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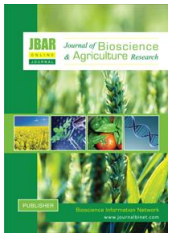

Management of ecto-parasitic mite, *Tropilaelaps mercedesae* Anderson and Morgan in honeybee, *Apis mellifera* Lin. colonies in the mid-hill areas of Nepal

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ABSTRACT

The ecto-parasitic mite, Tropilaelaps mercedesae Anderson and Morgan is one of the major limiting factors for both subsistence and commercial Apis mellifera Lin. beekeeping in Nepal. Its management by the beekeepers with chemical application regularly causes resistance in mite and contamination in bee products. So, an investigation was undertaken to explore the acaricidal properties of commonly available organic acid, plant and animal products as alternative methods for honeybee mite management. The ten-frame Langstroth colonies [7-frame honeybees with 5-frame brood] were randomly selected and divided into five groups (treatments) with four colonies in each treatment. The treatments were: formic acid (FA) (65% @ 3 ml/frame), glucose powder (GP) (@ 4 g/frame), neem seed powder (NSP) (@ 3 g/frame), 100% cow urine solution (CUS) (@ 5 ml/frame), and control from third week of May to first week of July in the mid-hill areas of Lalitpur district for two consecutive years, 2017-018. The honeybee mite infestation was determined in randomly selected fifty sealed worker brood cells on 1st, 3rd, 6th, 10th and 15th day. The parameters as percent brood infestation by honeybee mite, percentage reduction of mite infestation, efficacy of different treatments, median lethal time in days (LT_{50}) of the treatments and survivable of honeybee mites in different treatments were recorded. The population of mites before the treatment application was found non-significant, while after treatment application mite population varied significantly among the treatments. However, the interaction between year and treatments during entire observation dates were non-significant. In case of honey yield, it was highly significant among the treatments with the highest yield in FA treated colonies. The highest honeybee mite count on brood cells was observed on control colonies (37.37) and the lowest on FA treated colonies (3.62) followed by CUS (7.87), NSP (11.0) and GP (23.38), respectively, on the 15th day after the treatment. Similarly, the percent reduction of honeybee mite was noticed the highest in FA (91.01%) followed by CUS (81.02%), NSP (71.72%) and GP (40.85%) treatments, respectively. Among the acaracidal material evaluated against honeybee mite, the efficacy of FA was found better which increased from 43.08% on the 1st day to 90.52% on the 15th day of observation. All these evidences indicate superiority of FA over other materials investigated for the management of honeybee mite. This was further strengthened with the lowest LT_{50} value of FA (0.72 days) as compared to other treatments, NSP (0.80 days), CUS (1.34 days) and GP (2.56 days). All these evidences elucidate the use of FA for the sustainable management of honeybee mite, T. mercedesae in A. mellifera colonies, whereas

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application of CUS and NSP are also advisable in the mid-hill areas. FA does not contaminate honey as it is volatile, but precaution should be taken during its application.

Key Words: *Beekeeping, Apis mellifera, Honeybee mite (Tropilaelaps mercedesae), Management and Non-chemical control*

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I. Introduction

Beekeeping in Nepal is a highly potential sector, as income generation, employment opportunity and exportable commodity. The occurrence of diversified floral resources and suitable climatic conditions has shown tremendous possibility for honeybee enterprises. The presence of both domesticated honeybee species, *Apis cerana* Fab. and *Apis mellifera* Lin. as well as other wild native species *Apis florea* Fab. *Apis dorsata* Fab. and *Apis laboriosa* Smith have generated opportunity at various levels. A study has estimated that that Nepal could produce more than 10,000 mt of honey annually from one million honeybee colonies (Singh, 2012; Pokhrel et al. 2014). Currently the country possesses about 240,000 (including estimated natural honeybee colonies) colonies with 3,950 mt of annual honey production (MoALD, 2017), of which, about one-fourth amount of honey is consumed at domestic market (Singh, 2012) with considerable amount used in traditional natural medicine, sweetening material as well as during numerous religious occasions. In addition, there have been exports of both processed and unprocessed natural honey (MoALD, 2017). Not only honey, other hive products, such as propolis, pollen, beewax, royal jelly also have important role in uplifting the economic level of the farmers. The pollination is also one of the fundamental issues which have been in large scale mitigated by honey bee population.

The traditional beekeeping with native honeybee, *A. cerana* is a common practice among Nepalese farmers, but the industry flourished with the introduction of European honeybee, *A. mellifera* which was officially introduced in April 1994 (Bista and Shivakoti, 2001). The *A. mellifera* colonies possess many desirable honey production traits and are well-acclimatized from mid-hills to sub-tropical plain areas. Besides retaining the basic requirements for *A. mellifera* cultivation as favorable climate with rich and varied bee floras, but the national productivity is far low behind anticipated. The annual honey productivity at national level is 25 to 30 kg/colony from its production potential of 70 to 80 kg/colony (Kafle, 2012; Singh, 2012; Pokhrel et al. 2014). Among the various factors responsible for this, prevalence of honeybee pests and lack of appropriate management practices are the major ones. Two species of mites, *Varroa* and *Tropilaelaps* are identified as major pests of honeybees in Nepal and many authors have advocated for their timely management for the survival of colonies (Bista and Shivakoti, 2001; Dawadi, 2003; Thapa and Dawadi, 2004; Bista et al. 2004; Pokhrel, 2008; Neupane, 2009; Kafle, 2012).

Burgett and Akratanakul during 1985 predicted that in the coming years the destruction on commercial beekeeping by *Tripolaelaps clareae* Delfinado and Baker will be far greater than caused by other two mites, *Varroa jacobsonii* Oudemans and *Acarapis woodi* Rennie. The major reason behind this statement may be the biological factor that differs between *Tropilaelaps mercedesae* Anderson and Morgan (Arachnida: Mesostigmata: Laelapidae) and *Varroa destructor* Anderson and Trueman. *T. mercedesae* mites are phoretic on adult bees for 1-2 days (Woyke, 1987) whereas much longer phoretic phase (average 27 days or more) occur on *V. destructor* mites (Ruijter, 1987) giving faster reproductive cycles and as a result higher population growth rate in *T. mercedesae* than *V. destructor* (DEFRA, 2005). After the introduction of A. mellifera into Asian region, the cross-infection of *Tropilaelaps* mite occurred from its original host, *A. dorsata* that resulted in considerable losses to commercial beekeeping (DeJong et al. 1982; Bailey and Ball, 1991). Heavy losses of colonies and decline in honey production has been reported from neighboring countries, China (Youguan et al. 2000), Pakistan (Mahmood et al. 2014), India (Atwal and Goyal, 1971), and Afghanistan (Woyke, 1984) demanding appropriate control

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measures. Many authors in Nepal, also reported the highest incidence and colony losses due to *T. mercedesae* and referred as major threat to *A. mellifera* beekeeping (Dawadi, 2003; Bista et al. 2004; Pokhrel, 2008; Neupane, 2009).

