




Seasonal prevalence of Asian honeybee ectoparasitic mite *Varroa destructor* Anderson and Trueman, 2000 in Madanpokhara Apiaries, Palpa, Nepal

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Abstract

The present study highlights the prevalence and seasonal variation of *Varroa destructor* Anderson and Trueman, 2000 in *Apis cerana* colonies at two apiaries (site-I and site-II) in Madanpokhara, Palpa district, Nepal. Altogether 498 varroa mites were collected from February to October 2017. The highest and lowest number of varroa mites were observed in March and September, respectively. The mites were observed higher in brood cells (54.84% and 52.51%) and lower in adult bees (10.39% and 9.59%) in site-I and site-II, respectively. The inner hive and outer temperature in site-I and II were ranged from 30°C to 34°C and 24°C to 32.4°C, 30°C to 33.5°C and 25.1°C to 32.3°C, respectively. Similarly, inner and outer hive humidity ranged from 41% to 61% and 40% to 68%, 40% to 60% and 41% to 65% at site-I and II respectively, differed significantly and correlated positively with mite population. Concern for honeybee health and conservation along with Nepali apiculture are also discussed in this paper.

Keywords: *Apis cerana*, Climatic factors, Honeybee, Population variations, Varroa mite

1 | Introduction

The ectoparasitic honeybee mite *Varroa destructor* Anderson and Trueman, 2000 is primarily confined to the Asian honeybee *Apis cerana*, which is also one of the most important causes of colony failures in the European honeybee *A. mellifera* (Sanford et al. 2007). The Asian honeybee affected by the other species *V. jacobsoni*, switched to *A. mellifera* causes its colonies decline. Originally *V. destructor* was described from *A. cerana* as well as the new host *A. mellifera* which as previously known as *V. jacobsoni* (Anderson 2000, Anderson & Trueman, 2000). There are eighteen haplotypes among *V. jacobsoni* throughout the Asian mainland (Anderson & Trueman 2000), one of them is the *V. destructor* identified as a distinct species which is also reported from Nepal (Anderson & Trueman 2000, Neupane 2009, Shrestha et al. 2020). Adult varroa mites contribute as new transmission routes of some naturally harmless honeybee viruses (Sumpter & Martin 2004). Nevertheless, *V. destructor* has been infesting colonies leading to failures, attributive to viruses and described as bee parasitic mite syndrome (Shimanuki et al. 1994). It is a vector of honeybee viruses by sucking hemolymph of pupae and

adults from one host and then inject viruses into another healthy individual in phoretic life stage (Ball, 1989), spread the viruses rapidly (Bernardi & Venturino 2016), trigger high virus load (Tentcheva et al. 2004), virus replication through penetrating cuticle or by injecting external proteins into the insect hemolymph (Dandeu et al. 1991). Some empirical evidences are available highlighting impacts of varroa mites on brood, hive bees and foragers (Khoury et al. 2011) especially *V. destructor* in workers and drones populations (Tentcheva et al. 2004, Maidana et al. 2005, Jamshidi et al. 2009, Neupane 2009) including prevalence and reproduction of *Tropilaelaps mercedesae* and *V. destructor* in *A. mellifera* (Buawangpong et al. 2015, Shrestha et al. 2020). Low varroa mite infestations can reduce the expression of antimicrobial peptides, reduce immunity function, facilitate virus amplification, and also affect behavior (Gregory et al. 2005, Yang & Cox-Foster, 2005, Navajas et al. 2008). High infestation can lower pupal and adult weight, reduce reproductive ability of drones and foraging capabilities of workers (Bowen-Walker & Gunn 2001, Duay et al. 2003) which may increase sensitivity to insecticides through lowering the titer of vitellogenin in the hemolymph of infested bees (Amdam et al. 2004).

