SEASONAL VARIATION IN ECTOPARASITIC MITE *Tropilaelaps mercedesae* Anderson and Morgan, 2007 IN APISTAN TREATED AND UNTREATED COLONIES OF *Apis mellifera* IN KATHMANDU, NEPAL

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Submitted to

Central Department of Zoology
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Kirtipur, Kathmandu
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January, 2021

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATIONS

This is to recommend that the thesis entitled "**SEASONAL VARIATION IN ECTOPARASITIC MITE *Tropilaelaps mercedesae* Anderson and Morgan, 2007 IN APISTAN TREATED AND UNTREATED COLONIES OF *Apis mellifera* IN KATHMANDU, NEPAL**" has been carried out by Ms. Jamuna Kafle for the partial fulfillment of Master's Degree of Science in Zoology with special paper Entomology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor, Dr. Ishan Gautam, this thesis submitted by Ms. Jamuna Kafle entitled **"SEASONAL VARIATION IN ECTOPARASITIC MITE *Tropilaelaps mercedesae* Anderson and Morgan, 2007 IN APISTAN TREATED AND UNTREATED COLONIES OF *Apis mellifera* IN KATHMANDU, NEPAL"** is approved for the examination [redacted] in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Entomology.

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ABSTRACT

This study carried out for a period of six months from May 2017 to October 2017 describes the development of *T. mercedesae* infestation in the brood and foraging adults along with debris of *A. mellifera* honey bee colonies in Kathmandu, Nepal. *A. mellifera* colonies were divided into two groups: group A (treated with Apistan) and group B (untreated) that were naturally infested with *T. mercedesae*. Fortnightly observation of mites by "Sugar shake" method on adult bees and by opening the capped cells from the honey comb for broods was done. To inspect the number of dead mites in debris, formica sheets were placed at the bottom of hives. Group A colonies showed fluctuation in number of mites within study period with highest rate of infestation during October month (0.625% in adults, 8.375% in brood and 25.75 in debris) while the least rate of infestation during May (0% in adult, 2% in brood and 3.25 in debris). In Group B colonies, the highest infestation was observed in October in adult and debris (2.5% and 67.625 respectively) while it was 21.75% in brood in September while the least infestation was observed in May (0.5625% in adult, 2.75% in brood and 3.25 in debris). The difference between the mean values of the mites in broods of two colonies wasn't found to be significant with p value 0.089 (df, 6). The Apistan was found to be effective in controlling the mites in adult bees with p-value 0.0067 (df, 6). The population of the *T. mercedesae* mites was found to be positively correlated with humidity while it was found to be negatively correlated with the temperature. Therefore, during summer season, the number of mites remained low while the number increased during autumn when there was much brood rearing.

Key words: *A. mellifera*, *T. mercedesae*, Apistan, fluctuation, infestation

1. INTRODUCTION

1.1. Background of the study

Apis mellifera is a European honey bee. Though it isn't native to Asian countries, is preferred because of its high productivity (Singh et al. 2019).This honey bee is larger than *A.cerana* and has golden yellow colour, has dark brown-coloured legs and like other honey bee colonies, *A.mellifera* also contains the queen, drones, worker bees and the developing larvae and pupae.

The honeybees are vulnerable to several types of infections and infestations such as microbial, protozoan, fungal, viral, parasitic diseases, insects and other different vertebrates. For instance, American foul brood, European foul brood, Chalk brood, Sacbrood, Nosema disease, Varroasis, Acarapidosis etc. (FAO 2006).

One of the major ecto-parasitic mites of the honeybee *A. mellifera* (Laigo and Morse 1968), *Tropilaelaps* spp. hinders its development and is detrimental to beekeeping industry. These are native of Asia parasitizing *Apis dorsata* Fabricius, 1793 and are also reported to occur in *A. cerana*, *A. laboriosa*, and *A. breviligula* (Laigo and Morse 1968).

Tropilaelaps mites belong to family Laelapidae and four species have been known so far (Chantawannkul et al. 2016). Among the other species, *Tropilaelaps clareae* was discovered for the first time from the field rats and *Apis mellifera* specimens in Phillipines (Delfinado and Baker 1961). Later in 1963, Delfinado reported it in association with *Varroa jacobsoni* in aparies in Hongkong (Laigo and Morse 1968) and twenty years later, *T. koenigerum* was discovered from *Apis dorsata* in Srilanka. Anderson and Morgan (2007) described two species of *Tropilaelaps* and they are *T. mercedesae* and *T. thaii*. *T. mercedesae* was discovered from *A. mellifera* and *A. dorsata* in mainland Asia while *T. thaii* from *A. laboriosa* in the Himalayas. *T. mercedesae* was initially thought to be *T. clareae* and described accordingly before Anderson and Morgan's job (Chantawannkul et al. 2016). In Nepal, *T. mercedesae* has been reported from *A. dorsata* and *A. laboriosa* (de Guzman et al. 2017) and *A. mellifera* (Shrestha et al. 2020), *T. clareae* from Chitwan district (Neupane 2009)as well as *Varroa destructor* from *A. cerana* (Shrestha and Gautam 2020).*T. koenigerum* and *T. thaii* are limited to Asian bees, *A. dorsata*, and *A. laboriosa* respectively and harmless to *A. mellifera* (Anderson and Roberts 2013). *Tropilaelaps* spp. found on

either giant Asian honeybees and/or European honey bees in Philippines (except on Palawan Island) was more probability of being *T. clareae*. Similarly, those would be *T. mercedesae* found on European honey bees in other parts of Asia (Anderson and Roberts 2013).

Tropilaelaps spp. are similar to *Varroa* in appearance and both resemble in many ways but the difference is the body shape which is much longer than wider unlike *Varroa* spp. They have similar lifecycle as that of *Varroa* mites but differ in being shorter. Just before capping the brood by worker bees, the foundress lays three to four eggs inside brood cells (Sammataro et al. 2000). They pass through five stages of development (egg, six-legged larva, protonymph, deutonymph and adult). Adult male *Tropilaelaps* do not feed as they do not have feeding apparatus. The piercing apparatus i.e., chelicerae is modified to transfer the sperms. The adult female and immature feed on the haemolymph of the honey bee brood (FAO 2006) only on the soft tissues.

Because their mouth parts (chelicerae) are modified accordingly i.e., piercing soft tissues unlike that of Varroa mites that are of tearing and sawing type (Sammataro et al. 2000) and because their mouthparts cannot penetrate the cuticle of adult bees,they die within two days. That's why the mites can't survive more than 3 days on adult honeybees (Kavinseksan and Wongsiri 2016) including *A. dorsata* (Rinderer et al. 1994).Unlike *Varroa*, *Tropilaelaps* doesn't stay outside the brood cells longer (Woyke 1987). In an experiment, Wokye (1987) suggested the average stay of *Tropilaelaps* outside the brood cell is 1.3 days in average while it is 13 days for *Varroa*. It suggests the phoretic phage of lifecycle which occurs in life cycle of *Varroa* is less common in case of *Tropilaelaps clareae.*

The life cycle of the mite is synchronized with the lifecycle of the honey bee. After feeding the haemolymph and soft tissues of developing larvae for about one week, the adult mites emerge with the emergence of deformed adult bee. This usually includes a male and several females (Sammataro et al. 2000). When heavily infested, they can be seen running rapidly over the honey bee comb and also on the body of adult bees.

As mentioned above, *Tropilaelaps* spp. causes harm by direct feeding on the haemolymph of the developing brood. With the help of pointed chelicerae, the adult female and offsprings pierce the soft intersegmental membrane of the bee and suck the haemolymph causing mainly damage to the brood leading to colony failure. Decaying of the brood and the deformation of the bees are followed by infestation of honey bee brood. Deformation includes shortened abdomen and deformed wings. Also reduced

body weights of bees are symptoms of its infestation. Infestation with a single mite may not produce visible symptoms but it decreases the life span of bee, disturbance in the behavior of the bee such as in orientation and foraging (FAO 2006). Usually, fat body is reduced so that the functioning of gland is reduced and susceptibility to pesticides is increased. Unfavorable climatic conditions or insufficient stock of pollens and nectar can increase the process of disintegration (FAO 2006). Infestation results to deformed or wrinkled wings, short abdomen and missing legs (Warrit and Lekprayoon 2011). Malformation of brood and bees crawling due to inability in flying are other symptoms of *Tropilaelaps* infestation, irregular brood pattern, dead or malformed wingless bees at the entrance of hive, fast running brownish mites seen running on the combs (Sammataro et al. 2000) are the symptoms of *Tropilaelaps* infestation. Since, *Tropilaelaps* mites have faster rate of development than *Varroa* and much more mobile (Anderson and Roberts 2013) making *Tropilaelaps* more dangerous pest of *A. mellifera* than *Varroa* (Sammataro et al. 2000).

In Asia, *Tropilaelaps* spp. is the most serious pests in *A. mellifera* colonies (Burgett et al. 1983). In Thailand, beekeepers take them to be more serious pests than *Varroa* mites but easier to control than *Varroa* mites (Burgett et.al 1983). In Pakistan, ectoparasitic mites' infestation was the cause of heavy loss of honeybee colonies from 1992 to onwards (Mahmood 2012). This species causes severe damage to beekeeping with *A. mellifera* in Nepal (Neupane 2009).

Non-chemical controlling method being practiced is its nature i.e., its inability to feed adult bees resulting less survival outside the sealed brood cells (Sammataro et al. 2000). Brood removal is one of the resistance mechanisms in *Tropilaelaps* (Khongphinitbunjong et al. 2013). For the chemical treatment of this mite fluvalinate, sulfur, formic acid, chlorobenzilate are used. Most of the popular means of chemical control are formic acid, oxalic acid, lactic acid, etheric oil (thymol) and pyrethroid (FAO 2006).

Apistan is a synthetic pyrethroid which is a miticide especially used to control Varroa mites. Keeping this in mind, the efficacy of Apistan was analyzed against *Tropilaelaps* in the present study to seek new avenues for *Tropilaelaps* prevention and control. Therefore, the present study aims to investigate seasonal fluctuation of *T. mercedesae* in *A. mellifera* colony set up for SMARTBees Project at Natural History Museum premises, Swayambhu, Kathmandu, Nepal encompassing the comparison between mites in Apistan treated and untreated *A. mellifera* colonies in different seasons.