Currently, the management of honeybee mite complex in Nepal relies on chemicals, like sulphur, some antibiotics and formic acid, while most of the commercial beekeepers use synthetic pyrethroid acaracides as Apistan (a.i. fluvalinate) and Bayvarol (a.i. flumethrin) (Bista and Shivakoti, 2001; Thapa and Dawadi, 2004; Pokhrel, 2008; Pokhrel et al. 2014). The continuous application of these chemicals in the colony contaminates bee products and its side effects can create new problems. The use of sulphur may cause sub-lethal residues to accumulate in honey and beeswax (Putatunda et al. 2001). Also, the development of resistance in mites by these chemicals have been reported from many parts of the world (Lodesani et al. 1995; Pettis, 2004) thereby significantly decreasing the efficacy of these chemicals for the management of honeybee mites (Lodesani et al. 1995). Furthermore, the considerable amount of pesticide residues by these chemicals also has been detected from honey, beeswax and other hive products (Bogdanov et al. 1998; Wallner, 1999). These circumstances have created major concern worldwide on the trade of honeybee products affecting the beekeeping industry.

So, there is an earnest need to find out the sustainable options for the management of these parasitic mites in honeybee colonies. Many reports have advocated that this could be conceived through the utilization of natural products as organic acids, essential oils and plant extracts. The organic acids and essential oils are relatively safe as they cause lower health hazards to both honeybees as well as to human beings (Bogdanov, 2006), also nowadays there is an increasing interest in the utilization of natural pesticides derived from plants, animals and micro-organisms (Isman and Machial, 2006) because they are largely safer as compared to the synthetic insecticides. So, this investigation was undertaken to explore the acaricidal properties of some commonly available organic acid, plant and animal products against parasitic honeybee mite.

II. Materials and Methods

This investigation was carried out from third week of May to first week of July at Lalitpur district, representing central mid-hill areas of Nepal. The study was conducted for two consecutive years, 2017 and 2018 on European honeybee, *Apis mellifera* Lin. in ten-frame Langstroth beehives.

Study area: The study was undertaken in beekeeper's apiary situated at Dhapakhel, Lalitpur Metropolitan City-24 (1343-masl; N-27°38.17" and E-085°19.39"), mid-hill areas of Lalitpur district, Nepal. The location is surrounded by agricultural land with running water and natural vegetation with settlement areas nearby. *A. mellifera* is the dominant species, while few beekeepers also rear *A. cerana* colonies. Due to accessibility of transport, migration of colonies is common during autumn and early spring season. While during rainy season, most of the beekeepers place their colonies nearby their residence. Mustard, maize, buckwheat, horticultural trees, ornamental plants and some plantation trees are major honeybee floras available around the areas.

Arrangement of study materials: The *A. mellifera* honeybee colonies for the experiment were randomly selected from the apiary during the 2nd week of March both years. Twenty-five colonies were kept at least in 5m distance facing eastern side, re-queening was done on the 2nd week of March and 7 frame honeybees with 5 frame broods were maintained at the starting of experiment both years. An additional five colonies were kept on the side of apiary without any treatment for natural infestation of honeybee mite, which were used as source of mite infestation in the experimental colonies.

The investigation was carried out during early summer, after spring honey harvesting, from the 21st May to 4th July in 2017 and from the 22nd May to 5th July in 2018, which is one of the major mite incidence period (Thapa and Dawadi, 2004). The colonies were divided into five groups (treatments) with four colonies (replications) per treatment. The 1st group of four colonies was treated with 65% formic acid (@ 3 ml/frame); the colonies of the 2nd group received glucose powder (@ 4 g/frame), the 3rd group was

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treated with neem seed powder (@ 3 g/frame) while the four colonies of the 4th group was sprayed with 100% cow urine solution (@ 5 ml/ frame) and the 5th group served as control (untreated).

Preparation of treatments and mode of application: Formic acid was purchased from market, the measured quantity of formic acid (FA) was poured in a glass vial closed with cotton plug and placed at the bottom board of the experimental colonies. Glucose powder (GP) was purchased from market and 4 g of powder was dusted at the top part of each frame. Cow urine (CUS) was collected from local breed of cow, filtered and the measured quantity was sprayed (100% pure) using plastic sprayer at the top of honeybee frames uniformly. Neem seed powder (NSP) was prepared by grinding the collected neem seed and 3 g powder was dusted at the top part of each frame. In control colonies, no treatment was applied. All the treatments were applied during evening time.

Field experiment: On the first day of experiment, acaricide Apistan (a.i. fluvalinate) at the rate of one strip per colony was placed in all experimental colonies for three days to control prevailing honeybee mites. On the fourth day, the acaricide (Apistan) was removed and colonies were left for next five days to maintain uniformity. Then honeybee mite infested bee-frame (@ 1 frame/experimental colony) was placed at the center on the 9th day. Thereafter all the colonies were left for next twelve days for population build-up of honeybee mites.

On the 21st and 23rd day, the pre-treatment assessments of *T. mercedesae* mite were carried out in all experimental colonies, and the treatments were applied on the next day. The treatments on all experimental colonies were applied three times at three days interval (i.e. on 24th, 27th and 30th day). The presence of *T. mercedesae* mite on honeybee brood was determined five times post-treatment (i.e. on 32 th, 34 th, 37 th, 41th and 46th day).

Observation of honeybee mite: The infestation of honeybee mite, *T. mercedesae* in the studied colonies during the experimental period was determined in worker honeybee brood cells. For sampling the mite population, two frames with sealed brood were selected from each colony 50 sealed worker brood cells were randomly selected and thoroughly examined (Anderson and Roberts, 2013).