The rapid spread of *V. destructor* in *A. mellifera* colonies is one of the main causes of colony failures. The varroa mite is widespread in Europe, North Africa, the Americas, and Asia. The colony death occurs in two years if the mite infestation is not controlled by acaricide treatments (Bernardi & Venturino 2016). In Nepal, another honeybee mite *T. clareae* was noticed in *A. mellifera* and *A. dorsata* colonies whereas *V. destructor* was observed in *A. cerana* and *A. mellifera* colonies in Chitwan considering threat to *A. cerana* bees in the Nepalese context (Neupane 2009, Shrestha et al. 2020). Also, a collection of *V. jacobsoni* from the nest of *A. cerana* from Nepal yielded a new species, *V. underwoodi* (Delfinado-Baker & Aggarwal 1987).

In Nepal, the activities such as importation of queen bees from varroa mite infested areas, movement of infested colonies for pollination purpose have admitted rapid spread of mites, diseases and pests (Neupane 2009). Unchecked mite infestation leads to

and seasonal variations of *V. destructor* on honey bee colonies are scanty in Nepal. Therefore, spread of several viruses through varroa parasitism which may have deleterious effects such as mortality and decline of honeybee colonies strongly necessitate this study. The present study reports the prevalence and seasonal occurrence of *V. destructor* in the apiaries of Palpa district, Nepal.

2 | Materials and methods

2.1 | Study area

This study was conducted in Madanpokhara of Tansen Municipality, Palpa district, Nepal (Fig. 1). The elevation and climate vary from 213 m (tropical) to 1900 m (subtropical). The average maximum and minimum temperature recorded was 31.02°C in June and 6.56°C in January. The district receives average annual rainfall of 0 mm. to 388.72 mm. Study was conducted at two different established and managed apiaries (site-I and site-II) having 20 beehives each. Both sites (I and II) were two kilometers apart, lying between 27°34'-27°57'N and 83°15'-83°22'E. An apiary at site-I possessed two years old bee hives and another site-II had both old and new bee hives.

2.2 | Data collection

Sampling was carried from February to October 2017. Mites were collected from both worker and drone cells and base, brood and adult honeybees during 10 AM to 4 PM. The debris presented on the bottom board of bee hive was collected regularly and was floated in 70 percent alcohol. The mites and pieces of chitin floated while wax and other heavy materials were drained off in separate petri dishes and the mite species were picked up with the help of a fine camel hair brush (Ritter & Ruttner 1980). Analysis of brood mites was performed by examining 50-100 capped brood cells of both drone and worker bees with perforations caps or sunken caps (Aggarwal 1988, Wongsiri et al. 1989). Adult honeybee mites were examined by direct visual observation in every frame of the hive. The thorax and legs of honeybees were carefully noticed and mites were collected using brush and forceps. Permanent slides of the mites were prepared and were deposited at Natural History Museum, Tribhuvan University. The outside (ambient) and inside hive temperature and outside (atmospheric) along with inside hive relative humidity of each hives were recorded using Thermohygrometer HTC-01.