1.2 Objectives

General Objective

Determination of seasonal changes in ecto-parasitic mite *T. mercedesae* in Apistan treated and untreated colonies of *A. mellifera* in Swayambhu, Kathmandu, Nepal.

Specific Objectives

1. To examine the mite populations periodically in brood, adult bees and debris samples in two sets of *A. mellifera* colonies.
2. To observe the mite infestations under controlled and Apistan treated conditions.
3. To determine the relationships among various meteorological parameters such as temperature, humidity and precipitation to the population of *T. mercedesae* in Apistan treated and untreated conditions.

1.3 Significance of the study

As mentioned above, the honeybees are prone to several infections and infestations. *Tropilaelaps* mites are taken to be serious pests causing severe damages to *A. mellifera* beekeeping worldwide including Nepal (Sammataro and Avitabile 1998). They have faster development rates and feed on the soft tissues as well as haemolymph of developing brood resulting to deformation of brood. This causes loss of colony and consequently affects those who are associated with beekeeping.

Apistan is the trade name for a pyrethroid. Pyrethroid is a man-made organic compound and is similar to natural pyrethrins produced by the flowers of pyrethrums, are very poorly soluble in water, non-persistant, lack photostability, are less toxic to birds and mammals, are active and effective even at low doses (Clark and Matsumura, 1987) which can be toxic to arthropod disrupting the nervous system which causes the weakening leading to death. They have shown to inhibit Na-Ca ATP hydrolysis. Also, Ca-Mg hydrolysis is caused by the pyrethroid (Clark and Matsumura, 1987) affecting sodium channels (neurotoxins) and eventually muscular paralysis occurs which is followed by death of insects (Gusovsky et al. 1986).

In Nepal, very little study on *Tropilaelaps* and their control have been carried out so far. Keeping this in mind, this study depicts the *T. mercedesae* infestation in the

Apistan treated and untreated *A. mellifera* colonies having brood and foraging bees in Kathmandu, Nepal. It is an attempt in the field of *T. mercedesae* research stepping towards new horizon. Further, these observations will help to explain effects of various physical parameters in the fluctuation of *T. mercedesae* population in the colonies of *A. mellifera* in Kathmandu, Nepal.

1.4 Limitation of the study

- The population of honey bees declined due to the hornets attack.

2. LITERATURE REVIEW

Apis mellifera is prone to various types of infections and diseases. One of them is *Tropilaelaps* infestation. *Apis mellifera* commonly known as western honeybee, native to Europe, western Asia and Africa was introduced to other continents in 17th century since when, it has been found in remaining parts of the world like east Asia, Australia, North and South America also (Sammataro and Avitabile 1998). In Nepal, it was introduced in early 1990s after a massive colony loss of *Apis cerena* from entire Hindu Khus Himalayan regions due to Thai sacbrood virus. This was introduced by commercial beekeepers in Nepal (Thapa et al. 2000).

There are few publish works on the natural history of *Tropilaelaps* mites probably because this mite is presently limited to Asia and internationally most of the studies are focused on *Varroa* (Baker et al. 2005).Much remains to be studied about *Tropilaelaps* mites in many countries (Anderson and Roberts 2013) including Nepal.

2.1 Range of distribution

The *Tropilaelaps* species were originally parasites of *A. dorsata*, but later became affiliated to the Western honey bee, *A. mellifera* also (Anderson and Morgan 2007).Among other species of *Tropilaelaps*, *T. clareae* was first reported from *A. mellifera* in Philippines and then in *A. breviligula* in Philippines and Sulawesi Island inIndonesia (De Guzman et al. 2017) .*T. clareae* was widely distributed and restricted to Asia and had been recorded from Iran to Papua New Guinea (Baker et al. 2005) while *T. mercedesae* was reported to have parasitized *A. dorsata* and *A. mellifera* in the southern mainland Asia and Indonesia, (apart from Sulawesi Island). This species had also been reported to have parasitized *A. laboriosa* in the Himalayas (De Guzman et al. 2017). Further, *T. koenigerum* was found to be parasite of *A. dorsata* in Indonesia (apart from Sulwasesi) and Borneo, Sri Lanka and mainland Asia and was reported co-infesting with *T. mercedesae* in Borneo and Thailand in *A. dorsata* colonies. It was observed on *A. cerana* brood in India also (De Guzman et al. 2017).*T. koenigerum* were known from Borneo, Nepal, Sri Lanka and Thailand (Baker et al. 2005) also.

2.2 Morphology and lifecycle

Mites of the genus *Tropilaelaps* belongs to the family Laelapidae. These are ectoparasites of honey bees. Morphologically, *Tropilaelaps* spp. are reddish brown in

colour, smaller and are almost half as wide as *Varroa* (Fig. 1a) giving elongated or oval appearance (FAO 2006). They can be easily seen with unaided eye. Adult *Tropilaelaps* female mites have reddish brown-colour. They appear oval-shaped with body length about 0.96 mm and the width is about 0.55 mm. Entire body of the adult female is covered with short setae. A red streak runs longitudinally on the ventral surface of the body of the adult female mite.

Fig.1a: *Varroa* (Dorsal view)

Source: Shrestha and Gautam (2020)

Fig.1 b*: T. mercedesae* (Dorsal view)

Fig. 1c: *T. mercedesae* (Ventral vew)

Fusion of the epigynial and anal shields occurs in adult female. Males are similar but less sclerotized than female *Tropilaelaps* mites (FAO 2006). Adult *Tropilaelaps* mites hold their first pair of legs upright which resembles antennae. Males are usually less common in any specimen collection. More common are female mites. Major identifying character of adult male is chelae spermatodactyl which is a sperm transfer organ. This is in the form of spirally coiled apex in *T.mercedesae* and *T. clareae*. In *T. koenigerum* it is short and at the apex of which lies 'pig-tail' loop. The brilliant white-coloured nymphs are easily noticeable even with naked eye. Adult females are easily found inside the capped brood cells (Anderson and Roberts 2013). *Tropilaelaps* is elongated with a heavily sclerotised holoventral or similar shield (Anderson and Morgan 2007). Its body is longer than wide unlike *Varroa* assisting in high mobility on combs and on the body of adult bees (De Guzman et al. 2018). Large ambulacra along with claws help in attaching to host's body and during copulation. Legs 1 are long and slender (antennae-like), and appear to perform a sensory function (De Guzman et al. 2018).

In *T. mercedesae*, the apex of the epigynial plate is bluntly pointed to sharply point. It is parallel to flanged sided (Fig. 1b and 1c). Subapical tooth is present on the female chelicerae. The male chela spermodactyl is long and attenuate. The apex of male chela spermodactyl distal spirally coiled (Anderson and Morgan 2007).

Breeding of *Tropilaelaps* resembles with that of *Varroa*. The mated mature females after entering the brood cell containing the mature larva of bees, lay eggs before it is capped by wax covering by worker bees. The mother mites and several off springs remain safely concealed, feed on the haemolymph of developing brood and eventually emerge when the adult bees come out of the brood cell (Anderson and Roberts 2013). After a brief phoretic phase, they move about the comb, mating may occur and spend time on adult bees, before commencing a new reproduction phase. Therefore, for the collection of large number of mites, capped broods should be opened. Dispersion of *Tropilaelaps* mites is restricted because of the 'limited food source' (Anderson and Roberts 2013). Koeniger and Muzaffar (1988) concluded the different time period of survival of mites in different species of mites outside the brood cells. They survived for 25 hours in the cages of *A. mellifera* adults. Similarly, in case of *A. cerena* adults it was 27 hours while in *A. dorsata*, they survived up to 57 hours. The survival period was 30 hours in an empty glass vial while in similar vials with pupa of *A. mellifera*, the mites could survive as long as 5 days (Koeniger and Muzaffar 1988). *T. clareae* could easily survive even the longest of international airline flights (Rinderer et al. 1994).

2.3 Nature of damage and symptoms of infestation

As already mentioned, the mites cannot feed on adult honey bees so their phoretic phase is brief and much of their life period is spent within brood cells feeding on the haemolymph and soft tissues of developing brood. The adults act as means of transportation not as food source (Wokye 1984) for the mites. Because of this feeding behaviour, certain symptoms of infestation are seen in honey bees such as missing legs in the adults, distorted abdomen, bees crawling then flying and stubby wings (Baker et al. 2005). Brood malformation and even the death of the larva and pupa are the symptoms of infestation by *Tropilaelaps* mites (Baker et al. 2005). In an experiment in Kunming (China), clinical symptoms of Deformed Wing Virus were also found to be associated with *Tropilaelaps* mites in *A. mellifera* colonies (Dainat et al. 2009).

Low honey yield, considerable morphological and physiological deformities inflicted by these parasites on infested bees, susceptibility to wax moth infestation because of a declining bee population leading outright colony death , reduction of weight and longevity of infested adult bees, reduction of total protein concentration of infested pupae, alteration of immune responses and viral infections in parasitized pupae, irregular brood pattern, opened and bald brood are important damages seen in *A.*

mellifera colonies as a result of *Tropilaelaps* infestation (Khongphinitbunjong et al. 2015).

2.4 Control measures

Non chemical control method depends on its inability to feed adult bees resulting less survival outside the sealed brood cells (Sammataro et al. 2000) and interrupting the brood cycle and removal of all broods for 2-5 days can kill all the mites. Acaricides used to control *Varroa* mites can also be used as controlling agent for *Tropilaelaps* mites (Anderson and Robert 2013). For the treatment of these mite chemicals such as Fluvalinate, sulfur, formic acid, chlorobenzilate, thymol can be used (Sammataro et al. 2000).

2.5 Seasonal population dynamics

Kabul, Afganistan has cold and semiarid climate. In such climate in 1984, 20% of the infestation was observed in July with no infestation during October. But 24% and 37% infestation were detected in May and September respectively (De Guzman et al. 2018). Observations in *A. dorsata* colonies in Hisar, Haryana, India during the experimental period of March 1982 to April 1985, revealed highest infestation rate of *T. clareae* during March and April 1984 with a minimum infestation (almost zero) in August. Infestation levels (20-40%) observed during September and October in the same experiment was also relatively higher (Aggarwal 1989).