Each selected sealed brood cell was uncapped using pointed forceps and the immature stages inside was carefully placed at the petri-dish using forceps and fine brush. The adult mites present on the immature stages examined and noted. Also, the sides of the brood cells and removed caps were inspected for the presence of mite. The counted mites were kept at glass vial containing 70% ethyl alcohol using fine brush and brought to Entomology Division taxonomy laboratory, NARC for further study. The studied honeybee frames were immediately returned to the colonies after being examined. At the final stage of experiment, the honey stored at the colonies was harvested on 48th day with the help of manual honey extracting machine to compare the honey yield among different treatments.

Data processing and statistical analysis: The average count of honeybee mite on the honeybee brood during pre-treatments and post-treatments as well as the honey produced during both years were calculated. Then percent brood infestation by honeybee mite at different observation dates was calculated following the formula given by Tiwari and Mall (2011). Similarly, the percentage reduction of honeybee mite infestation over control due to the effect of different treatments was assessed following the equation given by Henderson and Tilton (1955). The efficacy of different treatments on percent brood infestation by honeybee mite, *T. mercedesae* on *A. mellifera* colonies was evaluated as mentioned in the guidelines on assessment of parasitic drugs (Woyke, 1985).

The data were square root transformed wherever necessary and ANOVA was performed to compare the reduction of honeybee mite population and the efficacy of different treatments applied and the interactions between them. Significance means of the honeybee mite population as efficacy of treatments both years were separated using Tukey's Studentized Range Test (HSD) at 0.05% significance level (SPSS 16.0, SPSS Inc. Chicago, IL, USA). The median lethal time in days (LT_{50}) at 95%

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confidence limit of the honeybee mite population treated with different materials was calculated by probit analysis (SAS Institute, 2013).

III. Results and Discussion

Efficacy of treatments

The average honeybee mite, *T. mercedesae* population on *A. mellifera* brood was assessed before and after the application of different commonly available acaricidal materials. The honeybee mite population of both pre-treatments counts, and their interactions were not significantly different ($0.05 \leq p$). The population of mites before the treatment application was found nearly homogenous at first ($F_{4, 40}=0.261$, p=0.900) and second ($F_{4, 40}=2.209$, p=0.092) counts, while mite count was found highly significant on the 1st ($F_{4, 40}=22.26$, p=<0.001), 3rd ($F_{4, 40}=57.327$, p=<0.001), 6th ($F_{4, 40}=169.76$, p=<0.001), 10th ($F_{4, 40}=209.80$, p=<0.001) and 13th ($F_{4, 40}=148.43$, p=<0.001) day after treatment. Treatment effects on mite population between the years after 1st and 3rd day of application was found to be non-significant while in remaining days it varied significantly. In contrast, the interaction between year and treatments during entire observation dates were found non-significant (Table 1). In case of honey yield, it was observed highly significant among the treatments ($F_{4, 40}=36.89$, p=<0.001) but the interaction between the years and treatment was found non-significant ($F_{4, 40}=0.31$, p=0.872) (Table 01).

Table 01. Mean number of honeybee mite, *T. mercedesae* in *A. mellifera* brood as pre- and post-treatments of different acaricidal materials and honey yield in the mid-hill areas of Lalitpur district, Nepal

Treatments	Pre-treatment mite (No)		Post-treatment mite (No)					Honey yield (kg/colony)
	First	Second	Day 1	Day 3	Day 6	Day 10	Day 15	
Formic acid (FA)	29.25 ± 1.75	32.88 ± 0.95	16.75 ± 0.59 c	9.38 ± 0.77 d	5.25 ± 0.59 d	3.12 ± 0.58 d	3.62 ± 0.65 d	1.36 ± 1.09 a
Glucose powder (GP)	28.0 ± 1.92	31.5 ± 1.52	22.12 ± 1.07 b	19.62 ± 1.16 b	18.75 ± 1.12 b	21.37 ± 1.19 b	23.38 ± 1.60 b	0.64 ± 0.78 c
Neem seed powder (NSP)	27.88 ± 1.75	30.88 ± 0.97	20.0 ± 0.73 bc	14.37 ± 0.75 c	11.37 ± 0.75 c	10.5 ± 1.19 c	11.0 ± 1.76 c	0.97 ± 0.97 b
Cow urine solution (CUS)	28.75 ± 1.44	33.0 ± 1.41	21.5 ± 1.16 b	13.5 ± 1.43 cd	10.0 ± 0.70 c	9.25 ± 0.94 c	7.87 ± 0.58 c	0.98 ± 0.79 b
Control	26.88 ± 1.51	29.0 ± 0.94	29.62 ± 1.06 a	32.75 ± 1.38 a	33.0 ± 1.01 a	36.5 ± 1.19 a	37.37 ± 1.40 a	0.39 ± 0.35 d
Year	0.419 ns	0.167 ns	0.912 ns	0.433 ns	0. 014*	0.001**	0.002 **	0.834 ns
Treatments	0.900 ns	0.092 ns	<0.001 **	<0.001 **	<0.001 **	<0.001 **	<0.001 **	<0.001 **
Year x Treatments	0.903 ns	0.100 ns	0.835 ns	0.862 ns	0.919 ns	0.417 ns	0.315 ns	0.872 ns

Data pooled of two year (2017-2018). Means with same letter for mite incidence are not significantly different ($P \leq .005$) by Tukey's test; Honey yield of 25-experimental days calculated; ** = highly significant, *=significant, ns = non-significant; ± Standard error

The highest honeybee mite count on brood cells was observed on control colonies (29.62) and lowest on FA treated colonies (16.75) followed by NSP (20.0), CUS (21.5) and GP (22.12), respectively during the 1st day after treatment applications (Table 01). Similarly, during rest of the days of observation the presence of parasitic mite on honeybee brood was higher on control colonies followed by the colonies treated with glucose powder. Although the population of honeybee mite was somewhat lower in CUS treated colonies in comparison to NSP, but the result of mite infestation in both treatments were at par from 3rd to 15th days of observation. Among the treatments applied, the population of mite on honeybee brood was observed the lowest in the colonies treated with FA during all observation days.

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Similar result was obtained by Mahmood et al. (2011) in Pakistan where the application of FA control higher number of mite (33 to 142 with the mean of 94.12±7.21) than other treatments. Rademacher et al. (1995) applied daily on an average 17.5 g of 60% formic acid in Germany and recoded maximum mite mortality of 99.8% with no effects on queens. In Thailand, the commercial formic acid product (Mite-Away Quick Strips®) significantly reduced mite population up to eight weeks without any side effects (Pettis et al. 2017). Harold et al. (1989) found 94% of honeybee mites killed by FA after four treatments at four days intervals. Many authors have demonstrated that best results have been obtained using at least three applications of liquid FA per colony (Fries, 1989; Mutinelli et al. 1994; Eguaras et al. 1996; VanVeen et al. 1998), which is also confirmed in the present experiment with the same number of FA applied at three days interval.