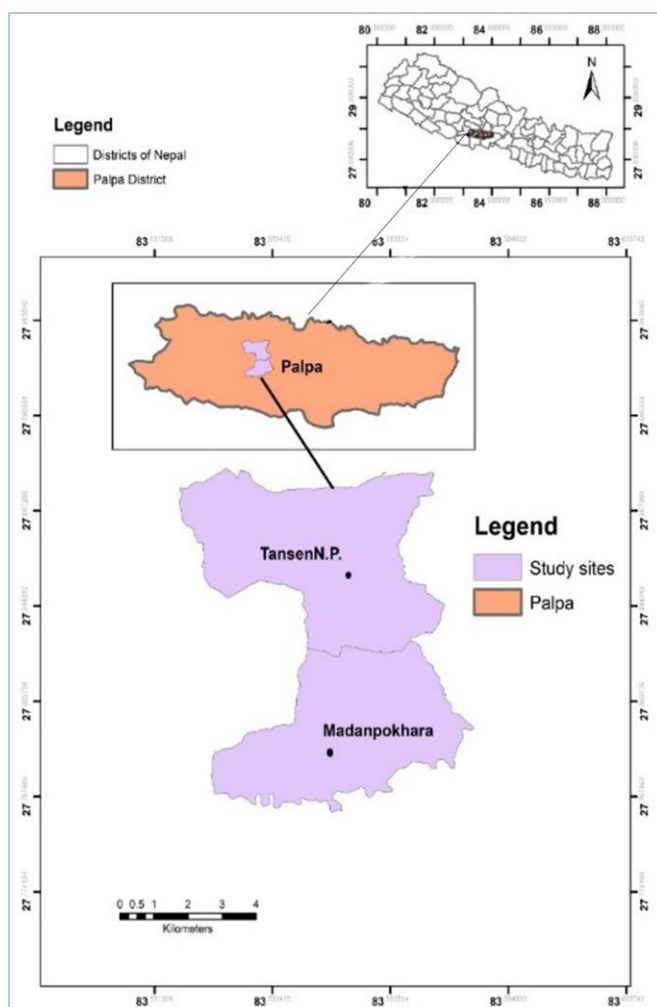


Figure 1. Map of the study area

colony collapse. Despite this fact, the study on the prevalence

2.3 | Identification of the mite

Out of eighteen haplotypes of *V. jacobsoni* reported, haplotypes from Nepal, China, Thailand, Vietnam, and Japan are known as *V. destructor*. In original description of Anderson and Trueman (2000) it is morphologically similar to *V. jacobsoni*, but larger. Mean body length is of 1167.3 μm ($\pm 26.8 \mu\text{m}$) and the mean body width is of 1708.9 μm ($\pm 41.2 \mu\text{m}$). The size, shape and colour of the collected mite match with the original description (Fig. 2).

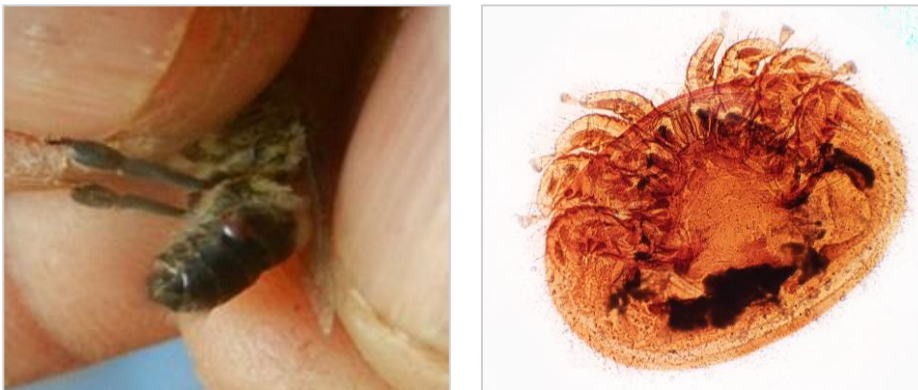


Figure 2. *V. destructor* collected from Palpa district, Nepal. Left: Female mite on adult *A. cerana*. Right: Dorsal enlarged view of the female mite

2.4 | Data analysis

Pearson's correlation coefficient (r) was calculated between the population of varroa mites observed and temperature and humidity records. The data was analyzed through ANOVA of two different sites of population of varroa mites in nine-month periods with MS-Excel 2016.

3 | Results

A total of 498 varroa mites were collected from site-I ($n = 279$) and site-II ($n = 219$). The prevalence of mite population was higher in March (site-I: 22.58%, site-II: 21%) and lower in September (site-I: 3.58%, site-II: 3.2%). Mites in brood cells were observed higher (site-I: 54.84%, site-II: 52.51%) than in adult bees (site-I: 10.39%, site-II: 9.59%).

The mite population was found higher in two years old bee hive (site-I) in comparison to mixed types of bee hives at site-II. The mite population was declined in April and May and increased in June. The monthly population of varroa mite was differed at site-I ($df = 2,24$, $P = 0.002$) and site-II ($df = 2,24$, $P = 0.002$). The prevalence of *V. destructor* is shown in Table 1.