Year round rearing of brood occurs in subtropical regions like northern Vietnam. In an experiment in 1987 A.D. in Vietnam, the highest level of prevalence of *Tropilaelaps* mites in brood cell was observed between August and November (around 23%) while lowest was during July and August (De Guzman et al. 2018).

In northern Thailand in 1987 A.D., prevalence of *Tropilaelaps* mites was in peak in September (10–15% brood cells infested) and the prevalence of the mites remained lowest in January (2%) and August (<1%) (De Guzman et al. 2018).

Treatment with Apistan resulted to comparatively better seasonal growth in Apistan treated colonies than in untreated colonies in Pakistan in the year 1994. In untreated colonies, the highest infestation in broods was observed in May and in October (autumn) and the mean natural mortality in debris reached maximum in March (3584) while decreased to minimum in September. The infestation on adult bees was always low indicating the short phoretic period in *Tropilaelaps* mites. Early treatment of

colonies with Apistan was suggested by Camphor et al. (2001) for the prevention of rapid growth of mites in favourable season.

In the Jammu region of India in 1997, the highest infestation by *T. mercedeseae* occurred in colony during March to May (~32% of colonies) and September to November (~23% of colonies) during which high brood rearing of *A. mellifera* occurred. The lowest was observed in January (1%) and June to August (~2%) (De Guzman et al. 2018).

In an experiment in 2000 in Karnataka studying the effect of *Tropilaelaps clareae* and *Varroa jacobsoni* on *Apis mellifera* colonies, the highest level of infestation on worker and drone brood was observed during March to April and September to October while minimum level of infestation was found during May to August (Nagaraja 2000).

In a study by Neupane (2005) in Chitwan of Nepal, population variation of different mites was studied. The study was carried out during autumn, winter, spring, summer and rainy season. *Tropilaelaps clareae* was observed in *A. mellifera* and *A. dorsata* colonies and identified as major threat to *Apis mellifera* in Nepali context. *Apis mellifera* colonies had high level of infestation (78 %) by parasitic mites than in other species. During rainy season, the level of infestation by *Tropilaelaps* clareae was lowest (6.4 per sample). Similarly, the highest level of infestation was observed during winter and spring (118.3 per sample).

In Nepal, the rapid spread of *Tropilaelaps* in *A. mellifera* colonies is one of the main causes of colony failures (Neupane 2009).

In an experiment in 2011, in China, infestation of *A. mellifera* apiaries with *Tropilaelaps* mites was found in the order autumn (86%), followed by summer (67%), spring (17%), and winter (15%) (De Guzman et al. 2018).

The mite is widespread and colony death occurs. Its infestation is not controlled by acaricide treatments. Import of queen bees from this mite infested areas and movement of infested bee colonies for pollination have allowed rapid spread of ectoparasitic mites *Varroa* and *Tropilaelaps* spp. in Nepal (Neupane 2009) and apiculture industry is being severely affected. Remaining unchecked, the infestation by mites leads to colony collapse. Despite this fact, the study on the seasonal variations of *Tropilaelaps mercedesae* in Apistan treated and untreated colonies of *Apis mellifera* suffer from a scarcity of data in Nepal. Therefore, spread of diseases and colony failure due to *Tropilaelaps* parasitism which may have deleterious effects such as mortality and decline of honey bees' colonies strongly necessitate this study.

A similar type of experiment was carried out in Pakistan in from November 1993 to June 1993. Two sets of *A. mellifera* colonies were selected and placed at the distance of 2 m from one another. The group of four low-infested colonies in set A was treated with Apistan two times: firstly, in November 1993 and then in June 1994. Another group of four heavily infested colonies were selected as Group B. The group B colonies remained untreated. Mite population was periodically examined in brood, adult bees and debris samples of honey bees in two sets (Camphor et al. 2001, Camphor and Martin 2009).

By measuring the brood area in each frame and estimating the number of bees, strength of each colony was determined. This was done on the last week of every month when bees were actively foraging.

The infestation level in adult bees was determined by taking 200-300 adult bees and the infestation level in the brood was observed by taking 100 brood cells from previously frozen brood combs both every two months. Formica sheets in the hives were used to examine the mite mortality. Mean infestation levels in bees and brood were (mites/100 bees and mites/100 brood cells) determined. In this study, the same methodology has been applied.

One of the standard methods for collection of *Tropilaelaps* mites is collection of a sample of about 200 adult bees from an infested colony and transferring them to a transparent container containing 70% ethyl alcohol (Anderson and Roberts 2015). Washing the adult bees in soapy water or 'sugar-shake' method can be applied to remove the mites from adult bees. Alternate method of dislodging the mites could be placing the bees into plastic bags, labeling them accordingly and freezing until the bees could be visually examined (Anderson and Roberts 2015). Collection of the mites and their preservation in the present study was done following the same.

3. MATERIALS AND METHODS

3.1 Study area

The study area was Natural History Museum (NHM), Swayambhu, Kathmandu, Nepal. Experiment was carried out in well-arranged apiary within the premises of NHM and laboratory as well during the period of six months from May 2017 to October 2017. An apiary with twenty hives for the purpose of SmartBees Project (collaborative project between Länderinstitut für Bienenkunde (LIB), HohenNeuendorfd.V., Germany and Natural History Museum, Tribhuvan University, Swayambhu, Kathmandu, Nepal) was set up at NHM premises (Fig. 2). The apiary had the hives of *A. cerana* and *A. mellifera*. Among them, eight infested hives (four Apistan treated and four untreated colonies) were chosen for this study.

Swayambhunath is cultural and religious site located at 27'714" N and 85'287" E geographic coordinates in the northwest of Kathmandu Valley. Known as "self-existentone" the stupa sits atop a hill and stands as a symbol of the religious harmony prevailing in Nepal. Since the place is equally important shrine for both Hindus and Buddhists in the country, it has also been listed in the World Heritage Site by UNESCO in 1979.

Generally temperate type of climate with mean temperature of 21°C and average annual rainfall of 1377 mm (61%), summer receiving plenty of rainfall and dry winter is found in this region and remains dry for 152 days of a year. UV-index of this area is 4 (Ranjitkar and Chaulagain 2004).

Ranging from 1350m to 1405m, the area has moderate climate and so is the vegetation i.e. sub-tropical flora. Needlewood (*Schima wallichii)*, Pine (*Pinus roxburghii)*, Wild Himalayan Pear (*Pyrus pashia)* and ground layers like Sticky snakeroot (*Eupatorium adenophorum*, *Lantana camara*, *Justica adhatoda* are found as the principle vegetation of this area (Ranjitkar and Chaulagain 2004).

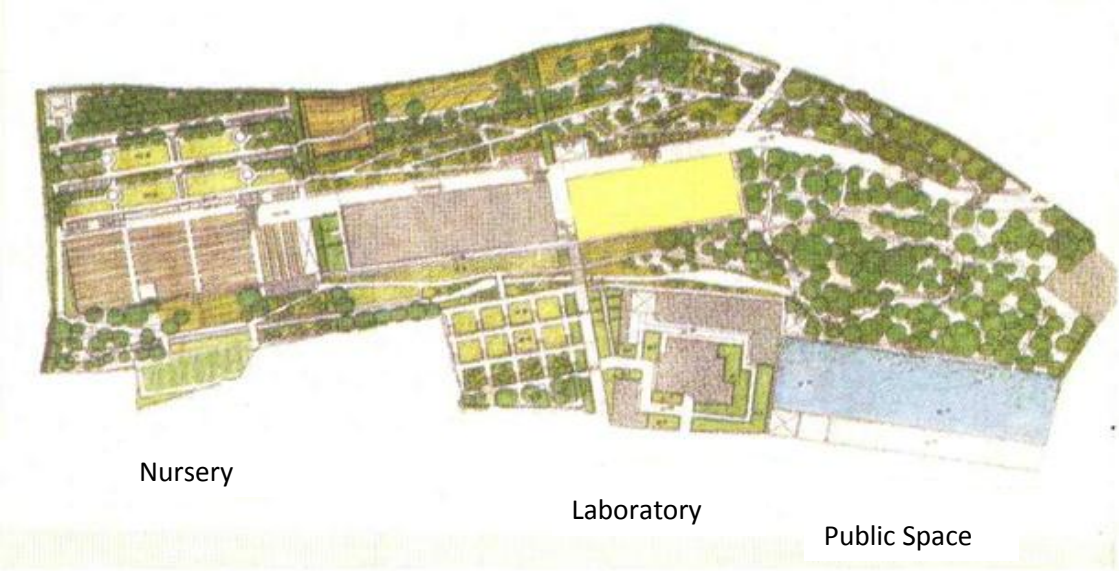

Fig.2. Study site

3.2 Materials and methods

3.2.1 Materials

Different tools used during the study have been listed as follows:

- Veil (with PPE),
- Hive tool,
- Forceps (pointed and blunt),
- Camel hair brush,
- Vials,
- Smoker
- 70% Ethyl alcohol
- Apistan sheets
- Sugar powder
- Trays
- Transfer boxes
- Petri dishes
- Microscope
- Camera
- Mounting Slides
- Test tubes etc.

3.2.2 Methods

3.2.2.1 Sampling design

This study was carried out for a period of 6 months from (May 2017 to October 2017). *A. mellifera* colonies naturally infested with *T. mercedesae* were selected. The methodology was adopted from Camphor et al. (2001).

For the determination of the seasonal occurrence of mites the two sets of colonies naturally *T. mercedesae* infested were selected. The mite infestation was observed under controlled (untreated) and Apistan treated conditions. A set of four infested colonies was treated with Apistan in group A, while the four infested colonies in group B was remained untreated in the same apiary. Populations of *T. mercedesae* were examined twice a month in brood, adult bees and debris samples in two sets of colonies in different seasons. Unlike the experiment carried out by Camphor et al. (2001), the present observation was made fortnightly. Samples of honey bees and brood (200 adult bees; 100 sealed brood cells respectively) were taken twice a month.

3.2.2.2 Mites observation

The lid of the container should be secured and should be shaken for about 1 minute. The mites collected at the bottom of the container should be collected into the container containing fresh 70% ethyl alcohol.

The mites from the adult bees were removed by 'sugar-shake method' (Anderson and Roberts 2015). The adult bee mites were examined by brushing 200 adult worker bees from an infested colony into transfer containers. Fine sugar was added from the sieve of the container and then was shaken vigorously for 1 minute. Then mites along with sugar were transferred into Petri dishes. The number of mites collected was then counted with the help of camel hair brush. For sampling fornightly debris, white Formica sheets, were placed in the hives and examined for mite mortality.