The urine from animal origin and neem tree (*Azadirachta indica* A. Juss) products has insecticidal properties and recommended for the control of different insect and mite pests. Melathopoulos et al. (2000), Schenk et al. (2001) and Peng et al. (2000) stated neem products as a novel and safe method for managing honeybee mites; Dimetry et al. (2005) found that neem oil spray killed 90 to 94% of honeybee mites in honeybee colonies. The studies carried out by Tiwari et al. (2014) at different locations of Uttarakhand, India for the management of the ecto-parasitic brood mite in *A. mellifera* colonies revealed that cow urine (desi breed) @ 100% significantly reduced brood mite infestation up to 75.60%. Also, Chand and Tiwari (2012) showed rapid recovery in bacterial disease infection of honeybees within 10-14 days of *local breed* cow urine spray with growth of bee brood area and also suggested not to apply before five days of honey extraction.

Among the applied treatments, FA demonstrated the highest efficacy during entire observation dates while the GP exhibited lowest efficacy for the management of honeybee mite. The result of other two materials, CUS and NSP were nearly similar on controlling the mite population. On the 1st day after treatment application, the NSP (31.65%) was superior in efficacy to CUS (26.61%) but in latter days of observation CUS was better than NSP (Table 01). Also, the efficacy rate of CUS increased in succession (26.61 to 78.94%) during entire observation dates while in other treatments its effectiveness slightly decreased at 15th days of observation. In general, the efficacy of FA (43.08 to 90.52%) was the best in reducing the percent brood infestation in *A. mellifera* colonies (Table 01) though its effect was somewhat lower on the last day of observation (91.66 to 90.52%). This might be due to the lower quantity of FA and other treatment materials used during the study. Mahmood et al. (2011) applied 20 ml of FA four times to get *T. clareae* mite control up to four weeks in *A. mellifera* in Pakistan. Similarly, Raffique et al. (2012) reported control of *T. clareae* till five weeks with the application of five doses of 25 ml FA. With the application of 15-20 ml of CUS per frame at Uttranchal, India, Tiwari et al. (2014) found 75.6% *Varroa* mite control up to four weeks. In our investigation, tested dose of FA, CUS and NSP have shown positive results for the management of honeybee mite.

The efficacy of FA in managing the ecto-parasitic mites *Tropilaelaps* and *Varroa* in *A. mellifera* colonies have been advocated by many authors (EFSA, 2013; Anderson and Roberts, 2013; Baker, 2010; Satta et al. 2005; Raffique et al. 2012). The efficacy reported in research findings ranges from 29.6 to more than 90% depending on doses, application modalities, experimental procedures and environmental conditions (Satta *et al.* 2005). FA achieves its control activity by fumigation and its efficacy depends on its evaporation rate inside the hive, factors influenced by colony environment, its size, beehives used, type of evaporation material and FA concentration (Campolo et al. 2017). Imdorf et al. (1990) reported that environmental temperature should be between 18 and 25°C with minimum night temp higher than 12°C for an effective use of FA for the control of *V. destructor* mite. These factors in the case of our study also might have played an important role on FA evaporation and thereafter minimization of honeybee mite population.

FA is a highly volatile compound and interferes with basic metabolic and respiratory processes of honeybee mite. These are water soluble chemicals and residues are more likely to occur in honey than beeswax which tends to dissipate with time and exposure to heat (Ellis, 2001). FA occurs naturally in honey that is why the chances of mite resistance against FA are minimum (Calderone, 2000). In the past few years, the interest of beekeepers and honeybee biologists increased in the use of FA as an acaricide for honeybee mite control due to the European regulation 1804/99 on organic production that

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authorized formic acid as a natural compound in the organic apiculture standard management (Satta et al. 2005).

In accordance to this, the honey yield (1.36 in FA application and 0.39 kg/colony in control) was also recorded higher in the colonies applied with FA (Table 1). A study conducted in Chitwan, Nepal by Thapa and Dawadi (2004) for the control of *T. clareae* mite on *A. mellifera* colonies using different materials also recorded the highest honey yield (11.94 kg) with formic acid followed by Apistan (10.43 kg), queen caged (8.1 kg), sulphur treated (7.0 kg) and control colonies (6.88 kg) per colony per season and stated lower honey yield in un-treated colonies mainly due to presence of highest number of honeybee mites. Similar result with *T. clareae* mite in Pakistan was concluded by Raffique et al. (2012) where the formic acid (25 ml) group yielded maximum honey (22.54 kg/colony) as compared to the thymol (25g) group (20.7 kg/colony). A recent study carried out by Islam et al. (2016) on the management of honeybee mite (*V. destructor*) with different essential oils and FA recorded high honey yield in FA treated colonies (14.2 kg) followed by thyme (13.7 kg), lemon grass (13.0 kg), mint (13.0 kg), rosemary (12.4 kg) and in control (6.5 kg honey per hive) and expressed their view that increased mite infestation on colonies resulted in low honey yield.

Percent reduction of honeybee mite population

Table 02. Percent reduction of honeybee mite, *T. mercedesae* infestation after application of different treatments in *A. mellifera* brood in the mid-hill areas of Lalitpur district, Nepal

Treatments	Post-treatment count control of mite (%)				
	1st day	3rd day	6th day	10th day	15th day
Formic acid	48.22 ± 3.55	74.06 ± 2.26	85.87 ± 1.58	92.35 ± 1.62	91.01 ± 1.94
Glucose powder	28.06 ± 6.62	42.04 ± 6.05	45.55 ± 4.58	43.79 ± 5.14	40.85 ± 3.94
Neem seed powder	34.06 ± 6.06	57.51 ± 3.38	66.70 ± 2.99	72.54 ± 3.23	71.72 ± 5.14
Cow urine solution	33.07 ± 5.98	61.94 ± 4.67	72.29 ± 2.17	75.85 ± 3.92	81.02 ± 0.84

Data pooled of two year (2017-2018); ± Standard Error.

