives | 251 | 28 | 11% |

### 4.10 SPECIES OR VARIETY OF HONEY BEES

During research work a total 251 hives were observed out of which 171 are *Apis cerana* and 80 are *Apis mellifera*. The results of the study show that the rate of infestation is high in *Apis mellifera* (26%) as compared to *Apis cerana* (4%) as described in the table 4.10.

**Table 4.10: Shows the infestation of ectoparasites in *Apis cerana* and *Apis mellifera***

| Species or variety | No.of hives observed | Infestation | %age |
|---|---|---|---|
| Apis cerana(local) | 171 | 7 | 4% |
| Apis mellifera(Non local) | 80 | 21 | 26% |
| Total hives | 250 | 28 | 11% |

### 4.11 INSPECTION OF HIVES

Some beekeepers do inspection of hives daily, weekly, monthly or rarely. The result of the current study highlight that the rate of the infestation is high in those high hives which were monthly inspected than daily inspection as shown in the table 4.11.

**Table 4.11: shows the infestation of Varroa mites with respect to inspection of hives.**

| Inspection | No .of hives observed | Infestation | %age |
|---|---|---|---|
| Daily | 3 | 0 | 0% |
| Weekly | 208 | 21 | 10% |
| Monthly | 33 | 6 | 18% |
| Rarely | 7 | 1 | 14% |
| Total hives | 251 | 28 | 11% |

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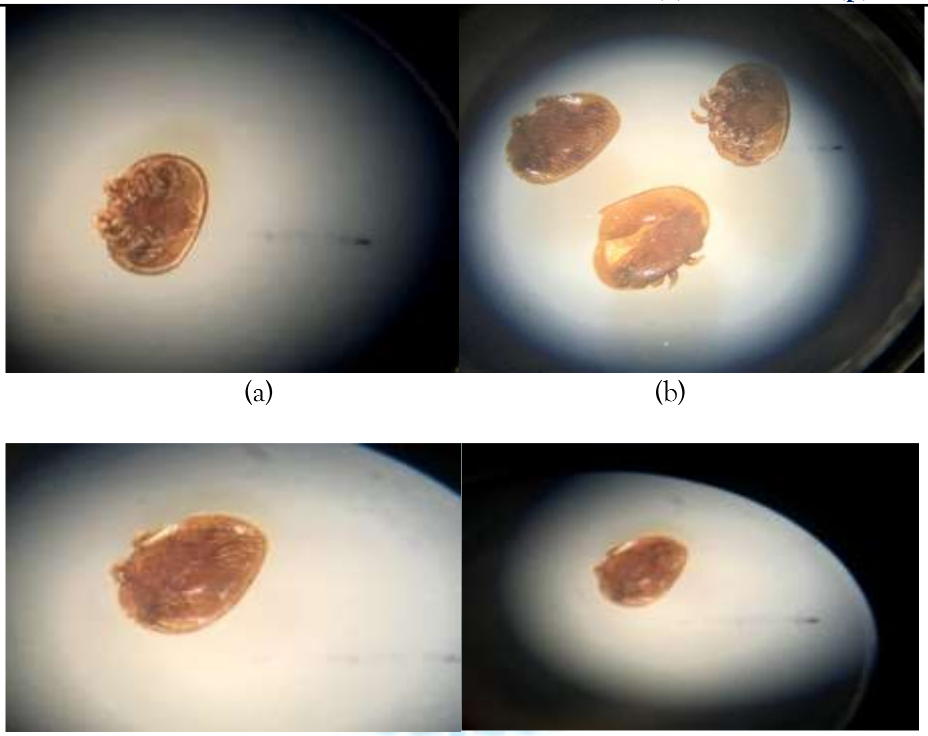

(a) (b)

(c) (d)

**Figure 4.1: (a), (b), (c) and (d) showing the Varroa mites collected across the tehsil Matta Swat.**

**Discussion**

A related study shows that the Varroa destructor is the parasitic mites of honey bees throughout the world. Some beekeepers use chemicals acaricides against the mites. Some honey bee's colonies show resistance against the mites especially sub-Saharan Africa and America. Also the scientist stated that mites in these regions do not show genetic differences with the mites in Europe but shows genetic differences with the other continents of world (Bearurepaire *et al*.,2022). The results of the current study show that Varroa mites is the major ectoparasites of honey bees in Tehsil Matta Swat. A total 251 hives were observed. Out of which 28 were infested and total infestation rate is 11%. Also this study shows some honey bees colonies to be found to show resistance to mites but no genetic difference and similarities were observed during the study.

According to Alshokri & Fadel (2010) the most common ectoparasites of honey bees is Varroa mites (Varroa destructor). They visited and observed about 10 apiaries in Benghazi area including AL-Jabal-Akhdar. The Varroa destructor was found in 88.78% of the total samples. The highest rate of infestation of mites were recorded in June (0.5317±0.0059) while the lowest in February (0.1717±0.0055). In recent research work a total 251 samples were visited and observed. Out of which 28 were infested with Varroa mites. The highest rate of infestation were record in July (28%) and lowest rate of infestation was in May (0%).