Mean infestation levels in bees and brood (mites/100 bees and mites/100 brood cells) were determined. Analysis of brood mites was done by examining 100 capped brood cells in each colony of *A.mellifera*. The metrological data were also collected by thermo hygrometer and data related to precipitation was also recorded.

3.2.2.3 Species identification and preservation

Identification features were adopted from (Anderson and Morgan 2007) and collected samples were preserved in 70% alcohol and further permanently mounted slides were deposited at the Central Department of Zoology Kirtipur, Kathmandu, Nepal.

3.2.3 Statistical analysis of data

To analyze the data, t test was performed with MS Excel 2016. The relation between mite's population with temperature and humidity were analyzed by correlation by using R studio.

4. RESULTS

The study was carried out from May to October 2017. The infestation of *A. mellifera* by *T. mercedesae* was observed for three seasons: Summer, Rainy and autumn. The highest infestation of *A. mellifera* colonies was observed during September-October i.e.autumn season in both Apistan treated and untreated colonies. In Apistan treated colonies, similar type of seasonal variation in mites was observed but the number of mites seemed to decrease. Following results were obtained for Apistan treated and untreated colonies of *Apis mellifera.*

Table 1: Mite Population in Apistan treated and untreated colonies

S.N.	Months	Percentage of Mites in Adult Bees		Percentage of Mites in Broods		Number of Mites in Debris		Temperature (ºC)	Precipitation (mm)	Relative Humidity (%)
		Set A	Set B	Set A	Set B	Set A	Set B			
1.	May	0	0.562	2	2.75	3.25	3.25	30	0	69
2.	June	0	1.312	3	4	6.875	16.12	31.5	1.4859	56.5
3.	July	0	0.812	2.25	3.75	14.75	31.12	30.5	8.411	65
4.	August	0.375	1	2	3.375	8.375	41	28.7	12.4	83
5.	September	0.5625	1.132	2.125	21.75	11.37	46.25	28.1	7.3	82
6.	October	0.625	2.5	8.375	18.87	25.75	67.62	29.1	2.4	79

4.1 Seasonal fluctuation of *Tropilaelaps mercedesae*

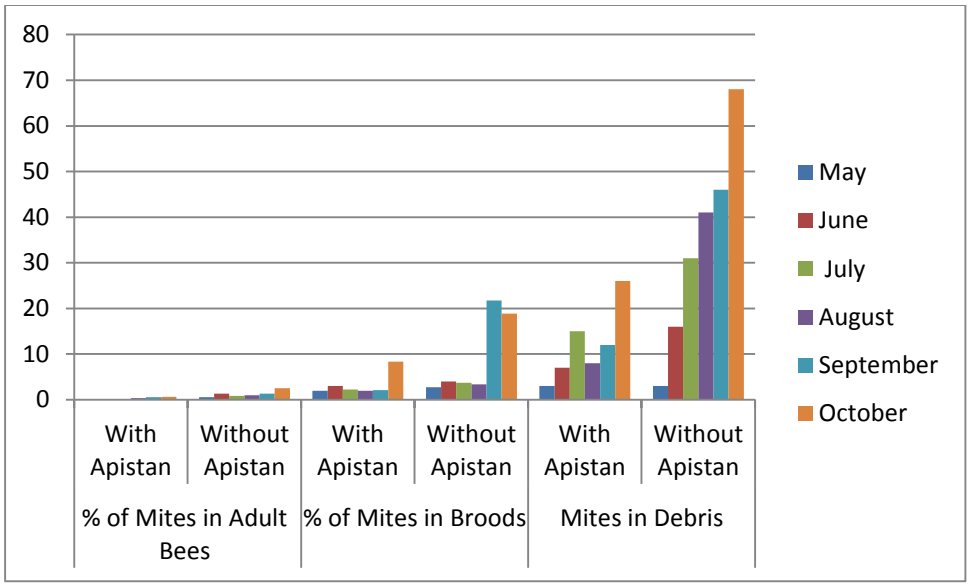

Fig. 3. Seasonal fluctuation of the population of T. mercedesae in Apistan treated and untreated colonies

Initially, in the Apistan treated colonies (Set A), the number of mites remained the lowest in all adult bees, broods and debris i.e., during May. The percentage of mites in adult honey bees remained the lowest continuously for three months May, June and July. No any mite was observed in adult honey bees during these three months. Then it started increasing and reached maximum in number in October. During May, the lowest percentage of mites i.e., 2% was observed in broods of *A. mellifera* colonies of Set A. The lowest number of mites in debris was also observed during May which was 3. A sudden rise in number of mites in debris was seen during July when the percentage of mites seemed to increase in brood also. However, no any increase in number of mites occurred in adult bees during July (Table 1 and Fig. 3).

The abundance of mites was found in October in all adult, brood and debris of honey bees of Apistan treated colonies of *A. mellifera*. In October, the highest percentage of mites was found in adult honey bees was 0.625%. Similarly, the highest percentage of mites found in broods was 8.375% during same month. The highest average number of mites in debris was found to be 26 during October (Table 1 and Fig. 3).

Hence, it can be stated that lowest infestation was observed during May i.e., Summer Season and the highest infestation was observed during October i.e., Autumn Season during the experimental period of six months. However, the number of mites in the

Apistan treated colonies seems to be lesser than that in untreated colonies (Table 1 and Fig. 3).

Among the six months of observation in untreated colonies, the number of mites was found to be the lowest during May in all adult bees, broods and debris. In adult bees, 0.56 % of mites were observed while 2.75 % of mites were observed in brood. Similarly, the least number of mites observed in debris in May was 3 (Table 1 and Fig. 3).

In case of mites in adult bees, the percentage remained almost same during the entire observation period reaching maximum (2.5%) in October. This percentage, however, was also a small number. The number of mites in adult bees and broods was observed to remain almost same with slight change in number up to August. But during September, it increased abruptly and reached maximum in brood. During October, the percentage of mites decreased to 18.87%. However, in case of debris, the number of mites was found to be increased increase continuously reaching maximum (27.75%) in October (Table 1 and Fig. 3).

The highest infestation in adult bees was found during October which was 2.5% in untreated colonies of *A. mellifera.* Similarly, the highest infestation in brood was found during September which was 21.75 %. During October, it decreased to 18.87% which was higher than other month's observation except September. The mites in brood seemed to decrease slightly in October. However, the highest number of mites in average in debris was found to be 68 which also were during October. It can be concluded that in the naturally infested colonies of *A. mellifera* by *T. mercedasae,* the highest infestation was observed during autumn season (Table 1 and Fig. 3).

Hence, it can be stated that in Apistan untreated colonies of *Apis mellifera*, the lowest infestation was observed during summer and highest infestation was observed during autumn.

4.2 Efficacy of controlling agent Apistan

The number of mites in adult bees of Apistan treated colonies was found to be lesser than that in untreated colonies. In observations made every month, the number of mites was lesser than that in untreated colonies. The percentage of mites in untreated colonies

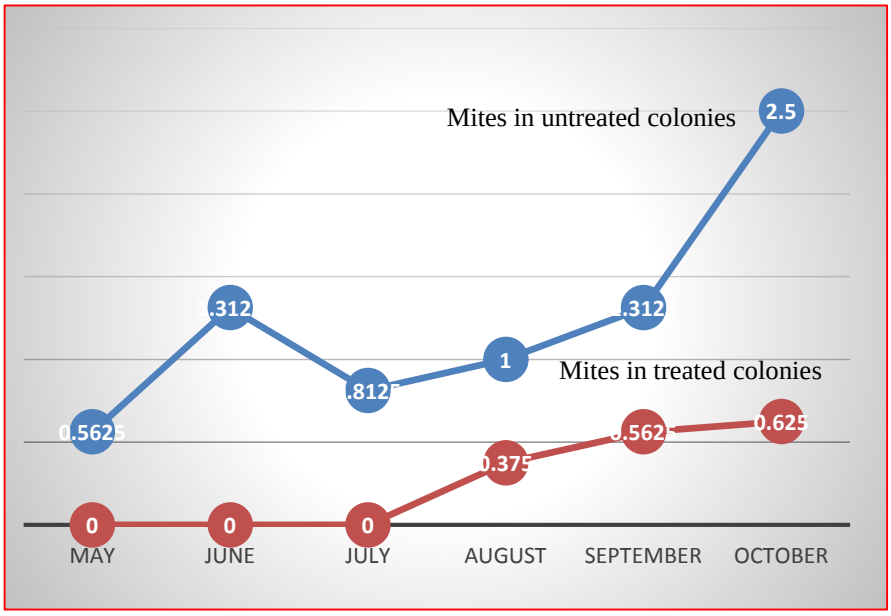

Fig. 4. Comparision of number of T. mercedesae in Apistan treated and untreated colonies of A. mellifera adult bees

were 0.562% while it remained zero in Apistan treated colonies during May. Then after the percentage of mites in Apistan treated colonies remained zero continuously for June and July when the percentage of mites in untreated colonies were 1.3125 and 0.8125 respectively. Similarly, the percentage of mites in untreated colonies was 1% in August whereas 0.375% in Apistan treated colonies. Likewise, the percentage of mites in untreated colonies during September was 1.3125% and it was 0.562% in Apistan treated colonies. When the percentage of mites in untreated colonies was 2.5 % in untreated colonies, it was 0.625% in Apistan treated colonies. Similarly, although the highest percentage of mites was observed during October in both apistan treated and untreated colonies, the percentage of mites in adult bees differ significantly in the Apistan treated and untreated colonies (p- value 0.0067 at 6 df, 0.05 level of significance) (Fig. 4).