The percent reduction of mite gradually increased from the day of treatment application to the later observation dates. In the colonies treated with FA and NSP, increment in percent reduction of mite population was found up to 10th day of observation while in GP treated colonies the increment remained only up to 6th day. The colonies applied with CUS showed successive increase of percent honeybee mite reduction (Table 02). Among the different treatments applied, the percent reduction of honeybee mite was noticed the highest in FA treatment followed by CUS, NSP and GP, respectively. The maximum reduction in mite population was observed in FA applied colonies at the 10th day (92.35 ± 1.62), whereas the lowest reduction was found in GP treatment colonies at the 15th day (40.85 ± 3.94) of observation (Table 02). Mutinelli et al. (1994) documented 65% FA applied at weekly interval caused 89.6- 94.3% mortality of mites during the second half of August and if applied up to 3 months could control mites up to 96.6% without side effects on honeybees.

Many authors have documented that FA has strong acaricidal effect and if applied in recommended dose will not adversely affect the colony health. The studies on colony health showed that long term FA treatment did not harm brood and young bees with no loss of queen and also did not limit colony development (Garg et al. 1984; Sharma et al. 1994; Bernie and Winston, 1998; Westcott and Winston, 1999). During our study we did not observe any losses of queen, damage on honeybee brood and adverse effect on honeybee behavior. Also, the colony development activities were normally functioning. These observations are further supported by Elzen et al. (2000) who found no damage in existing queen or queens in supersedure stages. Rashid et al. (2011) also reported no honeybee mortality with the application of 20 ml FA for four weeks period. Similarly, Mutinelli et al. (1996) in

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their study reported low or no mortality of honeybees in all tests of formic acid, lactic acid or Apilife-VAR®.

Median lethal times and survivability of honeybee mite

Table 03. Median lethal times in days (LT_{50}) at 95% confidence limit of the honeybee mite, *T. mercedesae* population treated with different materials in *A. mellifera* colonies in the mid-hill areas of Lalitpur district, Nepal

Treatments	LT_{50} (Day)	95% confidence limit		χ2	df	*P*-value
		Lower	Upper			
Formic acid	0.715	0.301	0.925	5.217	9	<0.0001
Glucose powder	2.565	1.39	7.422	11.02	34	<0.0001
Neem seed powder	0.799	0.149	1.449	8.07	15	<0.0001
Cow urine solution	1.343	0.681	2.016	11.78	15	<0.0001

Data pooled of two year (2017-2018); *n*=1490.

The median lethal time of honeybee mite population in *A. mellifera* brood area was evaluated after application of different materials with acaricidal properties. The investigation described the lowest median lethal time in formic acid applied honeybee colonies (0.72 days) closely followed by neem seed powder treated colonies (0.80 days). The LT_{50} value was observed the highest in glucose powder treated colonies (2.56 days), while the cow urine solution applied colonies (1.34 days) displayed intermediary result between neem seed powder and glucose powder treated honeybee colonies (Table 03). This once more illustrated the superiority of formic acid treatment in comparison to other materials investigated for the management of honeybee mite, *T. mercedesae* in *A. mellifera* colonies.

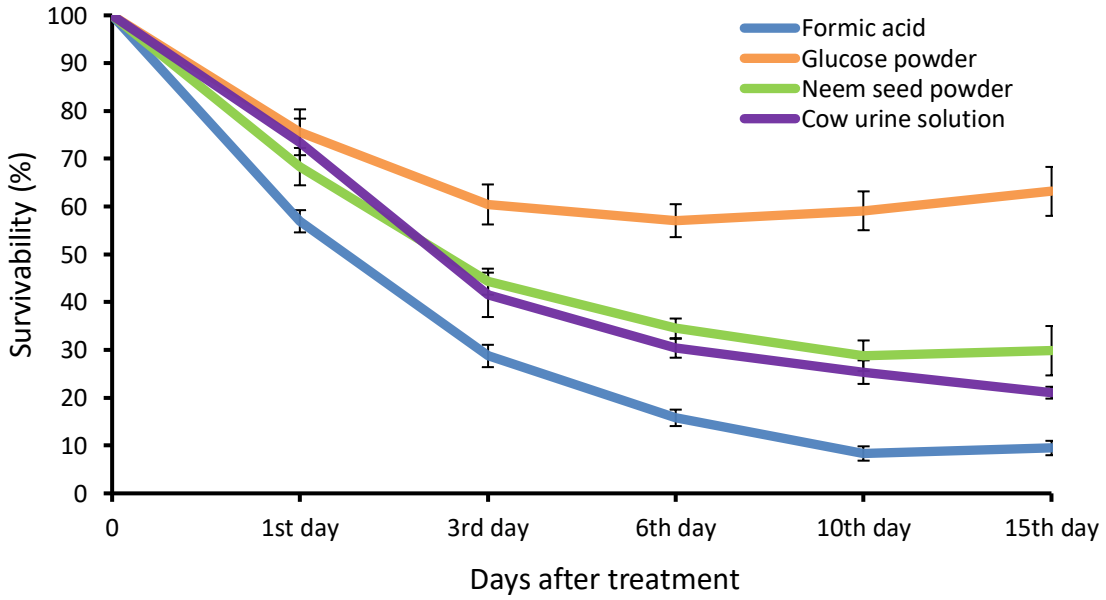

Figure 01. Survival curve of honeybee mite, *T. mercedesae* in honeybee, *A. mellifera* colonies (broods) after application of different treatments up to 15 days (Data pooled of two year, 2017-2018; Bar represents standard error)

The survival of honeybee mite after application of different acaricidal treatments on honeybee brood was assessed up to 15 days. The colonies treated with GP (63.17%) showed the highest survivability of honeybee mite followed by NSP (29.84%) and CUS (21.06%) treated colonies, respectively up to 15th day of observation (Figure 01). The highest mortality of honeybee mite was found in FA applied colonies where the population declined to 56.92% on the 1st day and to 9.48% on the last day of observation. The survival of mite was lower in NSP treated colonies on the 1st day after treatment application than CUS, while in latter observations the survivability was observed higher with NSP application. Among all the treatments evaluated in the honeybee colonies, the survivability of honeybee mite was observed minimum in the FA applied colonies at all observation dates. The chances in increment of certain

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number of honeybee mite are always possible through drifting process during the study period which might influence the study results slightly. The ecto-parasitic mites of honeybee possess parasitic and phoretic stages in their life cycle. In comparison to the phoretic stages of *Varroa* (average 27 days or more), the *T. mercedesae* mite are usually phoretic on adult honeybee for 1 to 2 days only (Ruijter, 1987; Rath, 1991; Calatayud and Verdu, 1995). During its short life cycle of about a week, the *T. mercedesae* mite spends most of its time as parasitic stage in the sealed brood colonies (Woyke, 1987; Kavinseksan, 2003).