As shown in Figure 3, the inner hive temperature and outer temperature ranged from 30°C to 34°C and 24°C to 32.4°C, respectively. The relationship between population of varroa mite with inner and outer hive temperature did not differ significantly ($P = 0.64$ and $P = 0.16$, respectively). Inner hive temperature and outer temperature were found negatively correlated and positively correlated with mite population as $r = 0.18$ and $r = -0.5$, respectively at site-I. Similarly, the relationship between

population of mite with inner and outer hive temperature did not show significant difference ($P = 0.08$ and $P = 0.46$, respectively) at site-II. The temperature ranged from 30°C to 33.5°C in inner hives and 25.1°C to 32.3°C around outer hives. Inner and outer hive temperature were found negatively correlated to mite as $r = -0.08$ and $r = -0.27$, respectively.

At the site-I, population of varroa mite in relation to inner and outer hive humidity differed significantly ($P = 0.02$ and $P = 0.05$, respectively). The humidity ranged from 41% to 61% and 40% to 68%, and inner and outer hive humidity found positively correlated with mite population as $r = 0.74$ and $r = 0.66$, respectively. The mite population with inner and outer hive humidity showed P -value 0.14 and 0.39, respectively. Humidity inside the hive ranged from 40% to 60% and outside the hive 41% to 65%. Inner hive humidity and outer humidity were found positively correlated with mite population as $r = 0.53$ and $r = 0.33$ respectively at site-II (Fig. 4).

Table 1. Prevalence of *V. destructor* on base, brood cell and adult at site-I and II.

S.N.	Month	Base (%)		Brood (%)		Adult (%)		Total (%)	
		Site-I	Site-II	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
1.	February	21.65	8.43	20.26	13.91	34.48	14.29	22.22	11.87
2.	March	22.68	16.87	20.92	24.35	31.03	19.05	22.58	21
3.	April	4.12	3.61	5.88	6.09	0	0	4.66	4.57
4.	May	11.34	22.89	11.11	7.83	13.79	0	11.47	12.79
5.	June	6.19	21.69	15.69	11.30	3.45	9.5	11.11	15.07
6.	July	16.49	12.05	9.15	12.17	6.90	28.57	11.47	13.70
7.	August	4.12	2.41	7.19	13.91	0	0	5.38	8.22
8.	September	8.23	3.61	1.31	3.48	0	0	3.58	3.2
9.	October	5.15	8.43	8.50	6.96	10.34	28.57	7.53	9.59
Total		34.77	37.90	37.90	52.51	52.51	9.59	279	219