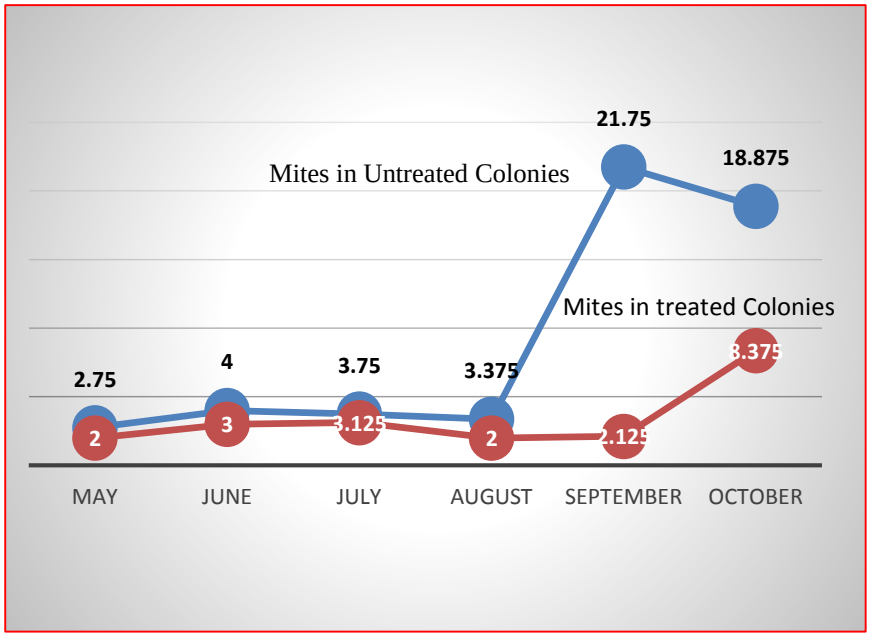

Fig. 5. Comparision of number of T. mercedesae in Apistan treated and untreated colonies of A. mellifera brood.

Although the number of mites in broods in Apistan treated colonies was also observed to be lesser than that of untreated colonies, from the t-test, no significant difference between the number of mites in Apistan treated and untreated colonies was determined with p value 0.089 (6 df at 0.05 level of significance) (Fig. 5).

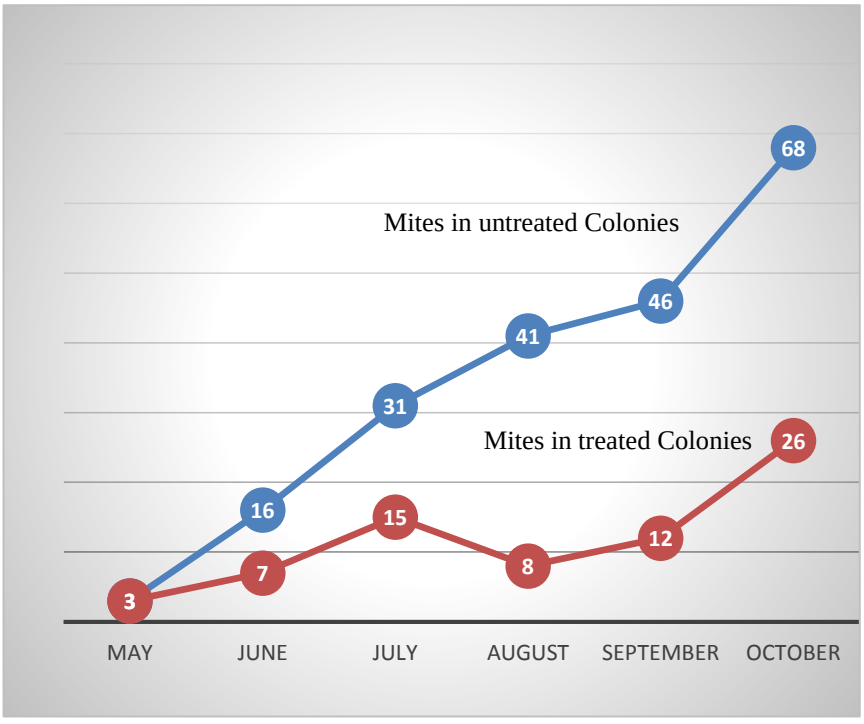

Fig. 6. Comparision of number of *T. mercedesae* in Apistan treated and untreated colonies of *A. mellifera* debris

In the Apistan treated colonies' debris, lesser number of dead mites was observed. The number of mites in debris was found to lower in each month's observation in Apistan treated colonies (Fig.6).

In Apistan untreated colonies, the average number of mites reached as much as 68 in the month during when maximum number of mites were obtained in adult bees and brood cells also.

Even though, the same month gave the highest number of mites in Apistan treated colonies also, the number of mites reduced significantly after treatment with Apistan in debris with p-value 0.032 (6 df, 00.5 level of significance) (fig. 6).

4.3 Relation between the population of mites and other physical parameters

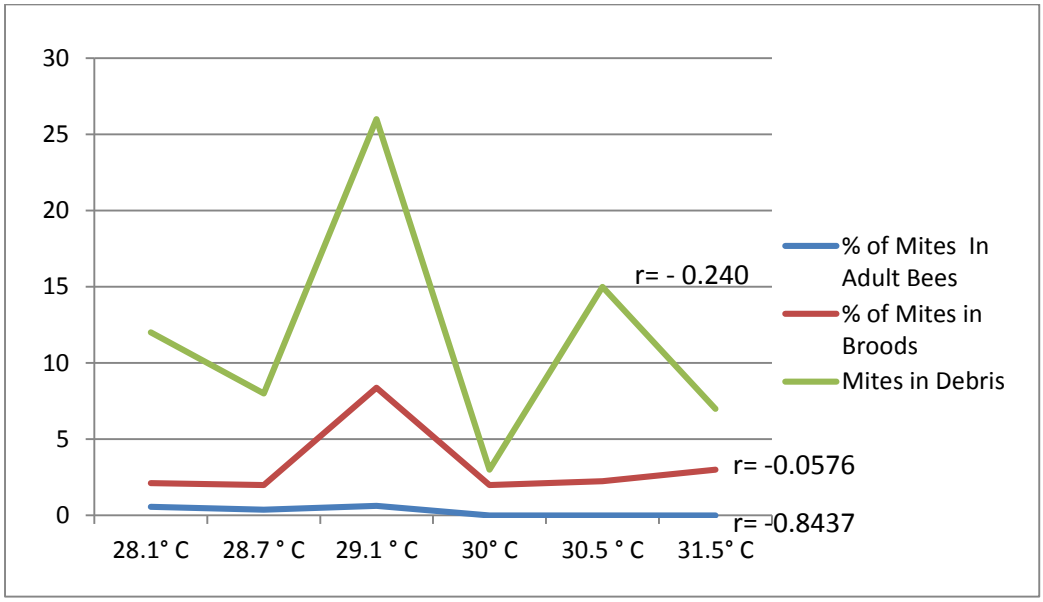

Fig. 7. Relation between the temperature and the number of mites in Apistan treated colonies

The correlation coefficient was -0.8437. This suggests that the number of mites on adult bees and temperature were highly negatively related in Apistan treated colonies. The correlation coefficient between the number of mites in broods and temperature was -0.05756 and that between the number of mites in debris and temperature was - 0.240. The linear coefficients thus obtained were found to be statistically significant from t-test. This shows that the number of mites in Apistan treated colonies and temperature were negatively correlated (Fig.7).

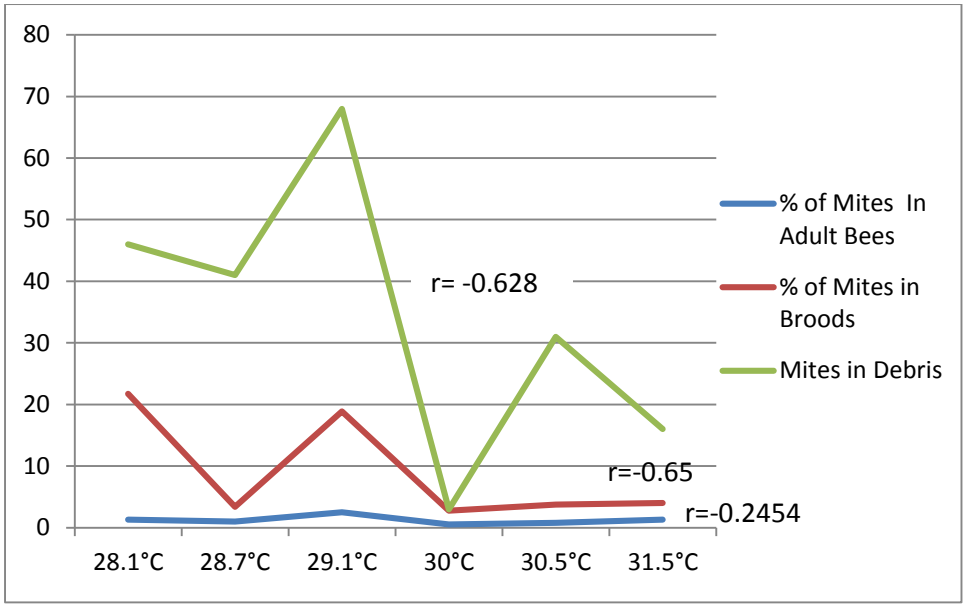

Fig. 8. Relation between the temperature and the number of mites in untreated colonies

The correlation coefficient between the number of mites on adult bees, broods and debris and temperature were -0.2454, -0.650 and-0.6283 respectively. In untreated colonies of *A. mellifera*, the number of mites on adult bees, broods and debris and temperature were found to be negatively correlated.

Since, the number of mites and temperature are found to be negatively correlated in both experimental colonies and control, it can be concluded that the temperature and number of mites are negatively correlated. The population of mites decreased with the increase in temperature and vice-versa.