Many studies have demonstrated that FA has a strong acaricidal effect. The main advantage of FA compared to other acaricides is that it kills honeybee mites both on adult bees and inside the capped brood (Calderon et al. 2000). The other benefits to the beekeepers with FA fumigation is that it also provides control for other honeybee parasites, including the honeybee tracheal mite, *A. woodi* (Wilson et al. 1993; vanEngelsdorp and Otis, 2001), *V. destuctor* (Hoppe *et al.* 1989; Sharma et al. 2003) and possibly nosema disease (*Nosema apis* Zander) (Hoppe et al. 1989; Sharma et al. 2003; Underwood and Currie, 2004). The efficacy of "soft" acaricides for the control of ecto-parasitic mites in honeybee colonies are generally recommended effective as well as with no detrimental effects on colony development and honeybee product quality. The formic acid, oxalic acid, lactic acid, thymol and plant essential oils are generally used as soft acaricides with varying degree of success for the management of honeybee mites. Among these acaricides, the formic acid is locally availability, low price as well as familiar to the beekeepers in Nepal. Are advantage

IV. Conclusion

Nepal is rich in honeybee diversity and the ecto-parasitic mites are associated with these native species since long time without exceeding their injury level. After the introduction of European honeybee, *A. mellifera*, the mites successfully transferred from its indigenous host to *A. mellifera*. As a result, the *Varroa* mite achieved a pest status worldwide and *Tropilaelaps* especially in Asia, but the victim in both the geographical regions is *A. mellifera*. The relatively rapid developmental period of *Tropilaelaps* establishment and reproduction in the brood cells concern many beekeepers and researchers because of its prompt population buildup that lead to the sudden collapse of the colonies. The use of chemical acaracides is the general control method applied by the beekeepers for the management of these ecto-parasitic mites in Nepal. The problem of continuous dependence on these acaricides resulted resistance in mite and residue in honey. For the sustainable management of parasitic mites in honeybee colonies, the control strategies should be directed in the use of natural products, organic acids, plant extracts and essential oils. The FA, GP, NSP and CUS were investigated against the honeybee mite, *T. mercedesae* for their efficacy in different parameters. Among these materials, FA reduced the *T. mercedesae* population at substantial level in the lowest median lethal time with the highest honey yield. The performance of CUS and NSP also provided significant results that could be used for the management of honeybee mite. Although the domestic and international markets are promising for honey, but to make it acceptable the quality must meet appropriate standards. This could be achieved through an integrated management practices for honey production where issues concerning honeybee mite management have to be addressed appropriately.

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Author Contributions

S.B. planned the research, conducted the study, analyzed the data and wrote the manuscript; R.B.T. G.B.K. S.B.P. and Y.N.G. assisted on planning and conducting the research work; S.A. assisted in analyzing the data and on preparing the paper.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

V. References

[1]. Anderson, D. L. and Roberts, J. M. K. (2013). Standard methods for *Tropilaelaps* mites research. Journal of Apicultural Research, 52(4), 1-16.

[2]. Atwal, A.S. and Goyal, N.P. (1971). Infestation of honeybee colonies with *Tropilaelaps clareae* and its control. Journal of Apicultural Research, 10(3), 137-142.

[3]. Bailey, L and Ball, B. V. (1991). Honey bee pathology, 2nd Edition. Academic, San Diego, USA.

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

[5]. Bernie, L. and Winston, M.L. (1998). Chemical acaricides in *Apis mellifera* (Hymenoptera: Apidae) colonies: do they cause non-lethal effects? The Canadian Entomologist, 131, 1-9.

[6]. Bista, S. and Shivakoti, G.P. (2001). Major constraints and management practices in the performance of *Apis cerana* F. and *Apis mellifera* L. in Kathmandu valley. In: Advances in agricultural research in Nepal, Proceedings of the 1st Society of Agricultural Scientists, Nepal Convention, March 29-31, 1999, Kathmandu, (Manandhar, H.K., Shrestha, C.L., Shrestha, R.K. and Pradhan, S.M. Eds.) Society of Agricultural Scientists (SAS), Nepal. pp. 190-194.

[7]. Bista, S., Aryal, S., Paneru, R.B. and Giri, Y.P. (2004). Performance study of European honeybee, *Apis mellifera* L. on different management practices. In: The Proceedings of 4th National Conference on Science and Technology, 23-26 March 2004. Nepal Academy of Science and Technology, Lalitpur, Nepal, Nepal. pp. 535-541.

[8]. Bogdanov, S. (2006). Contaminants of bee products. Apidologie, 37, 1-18.

[9]. Bogdanov, S., Imdorf, A. and Kilchenmann, V. (1998). Residues in wax and honey after Apilife VAR treatment. Apidologie, 29, 513-524.

[10]. Burgett, D.M. and Akratanakul, P. (1985). *Tropilaelaps clareae*, the little known honey bee brood mite. American Bee Journal, 125, 112-114.

[11]. Calatayud, F. and Verdu, M.J. (1995). Life expectancy of the mite *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) in colonies of the honey bee *Apis mellifera* L. during brood rearing periods. Invest. Agr. Prod. Sanid. Anim. 10: 153-161.

[12]. Calderone, R.A., Ortiz, R.A., Arce, H.G., van Veen, J.W. and Quan, J. (2000). Effectiveness of formic acid on *Varroa* mortality in capped brood cells of Africanized honeybees. Journal of Apicultural Research, 39, 177-179.

[13]. Calderone, N.W. (2000). Effective fall treatment of *Varroa jacobsoni* (Acari: Varroidae) with a new formulation of formic acid in colonies of *Apis mellifera* (Hymenoptera: Apidae) in the northeastern United States. Journal of Economic Entomology, 93(4), 1065-1075.

[14]. Campolo, O., Malacrinò, A., Laudani, F., Algeri, G.M., Giunti, G., Strano, C.P., Zoccali, P. and Palmeri, V. (2017). Field efficacy of two organic acids against *Varroa destructor*. Entomologia Generalis, 36(3), 251-260.

[15]. Chand, A. and Tiwari, R. (2012). Efficacy of cow urine against bacterial disease, European foulbrood, in honeybee, *Apis mellifera* (L.) colonies at different locations of Uttrakhand, an eco-friendly and novel approach. International Journal of Basic and Applied Sciences, 1(3), 179-189.