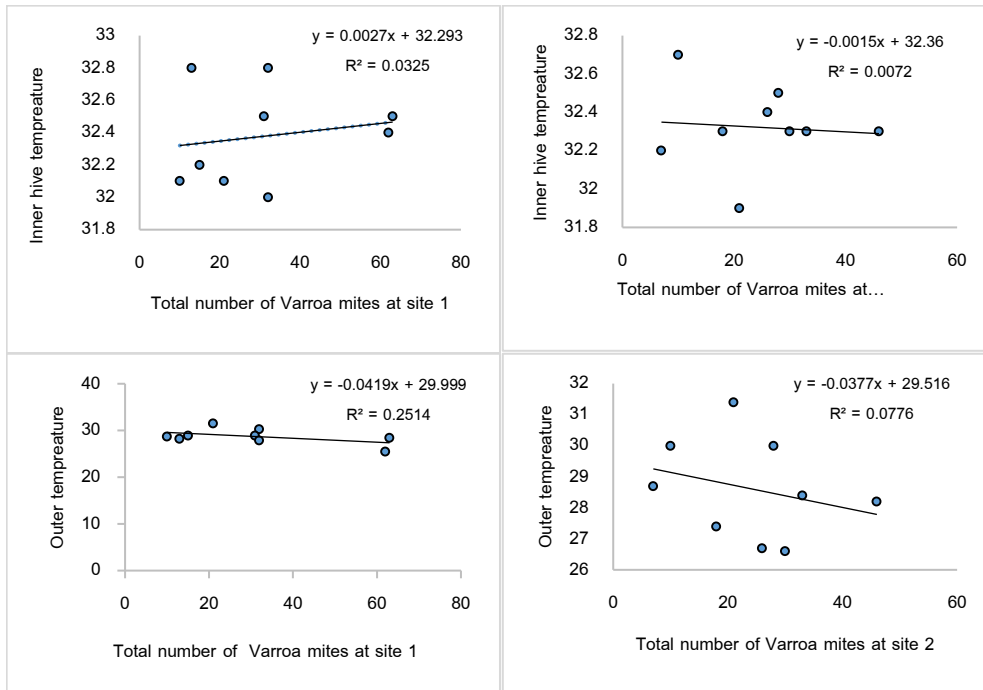


Figure 3. Population of *V. destructor* in relation to temperature

4 | Discussion

The Asian honey bee *A. cerana* is primarily parasitized by *V. destructor* and shifted from their primary host in north-east Asia (Navajas et al. 2010) infecting *A. mellifera* (Sanford et al. 2007) and is now a cosmopolitan species and causing serious threat to beekeeping industry. Despite this fact, some of the beekeepers were unaware of its impact in relation to its occurrence,

prevalence and climatic factors on apiculture. Finding of *V. destructor* in *A. cerana* bees in Madanpokhara is an alarm to beekeepers of the area. The similar threats in Nepal are already noticed (Woyke 1985, Neupane 2009, and Shrestha et al. 2020). The varroa mite damage less in *A. cerana* due to its defense mechanisms (Peng et al. 1987).

Neupane (2009) reported availability of *V. destructor* on *A. cerana* which is a natural host at the same ecological niches and region might attract this mite to brood in large number rather than in *A. mellifera*. Their study found the lowest number of *V. destructor* in rainy (4.0 per sample), and the highest in spring (27.7 per sample) season in *A. cerana* colonies. The population of mites began to multiply from autumn and reached at higher damaging level in winter season. Although, it is in contrast to what have been shown by Fries et al. (1994) and Tibor & Szabo (2003) who observed peak period of infestation by *Varroa* spp. between