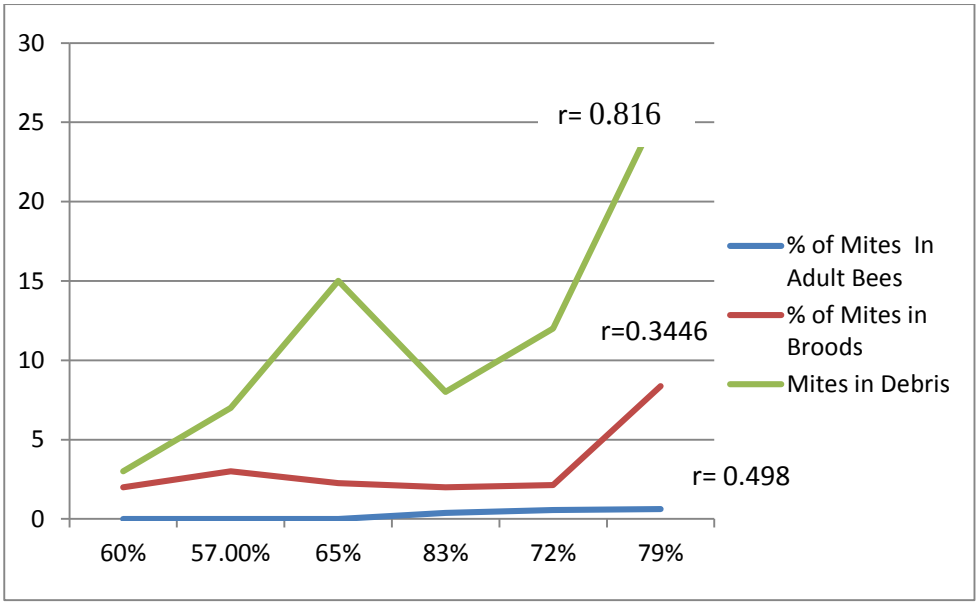

Fig. 9. Relation between the humidity and the number of mites in Apistan treated colonies

The number of mites on adult bees, broods and debris and humidity were found to be highly positively related in Apistan treated colonies (correlation coefficient = 0.816, 0.3446 and 0.498 respetively). In Apistan treated colonies, the number of mites on adult worker bees, in broods and debris were found to be positively correlated with the humidity (Fig. 9).

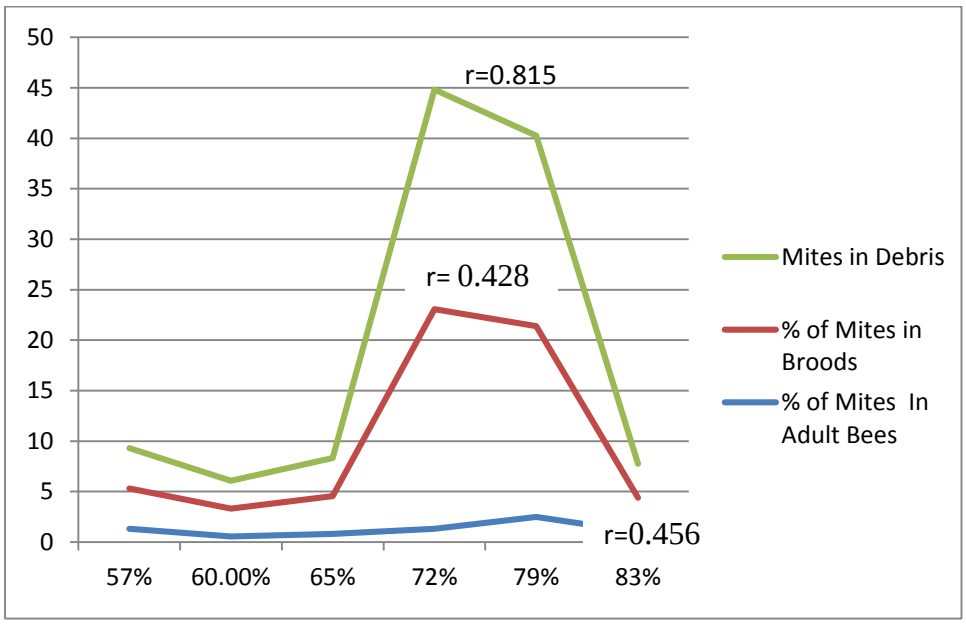

Fig. 10. Relation between the humidity and the number of mites in untreated colonies

The number of mites on adult bees, broods and debris and humidity were found to be highly positively correlated in untreated colonies (correlation coefficient = 0.456, 0.428 and 0.8151 respectively). In untreated colonies, the number of mites on adult worker bees, in broods and debris was found to be positively correlated with the humidity (Fig. 10).

In experimental colonies and control, the correlation between population of mites and the humidity was found to be positively correlated. It can be concluded that there was positive correlation between the population of mites and the humidity. Also, this result found statistically significant may be true for large population also.

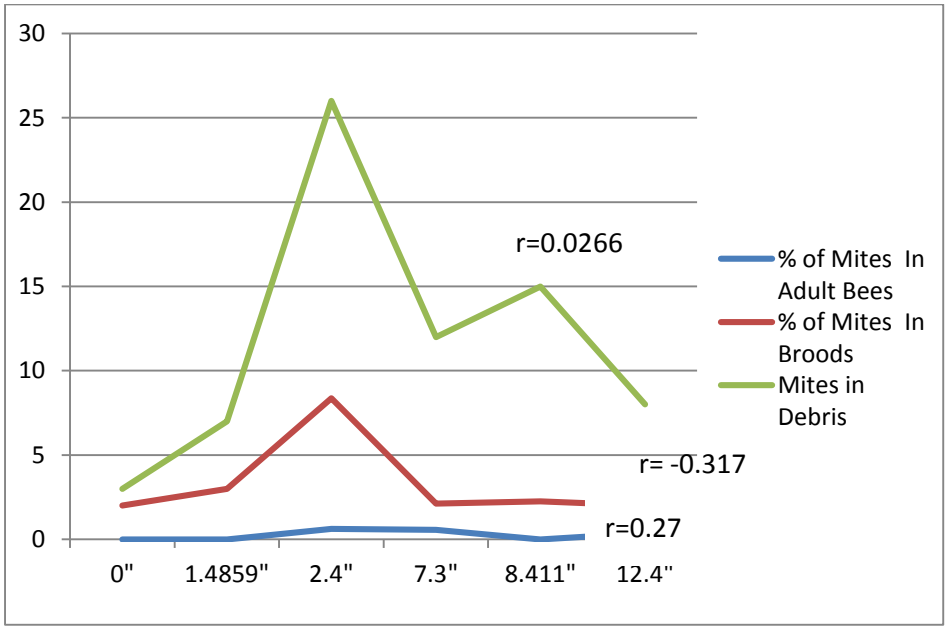

Fig. 11. Relation between the precipitation and the number of mites in Apistan treated Colonies

The precipitation were positively correlated in Apistan treated colonies (correlation coefficient = 0.27) in case of number of mites on adult bees whereas the correlation coefficient between the number of mites in broods and precipitation was found to be negatively correlated in Apistan treated colonies (r= -0.317). The number of mites on adult bees and precipitation were slightly positively related (r= 0.0266) in Apistan treated colonies (Fig. 11).

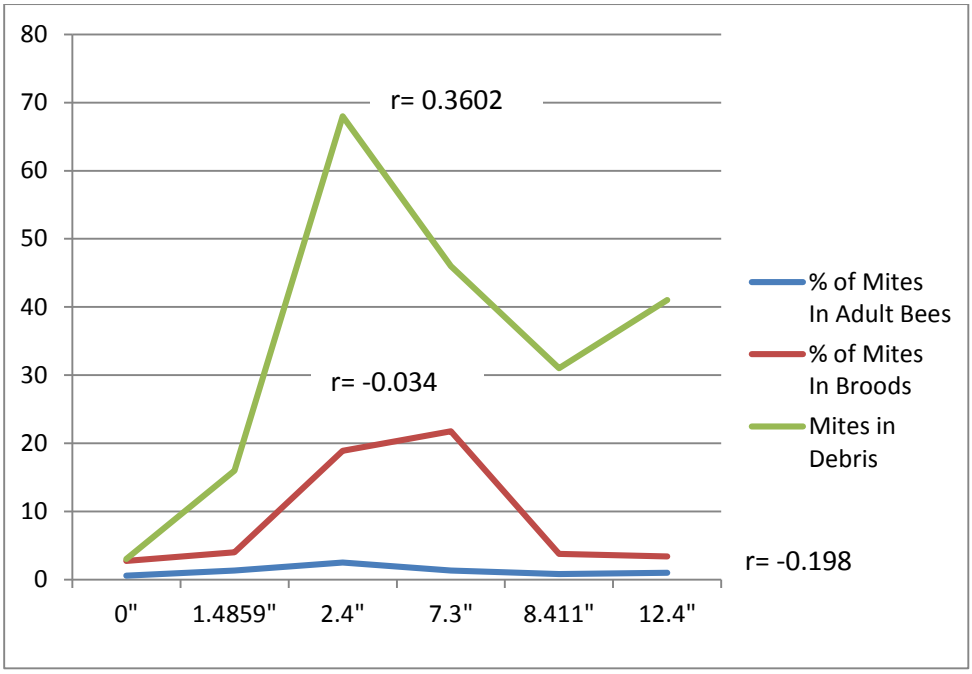

Fig. 12. Relation between the precipitation and the number of mites in untreated colonies

The number of mites on adult bees and broods and precipitation were slightly negatively correlated (r = - 0.198 and r = -0.0344 respectively) in untreated colonies whereas the number of mites on debris and precipitation were positively correlated (r = 0.3602) in untreated colonies. In untreated colonies, negative correlation was observed between the number of mites on adult bees and in broods while the number of mites in debris was found to be positively correlated with the precipitation (Fig. 12).

6. DISCUSSION

This study depicts an aspect covering effectiveness of Apistan against *T. mercedesae* encompassing seasonal occurrence which can be an important aspect acquiring long-term solution for controls.

The mites were found in higher number in brood. This may be because of the nature of the mites that they cannot feed on the adult mites so found inside the developing brood. The lesser number of mites in adult bees was also explained by Khongphinitbunjong et al. (2013). They stated that *Tropilaelaps* having short development period reach adulthood by the time of host emergence (nearly all). The number of mites on adult honey bees always remains lower than that in broods and in debris (Anderson and Roberts 2013). In present study, the higher number of dead mites was seen in debris which may be due to the reason that wastages get accumulated in debris including dead mites, dead bees and many other waste substances. The mites after death fall down to the debris and got accumulated (Anderson and Roberts 2013).

In untreated colonies of *A. mellifera,* the highest infestation in adult bees was found during October. Similarly, the highest infestation in brood was found during September. Thereafter in October, it decreased which was even higher than other month's observation except September. However, the highest number of mites in average in debris was found also during October.

The highest percentage of mites in Apistan treated colonies was found in adult honeybees, broods and debris during October. In the Apistan treated colonies of *A. mellifera*, the abundance of mites was found in October in all adult, brood and debris of honey bees.