[16]. Dawadi, D.P. (2003). Incidence and control of parasitic mite, *Tropilaelaps clareae* Delfinado and Baker in *Apis mellifera* L. M.Sc. Ag. Thesis. Tribhuvan University, Nepal.

[17]. DEFRA. 2005. *Tropilaelaps*: parasitic mites of honey bees. UK Department for environment, Food and Rural Affairs, London, UK. pp. 14.

[18]. De Jong, D., Morse, R.A. and Eickwort, G.C. (1982). Mite pests of honey bees. Annual Review of Entomology, 27, 229-252.

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[19]. Dimetry, N.Z., Abdel, T.E., El-Wahab and Zakaria, M.E. (2005). Effective control of *Varroa* mite *Varroa destructor* Anderson and Trueman infesting honey bee colonies *Apis mellifera* by some natural products. Bull Fac. Agric. Cairo Univ. 56, 295-308.
[20]. EFSA (European Food Safety Authority). (2013). Scientific opinion on the risk of entry of *Aethina tumida* and *Tropilaelaps* spp. in the EU. EFSA Journal, 11(3), 3128.
[21]. Egraras, M., Quiroga, S and Garcia, C. 1996. Organic acid in the control of *Varroa jacobsoni* Oud. Apiacta, 31, 51-54.
[22]. Ellis, M. (2001). Chemical control of *Varroa* mites. In: Mites of the Honey Bee, (Webster, T.C. and Delaplane, K.S., Eds.). Dadant and Sons, Hamilton, pp. 179-196.
[23]. Elzen, P.J., Baxter, J.R., Spivak, M. and Wilson, W.T. (2000). Control of *Varroa jacobsoni* Oud resistant to Fluvalinate and amitraz using coumaphos. Apidology, 31, 437-441.
[24]. Fries, I. 1989. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. Swed. J. Agric. Res., 19, 213-216.
[25]. Garg, R., Sharma, O.P. and Dogra, D.S. (1984). Formic acid: an effective acaricide against *Tropilaelaps clareae* Delfnado and Baker (Laelaptridae: Acarina) and its effects on the brood and longevity of honey bees. American Bee Journal, 124, 736-738.
[26]. Harold, H., Wolfgang, R. and Elizabeth, S.W. 1989. The control of parasitic bee mites: *Varroa jacobsoni*, *Acarapis woodi* and *Tropilaelaps clareae* with formic acid. Am. Bee J., 129, 739-742.
[27]. Henderson, C.F. and Tilton, W. (1955). Tests with acaricides against the brown wheat mite. Journal of Economic Entomology, 48, 157-161.
[28]. Hoppe, H., Ritter, W. and Stephen, E.W.C. (1989). The control of parasitic bee mites: *Varroa jacobsoni, Acarapis woodi* and *Tropilaelaps clareae* with formic acid. American Bee Journal, 129, 739-742.
[29]. Imdorf, A., Kilchenmann, V., and Maquelin, C. (1990). Optimal use of formic acid. Schweizerische Bienen-Zeitung, 113(7), 378-385.
[30]. Islam, N., Muhammad, A., Haq, E.U., Stephen, E. and Naz, F. 2016. Management of *Varroa destructor* by essential oils and formic acid in *Apis mellifera* Linn. Colonies. Journal of Entomology and Zoology Studies, 4(6), 97-104.
[31]. Isman, M.B. and Machial, C.M. (2006). Pesticides based on plant essential oils: From traditional practice to commercialization. In: M. Rai & Carpinella, M.C. (Eds): Naturally Occurring Bioactive Compounds: Advances in Phyto-medicine, 3, 29-44.
[32]. Kafle, G.P. (2012). Bees and beekeeping [Mauri Tatha Mauripalan (In Nepali language)]. Api-Net Nepal, Sanepa, Lalitpur, Nepal. (Pg 101) 294+9.
[33]. Kavinseksan, B. (2003). Defense mechanisms of *Apis dorsata* Fabricius and ARS Primorsky honey bee *Apis mellifera* Linnaeus to the bee mite *Tropilaelaps clareae* Delfinado and Baker. PhD dissertation in biological science, Chulalongkorn University, Thailand, pp. 28-67.
[34]. Lodesani, M., Colombo, M. and Spreafico, M. (1995). In effectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud. in several districts of Lombardy (Italy). Apidologie, 26(1), 67-72.
[35]. Mahmood, R., Wagchoure, E.S., Raja, S., Sarwar, G. and Aslam, M. (2011). Effect of thymol and formic acid against ecto-parasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies. Pakistan Journal of Zoology, 43(1), 91-95.
[36]. Mahmood, R., Mohsin, A.U., Asad, S., Raja, S. and Sarwar, G. 2014. Integrated control of *Tropilaelaps clareae* and *Varroa destructor* in *Apis mellifera,* L. colonies. Scholarly Journal of Agricultural Science, 4 (1), 32-37.
[37]. Melathopoulos, A.P., Winston, M.L., Whittington, R., Higo, H. and LeDous, M. (2000). Field evaluation of neem and canola oil for the selective control of the honeybee mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae*).* Journal of Economic Entomology, 93 (3), 559-567.
[38]. MoALD. (2017). Statistical information on Nepalese agriculture, 2016-17. Ministry of Agriculture and Livestock Development, Singha Durbar, Kathmandu, Nepal.
[39]. Mutinelli, F., Cremasco, S. and Irsra, A. (1994). Formic acid in the control of Varroatosis: a practical approach. J. Vet. Med. 41, 433-440.
[40]. Mutinelli, F., Cremasco, S., Isara, A., Baggio, A., Nanetti, A. and Massi, S. (1996). Organic acids and Apilife VAR in the control of Varroosis in Italy. Apicol. Mod. 87, 99-104.
[41]. 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.