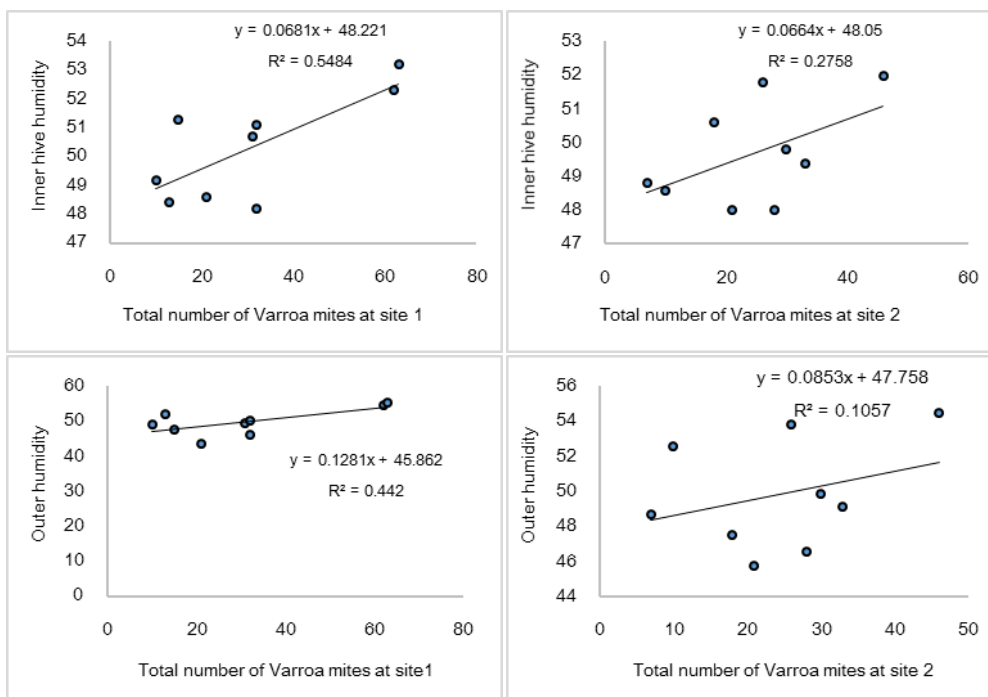


Figure 4. Population of *V. destructor* in relation to humidity

September to December in different parts of the world including Mattu and Sharma (2016) who concluded peak period in April. But, up to 90% of the varroa mites were observed into brood cells during the summer (Renz & Rosenkranz 2001). Furthermore, in some experimental conditions adult bees were found to be more favourable for varroa mites than larval stages (LeDoux et al. 2000, Zetlmeisl & Rosenkranz 1994) but in other studies more mites were

observed into the sealed brood cells in comparison to adult bees in natural conditions (Boot et al. 1993, Martin et al. 1998). In present study, the mites were observed higher in brood cells and lower in adult bees. Also, the mite population was found higher in two years old bee hives in comparison to mixed bee hives which possibly due to lack of management of old hives and mite's response to alteration in conditions of colonies. It is possible that variations in climatic conditions for countries other than Nepal are responsible for this discrepancy in seasonal fluctuation and prevalence of varroa mite. The reproductive process of *V. destructor* begins after abandoning adult worker bee or drone by adult females and then penetrating worker or drone brood cells (Gusman-Novoa et al. 1999). A few moments before cell operculation, the onset of mites in the cells of honeybees takes place when the bee brood reaches the last larval stage (De Jong 1997). Thus, varroa population is distributed among normal colonies with brood than adult bees. Drone brood about three to eight times more in comparison to worker brood is preferable to varroa (Fuchs & Langenbach 1989). Therefore, the infestation of varroa mites may be due to production of drone brood during the sampling period. Furthermore, De Guzman & Burgett (1991) explained that the infestation rate of the parasite cannot increase at the same rate as the host population.

The present findings showed humidity is correlated with mite's population whereas temperature was negatively correlated with mite's populations which indirectly corroborated the findings of Bonoan et al. (2014) with the observation, as there is a significant correlation between the densities of mite infection and climatic factors (temperature and humidity). The relationship between infestation rate of the mite in adult bees and environmental temperature was positively correlated (Pinto et al. 2015). In contrast, level of infestation of mite in colonies varied depending on the weather (season) and internal conditions of each colony (Koumad 2015). Opposite to Hou et al. (2016) who observed the mite infection in honeybee colonies was positively correlated with temperature but negatively correlated with humidity. In addition, the other influencing factors may be either equilibrium between mite receiver colonies from victim colonies more than one kilometer apart (Renz & Rosenkranz 2001) or grooming behavior of honeybees, and management practices of bee hives. The honeybee, *A. cerana*, is subject to many viral infections persistently in honeybee populations between individuals of the same colony and between different colonies due to varroa mites,

despite the lack of clinical signs. Environmental factors might play important role for disease outbreak resulting colony failure either through morbidity or mortality. It is worth to note that *V. destructor* might act as a vector or as an activator of replication of viruses. Therefore, further study is needed to understand the relationships among honeybees, varroa mites and viruses.

5 | Conclusions

The varroa mite population was reported higher in March and decreased in April and May and again increased in June. Mites were observed higher in brood cells than adult bees. Further, relationship among population of varroa mite with inner and outer hive temperature and humidity differed. varroa mites were not found detrimental to the colonies in this study but if remained unchecked, the population may become high enough to slow up the development of honeybees and honey production.

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Authors' contributions

Shrestha, N. performed the field study and analyzed the data. Both the authors wrote the manuscript and approved for the submission.

Conflicts of interest

Authors declare no conflict of interest.

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