One of the study reveals that in Hisar, Haryana, India scientist studied about 200 honey bees every month from a colony of 15,000 *Apis mellifera* bees between September 1994 and December 1996 in order to find the ectoparasites mites of honey bees. The highest number of infestation during February, March and April and reduced from May to August (Gupta, 1999). In contrast the current study highlights that the rate of infestation of mites is high from May to August in Tehsil Matta District Swat.

Some researchers studied over 400 colonies of honey bees across the three seasons fall, winter and spring in Ontario, Canada in order to evaluate the presence of mites in honey bees. This study highlights that the Varroa mites is the major reason of death of honey bees. In fall 75.7% of colonies were infested with Varroa mites and over 85% rate of infestation of

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Varroa mites in winter season. Also smaller population of honey bees were records in spring season (Guzmán-Novoa *et al*., (2010). The current study was carried out in summer season only. During research a total 251 samples were studied in tehsil Matta Swat .28 samples were found infested with Varroa mites which correspond to overall 11% infestation rate in Tehsil Matta District Swat.

According Nazzi and Conte (2016) stated that mites were strongly associated with environment and honey bees by using many signals and cues. Factors like temperature, touch, humidity matter, and chemical communication were the main way which guide key stages of the mite's life. The result of the current study shows that temperature is strongly association with Varroa mites' infestation. Average highest temperature at Tehsil Matta is 35°c, at this temperature total 77 hives were observed out of which 20 hives were infested with Varroa mites and average percentage is 25% while at 32$^0$C total 8 hives were infested out of 174 hives were observed and average percentage is 4.5%. So, its mean that the Varroa mites' infestation is increase with increase of temperature.

In recent study shows that treatment of bee was linked with Varroa mites' infestation. Those people who were not careful and didn't treated their bees hives have highly parasitized with Varroa mites' infestation. Current research work show that untreated hives have more infestation rate as compared to treated hives. The 7 infested untreated hives were observed out of 45 hives and their infestation rate is 15 % while 21 infested treated hives were obsreved out of 206 hives and infestation rate is 10%. So, the overall infestation rate of treated and untreated hives is 11% in Tehsil Matta. One of the related studies show that the mites usually cause infestation in honey bees' colony if the bees were not treated and also using chemical treatment, another way to naturally resistance bees breeding are also fight with mites (Mondet *et al*., 2020).

Chantawannakul *et al*. (2016) stated that at least nine species of honeybees are in Asia including the non-native *Apis mellifera*. Both *Apis mellifera* and *Apis cerana* were infected by Varroa mites because both of species live in same area, particularly in Southeast Asia. These mites reduced the honey bees colony which is the big problem of the worldwide, including in Asia. These mites increase pests chance and diseases cause between different species of honeybees. The current study shows that local (*Apis cerana*) were least infested than non-local (*Apis mellifera*). The 171 local (*Apis cerana)* hives were observed out of which 7 were infested with Varroa mites and average infestation rate is 4% while 21 hives of non-local (*Apis mellifera*) were infested out of 80 hives and average infestation rate is 26%.

A related study shows that Australia is free from Varroa mites it was detected to possible way the entry of these mites. Australia was started a project called "Sugar Sake Team". The aimed of the project to use this special modeling method for the detection of Varroa mites (Owen *et al*., 2021). During current research work the Sugar shake method were performed to determined Varroa mites infestation at Tehsil Matta Swat. The study was conducted in different location of Tehsil Matta. During the current research work total 251 honeybee's colony were inspected in Tehsil Matta out of which 28 sample were infested with Varroa mites. So the overall infestation rate is 11%.

**Conclusion**

This study assessed the prevalence of *Varroa destructor* mites in honey bee colonies across apiaries in Tehsil Matta, District Swat, during the spring-summer period of May to July 2025. Screening of 251 hives revealed an overall prevalence rate of 11.2% (28/251), establishing the presence of this significant ectoparasite within the local apicultural landscape.

The findings highlight critical risk factors associated with higher infestation rates. A significantly greater prevalence was documented in colonies of the non-native *Apis mellifera* compared to the native *Apis cerana*. Furthermore, infestation was strongly associated with modern beekeeping practices, including the use of moveable-frame wooden hives, hives located in urban or peri-urban settings, and the absence of acaricidal treatment. Supplemental feeding, suboptimal hive hygiene, exposure to higher ambient temperatures, and the peak summer month of July were also identified as factors exacerbating mite populations.

These results underscore a clear dichotomy: traditional apiculture practices using local bee subspecies in earthen hives within rural settings

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demonstrate a degree of resilience or lower susceptibility to Varroa infestation. In contrast, the shift towards intensive, commercial beekeeping with introduced species appears to elevate disease risk. This study, therefore, provides essential baseline data and evidence that sustainable Varroa management in the region should integrate both chemical control and fundamental modifications to hive management and species selection, tailored to the local ecology.

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