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[42]. Peng, C.Y.S., Trinh, S., Lopez, J.E., Mussen, E.C., Hung, A. and Chuang, R. (2000). The effect of azadirachtin on the parasitic mite, *Varroa jacobsoni* and its host honey bee (*Apis mellifera*). Journal of Apicultural Research, 39(3-4), 159-68.
[43]. Pettis, J.S. (2004). A scientific note on *Varroa destructor* resistance to coumaphos in the United States. Apidologie, 35, 91-92.
[44]. Pettis, J.S., Rose, R. and Chaimanee, V. 2017. Chemical and cultural control of *Tropilaelaps mercedesae* mites in honeybee (*Apis mellifera*) colonies in Northern Thailand. PLoS ONE, 12(11): e0188063.
[45]. Pokhrel, S., Shrestha, J.B. and Joshi, S.R. 2014. Suggested National Apiculture Policy, Strategy and Action Plan Nepal. 35p.
[46]. Pokhrel, S. (2008). The ecological problems and possible solutions of beekeeping in hills and terai of Chitwan, Nepal. The Journal of Agriculture and Environment, 9, 23-33.
[47]. Putatunda, B.N., Aggarwal, K. and Mathur, R. B. (2001). Honey bee mites (*Acarina*), a challenge to beekeeping industry-a review. Agriculture Review, 22(3/4), 205-214.
[48]. Rademacher, E., Polaczek, B. and Schricker, B. 1995. Control of varroosis using formic acid in applicator-II. Pszczellicze Zeszyty Naukowe, 39(11), 133-142.
[49]. Raffique, M.K., Mahmood, R., Aslam, M. and Sarwar, G. (2012). Control of *Tropilaelaps clareae* mite by using formic acid and thymol in honey bee *Apis mellifera* colonies. Pakistan Journal of Zoology, 44, 1129-1135.
[50]. Rashid, M., Wagchoure, E.S., Raja, S., Sarwar, G. and Aslam, M. (2011). Effect of thymol and formic acid against ecto-parasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies. Pakistan Journal of Zoology, 44, 45-51.
[51]. Rath, W. (1991). Investigation on the parasite mites *Varroa jacobsoni* Oud. and *Tropilaelaps clareae* Delfinado and Baker and their hosts *Apis cerana* Fabr., *Apis dorsata* Fabr. and *Apis mellifera* L. PhD dissertation, Fakulat der Rheinsischen Friedrich Wilhelms Universitat Bonn, Germany.
[52]. Ruijter, A. (1987). Reproduction of *Varroa jacobsoni* during successive brood cycles of the honey bee. Apidologie, 18, 321-326.
[53]. Sammataro, D., Gerson, U. and Needham, G. (2000). Parasitic mites of honey bees: life history, implications and impact. Annual Review of Entomology, 45, 519-548.
[54]. SAS Institute. (2013). SAS/STAT User's Guide: Statistics, Version 9.4. SAS Institute, Cary, N.C., USA.
[55]. Satta, A., Floris, I., Eguaras, M., Cabras, P., Garau, V.L. and Melis, M. (2005). Formic acid-based treatments for control of *Varroa destructor* in a Mediterranean area. Journal of Economic Entomology, 98(2), 267-273.
[56]. Schenk, P., Imdort, A. and Fluri, P. (2001). Effects of neem oil on *Varroa* mites and bees. American Bee Journal, 141(12), 878-879.
[57]. Sharma, S.D., Kashyap, N.P., Raj, D. and Sharma, O.P. (1994). Control of the ecto-parasitic mite, *Tropilaelaps clareae* Delfinado and Baker infestation with formic acid and sulphur. International Journal of Tropical Agriculture, 12, 96-100.
[58]. Sharma, S.D., Kashyap, N.P. and Desh, R. (2003). Efficacy of some acaricides against ecto-parasitic mite *Tropilaelaps clareae* infesting European honey bee *Apis mellifera.* Indian Journal of Agricultural Research, 37, 60-63.
[59]. Singh, R. (2012). Marketing network and trade scenario of natural honey. Paper presented at National Stakeholders' Participatory Workshop in Honey Value Chain, November 23, 2012, Kathmandu. Directorate of Industrial Entomology Development, Department of Agriculture, Lalitpur, Nepal.
[60]. Thapa, R.B. and Dawadi, D.P. (2004). Incidence and control of parasitic mite (*Tropilaelaps clareae* Delfinado and Baker) of the European honeybee (*Apis mellifera* F.). In: Proceedings of Fourth National Conference on Science and Technology, 23-26 March, 2004. Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal. pp. 2172-2176.
[61]. Tiwari, R. and Mall, P. (2011). Efficacy of different acaricides against honeybee mite, *Varroa destructor* (Anderson and Trueman) in *Apis mellifera* (Linnaeus) colonies in Uttarakhand. Journal of Insect Science, 24, 75-79.
[62]. Tiwari, R., Mathur, V. and Bisht, B. (2014). Efficacy of cow urine and plant products against honeybee mite, *Varroa destructor* (Anderson and Trueman) in *Apis mellifera* (Linnaeus) colonies in Uttarakhand-a novel approach. The Bioscan, 9(4), 1425-1429.
[63]. Underwood, R.M. and Currie, R.W. (2004). Indoor winter fumigation of *Apis mellifera* (Hymenoptera: Apidae) colonies infested with *Varroa destructor* (Acari: Varroidae) with formic acid is a potential control alternative in northern climates. Journal of Economic Entomology, 97, 177-186.

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[64]. vanEngelsdorp, D. and Otis, G.W. (2001). Field evaluation of nine families of honey bees for resistance to tracheal mites. The Canadian Entomologist, 133, 793-803.
[65]. VanVeen, J., Caledron, A.F., Cubero, A.M. and Arce, H. 1998. *Varroa jacobsoni* in Costa Rica: detection, spread, and treatment with formic acid. Bee World, 79, 5-10.
[66]. Wallner, K. (1999). Varroacides and their residues in bee products. Apidology, 30, 235-248.
[67]. Westcott, L.C. and Winston, M.L. (1999). Chemical acaricides in *Apis mellifera* (Hymenoptera: Apidae) colonies: Do they cause nonlethal effects? The Canadian Entomologist, 131, 363-371.
[68]. Wilson, W.T., Baxter, J.R., Collins, A.M., Cox, R.L. and Cardoso, D. (1993). Formic acid fumigation for control of tracheal mites in honey bee colonies. Bee Science, 3, 26-31
[69]. Woyke, J. (1984). Survival prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. Apidologie, 15(4), 421-434.
[70]. Woyke, J. (1985). *Tropilaelaps clareae* a serious pest of *Apis mellifera* in the tropics but not dangerous for apiculture in temperate zones. American Bee Journal, 25, 497- 499.
[71]. 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 Apiculture Research, 26(2), 104-109.
[72]. Youguan, L., Hairong, W. & Bangyk, K. (2000). A new record of parasitic mite on honey bee in China. Proceeding of the 7th International Conference on Tropical Bees and 5th AAA Conference, (March 19-25, 2000), Chiang Mai, Thailand.

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