In untreated colonies, the number of mites was the lowest during May in all adult, broods and debris. But in the treated colonies, not a single mite was observed in adult honey bees during three months (May, June and July). Then it started increasing and reached maximum in number in October. On the other hand, during May, the lowest percentage of mites was observed in broods and the the lowest number in debris was also observed during May. A rise in number of mites in debris was seen during July when the percentage of mites increased in brood also. However, number of mites in adult bees during July was not found to be increased.

The present study in untreated colonies, coincides with a study carried out in Karnataka (Nagaraja 2000) in which the minimum level of infestation was also found between

May to August and the highest level of infestation on worker and drone brood was observed September to October, 2000.

Hence, from the experiment, it can be concluded that the highest rate of infestation occurs during autumn. The highest infestation observed during autumn season and during the months of October is in accordance with the study carried out by Camphor et al. in Pakistan in 2001. In their study the mite population reaches maximum during autumn.

The findings of the present study coincide to some extent also with the study carried out by Camphor et al. (2001) in Pakistan where the highest mite death in debris for treated colonies started to decrease in April and reached minimum in September and in case of brood, it reached peak maximum in summer and autumn and lowest in winter and extreme in July. But in the contrary, the mite population was found to be nil in the treated colonies of adult bees, minimum in brood and debris in comparison to the same study.

According to de Guzman et al. (2018), the highest infestation of *A. mellifera* colonies by *Tropilaelaps* mites was observed during autumn in China. Similar type of observation was observed in Jammu region of India too (Guzman et al. 2018). The highest infestation occurred during September to November in Jammu region. The highest infestation of *A. mellifera* colonies by *Tropilaelaps* mites occurs during September in Kabul (Guzman et al. 2018). Similarly, in a study in Vietnam, the highest number of mites was observed during August to November and in a study in Thailand the time for the highest infestation was during September to November (De Guzman et al. 2018). The result of the present study coincides with the above-mentioned studies.

Further, the same month (October) gave the highest number of mites in Apistan treated colonies also but the number of mites was observed to reduce significantly after treatment with Apistan in adult bees and debris with the p value 0.0067 and 0.032 respectively (df 6 at 0.05 level of significance). Therefore, the chemical substance, Apistan is found to be an effective controlling agent for *T. mercedesae* mites in adult bees in this study because the number of mites remained low continuously for four months in Apistan treated colonies but with sudden rise in the number in debris during July. This suggests that if the colonies are treated early before the foraging time begins, the number of mites may be controlled. In addition, the number can be controlled even during the months when the infestation occurs at highest rate in untreated colonies which is in accordance with suggestions by (Camphor et al. 2001).

The difference between the percentages of mites in broods of two sets of colonies wasn't found to be significant. This might due to the nature of the mites that they remain inside the combs of bees as the parasites of the broods being much less exposed to the controlling agent used.

This study was also successful in finding the relationship between number of mites and physical parameters. Since, the number of mites and temperature were found to be negatively correlated in both experimental colonies and control. Therefore, it can be concluded that the temperature and number of mites were correlated negatively. The population of mites decreased with the increase in temperature and vice-versa. This explains why a smaller number of mites were observed on adult bees, in broods and in debris of both Apistan treated and untreated colonies during hotter months like July and August. Since in both experimental colonies and control, the relationship between population of mites and the humidity was found to be positively correlated, it can be concluded that there was positive correlation between the population of mites and the humidity. On the other hand, mixed result was obtained for the precipitation, the relationship between the number of mites and precipitation couldn't be predicted exactly. Hence it can be a matter of further study in the future.

During summer due to less foraging activities by honeybees lead to less brood rearing. This affects the number of mites. With the beginning of autumn availability of food increases which in turn increases the number of mites in brood (Camphor et al. 2001). Unlike the experiment carried out by Camphor et al. (2001), the mites in debris in this study increased with the increase in the number of brood and adult bees. This showed association of *T. mercedesae* mites with brood rearing. During the season when brood rearing is high, the number of mites is also high and when the brood rearing is less, the number of mites also decreases accordingly (Camphor et al. 2001).

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

The ectoparasitic mite, *T. mercedesae*, is becoming the largest threat to apiculture industry and honeybee health. Keeping this in mind, this study was carried out to adopt control measure using Apistan which is generally used for Varroa mites. In addition, the present study is expected to be useful in understanding the nature of infestation by *T. mercedesae* mites in *A. mellifera* colonies during different seasons. It has also been helpful in knowing the effect of physical parameters in the population fluctuations of the *T. mercedesae* mites.

It can be concluded from this study that the population of mites reaches maximum during the season when brood rearing occurs in autumn. Conversely, when the brood rearing is lesser, the number of mites also reduces in summer and rainy seasons. The population of the mites increased with the increase in humidity and decreased with the decrease in the humidity. Likewise, increase in the temperature of the hives caused the population of the mites to get lowered and vice versa. The effect of precipitation couldn't be understood clearly in present study so it can be a matter of further study in the future.

The findings obtained from the present study accept Apistan as an effective controlling agent for the *T. mercedesae* mites in adult bees of *A. mellifera* colonies foraging in Kathmandu district. The use of Apistan just before the foraging season begins may be more effective in controlling the mites.

Like Varroa, *T. mercedesae* mites are also serious pests of *A. mellifera*. To increase the productivity from apiculture, timely treatment of the hives by the controlling agents like Apistan is very essential. The use of the controlling agent becomes effective only when the pattern of change of population and effects of various physical parameters are understood.

7.2 Recommendations

On the basis of this study following recommendations can be done:

- Therefore, beekeepers should be more attentive during brood rearing periods.
- The effectiveness of Apistan depends on the stage when it is applied. Apistan can be used during early stage of brood rearing which restricts the number of mites throughout the year.
- Further research can be carried out to understand the fluctuation of number of mites throughout the year.
- The research can also be replicated to understand the effect of precipitation on the number of mites as this matter has remained unconcluded in this study.
- Dissemination of usefulness of Apistan as controlling agent should be done to make beekeepers aware of the fact that it is an effective control measure.
- Further, trainings can be organized for beekeepers about how the effectiveness of Apistan can be increased.

REFERENCES

ADW, 2015. *Apis mellifera* honeybee.http://animaldiversity.org/accounts/Apis_mellifera. accessed on 19 November, 2016.

Aggarwal, K. and Kapil, R.P. 1989. Seasonal population dynamics of *Tropilaelaps clareae* (Acari: Laelapidae) in *Apis dorsata* colonies. *In* Channabasavanna, G.P., Viraktamath, C.A. (eds.). Progress in Acarology, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India. p. 283-286.

Ahmad, K.J., Razzaq, A., Abbasi, K.H., Shafiq, M., Saleem, M. and Arshadullah, M. 2013. Thymol as control agent of mites (*Varroa destructor*) on honeybees (*Apis mellifera*). Pakistan Journal of Agricultural Research, **26**(4): 316-317.

Anderson, D.L. and Roberts, M.K.J. 2013. Standard methods for *Tropilaelaps* mites research. Journal of Apicultural Research, **52**(4): 6-10.

Anderson, D.L. and Morgan, M. J. 2007. Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): a new and re-defined species. xperimental and Applied Acarology, **43**:1-24.

Baker, R.A. 2010. The parasitic mites of honey bee past, present and future. Journal of Entomological Research**, 1**(4): 1-7.

Baker, R.A., Hick, A. and Chmielewski, W. 2005. Aspects of the history and biogeography of the bee mites *Tropilaelaps clareae* and *T. koenigerum*. Journal of Apicultural Science, **49** (2): 13-18.

Burgett, M., Akratanakul, P. and Morse, R. A. 1983. *Tropilaelaps clareae*: a parasite of honeybees in south-east Asia. Bee world, **64**(1): 25-18.

Camphor, E.S.W and Stephen, J.M. 2009. Population changes of *Tropilaelaps clareae* mites in *Apis mellifera* colonies in Pakistan. Journal of Apicultural Research**,** **48**(1): 46-49.

Camphor, E.S.W, Hashmi, A.A, Ritter W. and Bowen, I.D. 2001. Seasonal changes in mite (*Tropilaelaps clareae*) and honeybee (*Apis mellifera*) populations in Apistan treated and untreated colonies, Proceedings of the 37th International Apiculture Congress, 28 October -1 November 2001. Apimonida.

Chantawannakul P., de Guzman L.I., Li J. and Williams G.R. 2016. Parasites, pathogens and pests of honeybees in Asia. Apidologie, **47**: 301-324.

Chantawannakul P., Ramsey S., Engelsdrop D., Khongphinitbunjong, K., and Phokasem P. 2018. *Tropilaelaps* mite: an emerging threat to European honeybee. Current opinion in Insect science, **26**: 69-75.

Clark, J.M and Matsumura, F. 1982. Two different types of inhibitory effects of pyrethroids on nerve Ca- and Ca+Mg-ATPase activity in the squid, *Laigo pealei*. Pesticide Biochemistry and Physiology, **18**(2): 180-190.

Crane, E. 1999. The world history of beekeeping and honey hunting. London: Duckworth.

Dainat, B., Ken, T., Berthoud H. and Neuman P. 2009. The Ectoparasitic mite *Tropilaelaps mercedesae* (Acarilaelapidae) as a vector of honeybee viruses. Insects Social, **56**: 40-43.

De Guzman L.I., Williams G.R., Khogphinitbunjong K. and Chantawannkul P. 2017. Ecology, life history and management of *Tropilaelaps* mites. Journal of Economic Entomology, **110**(2): 319-332.

Delfinado, M. D. and Baker, E.W. 1985**.** The occurrence of *Tropilaelaps* mites in broodnests *Apis dorsata* and *Apis laboriosa* in Nepal, with description of nymphal stages. American Bee Journal, **125**(10): 703-706.

Delfinado, M.D. and Baker, E.W. 1982. New records for *Tropilaelaps* clareae from colonies of *Apis cerana*. American Bee Journal, **122**(5): 382.

Diana Sammataro, Uri Gerson and G Needham. 2000. Parasitic mite of honeybees: Life history, implications and impact. Annual Review of Entomology, **45**: 519-548.

Dietemann, V., Nazzi, F., Martin, S.J., Andersons, D.L., Locke, B. and Delaplane, K.S. et al. 2013. Standard methods for *Varroa* research. Journal of Apicultural Research, **52**(1): 4-5.

FAO. 2006. Honeybee diseases and pests: a practical guide, Vol. 4, Food and Agriculture Organization, Rome.

Featured Creatures. 2017. European Honeybee. http://entnemdept.ufl.edu/creatures/MISC/BEES/euro_honey_bee.htm#top .accessed on 25 November 2019.

Forsgren E., de Miranda, J.R., Isaksson, M., Wei, S., and Fries, I. 2009. Deformed wing virus associated with *Tropilaelaps mercedesae* infesting European honeybees (*Apis mellifera*). Experimental and Applied Acarology. **47**: 87-97.

Gulati, R. and Kaushik, H.D. 2004. Enemies of honeybees and their management- a review. Agricultural Reviews, **25**(3): 189-200.

Gusovsky, F., Secunda, S.I. and Daly, J.W. 1989. Pyrethroids: involvement of sodium chennels in effects on inositol phosphate formation in guinea pig synaptoneurosomes. Brain Research, **492**(1-2): 72-78.

Hoany L. M, and Hieu P.K. 2002. Study on the invasion and reproduction of *Varroa jacobsoni* and *Tropilaelaps clareae* in honeybees (*Apis mellifera*) in Dahlak province. Veterinary Sciences and Techniques, **9**(1): 42-45.

IFAS Extension, University of Florida. 2015. The Social organization of Honey bees. https://edis.ifas.ufl.edu/pdffiles/IN/IN110200.pdf. accessed on 25 November 2019.

Kavinseksan, B. and Wongsiri, S. 2016. Life history and control of the parasitic bee mite, *Tropilaelaps mercedesae* Anderson and Morgan (Cari: Laelapidae): A review. Basic Research Journal of Agricultural Science and Review, **5**(3): 56-71.

Koeniger, N. and Muzaffar, N. 1988. Lifespan of the parasitic honeybee mite *Tropilaelaps clareae*, on *Apis cerena*, *dorsata* and *mellifera*. Journal of Apiculture Research, **27**(4): 207-212.

Kotwal, S., Abrol, D.P., Shatinwazder A. and Gandotra A. 2013. Mite pests of honeybees (*Apis mellifera* L.) and their seasonal incidence in Jammu Division of Jammu and Kashmir, India. The Bioscan, **3**(2): 529-531.

Laigo F.M. and Morse R.A. 1968. The mites *Tropilaelaps clareae* in *Apis dorsata* colonies in the Philippines, Bee World, **49**(3): 116-118.

Nagaraja, N. 2000. Impact of ectoparasitic brood mite, *Tropilaelaps clareae* and *Varroa jacobsoni* on *Apis mellifera* beekeeping in Karnataka. Journal of Ecotoxicology and Environmental Monitoring, **10** (3): 205-210.

Neupane, K. R. 2009. Honeybee mites and their population variation in *Apis* spp. during different seasons, Chitwan Nepal. Journal of Institute of Agriculture and Animal Science, **30**: 159-166.

Pettis, J.S. and Chaimanee, V. 2019. The Survival of *Tropilaelaps mercedesae* on beehive products. Journal of Apicultural Research, **58**(3): 413-415.

Plos One. 2017. Chemical and cultural control of *Tropilaelaps mercedesae* mites in honeybees (Apis mllifera) colonies in Northern Thailand. https://doi.org/10.1371/journal.pone.0188063. accessed on 12 December, 2018.

Ranjitkar, S. and Chaulagain, B. 2004. Diversity and conservation practices in the Swayambhunath Hillock. Proceedings of IV National Conference on Science and Technolgy: 771-773.

Rinderer T.E., Oldroyd B.P., Lekprayoon C., Wongsiri S., Boonthai C. and Thapa R. 1994. Extended survival of the parasitic honey bee mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. Journal of Apicultural Research, **33**: 171-174.

Sammataro, D. and Avitabile, A. 1998. Beekeeper's Handbook 3rd ed. Cornell University Press, Ithaca, 4 p.

Shrestha, M., Wegener, J., Gautam, I., Singh, M., Schwekendiek, C. and Bienefeld, K. 2020. Individual-level comparisions of honey bee (Hymenoptera: Apoidea) hygienic behavior towards brood infested with *Varroa destructor* (Parasitiformes: Varroidae) or *Tropilaelaps mercedesae* (Mesostigmata: Laelapidae). Insects, **11**(8): 510-520.

Shrestha, N. and Gautam, I. (2020). Seasonal prevalence of Asian honeybee ectoparasitic mite *Varroa destructor* Anderson and Trueman, 2000 in Madanpokhara Apiaries, Palpa, Nepal. Nepalese Journal of Zoology, **4**(2): 61-67.

Singh, A., Chunneja, W.K., Singh, J. and Choudary, A. 2019. Population dynamics of ectoparasite mite (*Tropilaelaps clareae*) in European honey bee (*Apis mellifera*) colonies in north-western India. Journal of Entomology and Zoology Studies, **7**(1): 976–980.

Spivak, M., Masterman, R., Ross, R. and Mesce, K.A. 2003. Hygienic behaviour in the honeybee (*Apis mellifera*) and the modulatory role of octopamine. Journal of Neurobiology, **55**(3): 341-354.

Thapa, R., Shrestha, R., Manandhar, D.N. and Kafle, B. 2000. Beekeeping in Nepal. Proceeding 7th IBRA and 5th AAA Conference, 19-25 March 2000. Chiang Mai, Thailand: 409-413.

Woyke, J. 1984. Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. Apidologie, **15**(4): 421-434.

Woyke, J. 1985. *Tropilaelaps clareae*, a serious pest of *Apis mellifera* in the tropics but not in temperate zones. American Bee Journal, **125**(7): 497-499.

Woyke, J. 1987. Length of stay of the parasitic mite *Tropilaelaps clareae* outside sealed honeybee brood cells as a basis for its effective control. Journal of Apicultural Research, **26**(2): 104-109.

APPENDICES

Appendix 1: List of Observations

Month (2017 A.D)	Mites in adult bees (per 100 adult bees)		Mites in broods (per 100 broods)		Mites in debris	
	With Apistan	Without Apistan	With Apistan	Without Apistan	With Apistan	Without Apistan
May	0	0.5625	2	2.75	3	3
June	0	1.3125	3	4	7	16
July	0	0.8125	2.25	3.75	15	31
August	0.375	1	2	3.375	8	41
September	0.563	1.3125	2.125	21.75	12	46
October	0.625	2.5	8.375	18.875	26	68

Precipitation (inch)	Mites in adult bees (per 100 adult bees)		Mites in broods (per 100 Broods)		Mites in debris	
	With Apistan	Without Apistan	With Apistan	Without Apistan	With Apistan	Without Apistan
0.0 (May)	0	0.5625	2	2.75	3	3
1.4859 (June)	0	1.3125	3	4	7	16
8.411 (July)	0	0.8125	2.25	3.75	15	31
12.4 (August)	0.375	1	2	3.375	8	41
7.3 (September)	0.563	1.3125	2.125	21.75	12	46
2.4 (October)	0.625	2.5	8.375	18.875	26	68

Relative Humidity(%)	Mites in adult bees (per 100 adult bees)		Mites in broods (per 100 Broods)		Mites in debris	
	With Apistan	Without Apistan	with Apistan	Without Apistan	With Apistan	Without Apistan
60 (May)	0	0.5625	2	2.75	3	3
56.5 (June)	0	1.3125	3	4	7	16
65 (July)	0	0.8125	2.25	3.75	15	31
83 (August)	0.375	1	2	3.375	8	41
72 (September)	0.563	1.3125	2.125	21.75	12	46
79(October)	0.625	2.5	8.375	18.875	26	68

Temperature (ºC)	**Mites In Adult Bees (per 100 adult bees)**		**Mites in Broods (per 100 Broods)**		**Mites in Debris**	
	With Apistan	Without Apistan	with Apistan	Without Apistan	With Apistan	Without Apistan
30 (May)	0	0.5625	2	2.75	3	3
31.5 (June)	0	1.3125	3	4	7	16
30.5 (July)	0	0.8125	2.25	3.75	15	31
28.7 (August)	0.375	1	2	3.375	8	41
28.1 (September)	0.563	1.3125	2.125	21.75	12	46
29.1 (October)	0.625	2.5	8.375	18.875	26	68

Appendix 2: Identification Keys

There are 4 currently known species which can be morphologically and biogeographically separated as such:

S. N.	Characteristics	*T. mercedesae*	*T. clareae*	*T. thaii*	*T. koenigerum*
1.	**Known hosts**	*Apis dorsata dorsata, A. mellifera, A. laboriosa, A. cerana*	*A. dorsata breviligula; A. d. binghami; A. mellifera*	*A. laboriosa*	*A. dorsata dorsata; A. laboriosa*
2.	**Known localities**	Mainland Asia and Indonesia except Sulawesi; mountains near Himalayas; New Guinea	Philippines and Sulawesi (except Palawan Island), Luzon Island	Only known to date from Vietnam	Sri Lanka, Mainland Asia and Indonesia except Sulawesi
3.	**Size (Length x Breadth)**	Female: 0.978mm x 0.542mm Male: 0.920mm x 0.523mm	Female: Female: 0.881mm x 0.485mm Male: 0.856mm x 0.501 mm	Female: 0.890 mm x 0.4 91mm Male: male unknown	Female: 0.693mm x 0. 427mm Male:0.575mm x 0.384mm
4.	**Female Epigynial Plate**	Bluntly pointed to sharply pointed apex; parallel to flanged sided	Bluntly pointed apex, almost parallel sided	Bluntly pointed apex, slightly bell shaped sided	Rounded apex, pear shaped sided
5.	**Female Chelicerae Shape**	Subapical tooth present	Subapical tooth present	Lacks subapical tooth	Subapical tooth present
6.	**Male chela spermo dactyl shape**	Long and attenuate with distal spirally coiled apex	Long and attenuate with distal spirally coiled apex	Male unknown	Short and with pig-tailed-like end loop

Source: Anderson and Morgan (2007)

Appendix 3: Photograph of study site and collection of sample

Photograph 1: Study site Natural History Museum Premises, Swayambhu, Kathmandu, Nepal

Photograph 2: Applying "Sugar shake" method

Photograph 3: Adult honey bees covered with sugar powder

Appendix 4: Photographs of mites being obtained after and removal of brood

Photograph 4: Collection of mites from broods

Appendix 5: Photograph of mites being observed in debris on white formica sheet

Photograph 5: Collection of mites from debris