CHAPTER III: MATERIALS AND METHODS

3.1 STUDY AREA

Survey was carried out between January 2008 and December 2011 at monthly intervals in apiaries and migratory sites where *Apis mellifera* is established. These apiaries were selected randomly from different districts across Karnataka State, India in order to obtain a better representativeness of the entire State (Figure 4; Plate 4–10). These apiaries were with agricultural, forest and non-agricultural flora. The apiaries selected for the study were from the following locations:

1. Chikkegowdanapalya (Bangalore district)
2. Kantanakunte (Bangalore district)
3. Javagal (Hassan district)
4. Arasikere (Hassan district)
5. Hiriyur (Chitradurga district)
6. Devanuru (Chikkamagaloor district)
7. Bijjahalli (Ramanagaram district)
8. Ujjanahalli (Ramanagaram district)
9. Shivanahalli (Ramanagaram district)
10. Kanakapura (Ramanagaram district)
The colonies inspected for mites were with good brooding and young, healthy queen.

3.2 SURVEY OF BEEKEEPING AREAS FOR PREVALENCE OF MITE AND THE EXTENT OF LOSSES

For this purpose, random sampling of apiaries was done. Larvae, pupae, developing brood, adult bees and debris were collected from approximately 10 colonies from each apiary. Each sample consisted of a minimum of 50 larvae, pupae and adults and 50 grams of debris. The collected samples were analyzed in the laboratory for mites and associated pathogens if any, using a stereo zoom microscope.

3.3 IDENTIFICATION AND SELECTION OF APIS MELLIFERA COLONIES

At each site, bee colonies were selected at random to study the mite prevalence. The number of colonies selected at each site varied depending on the size of each apiary (Table 1).
3.4 STUDY OF MITE DENSITY ON ADULT BEES OF APIS MELLIFERA COLONIES

The following methods were employed to collect the mites present on adult bees:

3.4.1 EXAMINATION OF ADULT HONEYBEES

To study the mite count, three samples were collected from each apiary at monthly intervals. Approximately 50 adult bees were collected in a jar, weighed and stored in 80 % alcohol. Later, weight of the colony with and without bees was recorded (Plate 11). Using a stereo zoom microscope, the number of adult mites present on the collected adult bees was recorded and average number of mites present was calculated.

3.4.2 SUGAR SHAKE METHOD

Adult bees collected from the colonies were taken in a jar with a porous lid of 3–4 mm pore size. About a tablespoon of sugar powder was added in to the jar and mixed for few minutes. Sugar powder present in the jar along with bees was dusted through the holes in the lid on a white paper. The mites detached from the bees when the bees were shaken with sugar powder.
and they were counted using a stereo zoom microscope and stored in 80 % alcohol.

### 3.4.3 STICKY BOARD OR STICKY PAPER TRAP METHOD

A sheet of white paper coated with cooking oil or petroleum jelly was placed at the bottom of the hive and covered with 8 mm mesh screen. Mites falling naturally on the sticky paper were collected and analyzed. This method proved to be very useful during summer when the colonies are broodless.

### 3.4.4 ETHER METHOD

In this technique, adult bees were collected in a jar and anesthetized with ether. The bees were then rotated in a jar for about 5–10 sec. This dislodged the mites from their hosts. The bee sample was deposited on a white paper and spread around and the number of mites were counted and recorded.
3.4.5 DRONE BROOD UNCAPPING

It is one of the methods to detect the presence of Varroa mites in the honeybee brood. To examine the mites in the brood, approximately 50 sealed brood cells were uncapped using dissection needles and the pupae were carefully removed. The pupae were cautiously examined for the presence of mites on the body surface. The inside surface of the brood cells, especially the base, were also examined for any mites. Worker pupae were examined if there were no mites on drone pupae present in the hive.

3.4.6 NATURAL MITE FALL

Falling mites were collected on a tray that covers the hive floor and protected by a mesh screen. Mite fall was recorded for a period of approximately 2 weeks to one month. Mites were counted from the floor or separated from the debris before counting. Care was taken that there was no treatment in the colony while measuring natural mite drop. The number of mites and the number of days over which they were collected were recorded.
3.5 STUDY OF MITE DENSITY IN SEALED BROOD CELLS OF APIS MELLIFERA COLONIES

To determine the mite infestation rate in the sealed brood cells, the brood area was calculated by counting the number of sealed brood cells in one square inch area and the total brood area in two frames from the infected colonies (Plate 12). By uncaping 100 cells (50 cells per frame) with a needle or Varroa fork, all stages of mites present were collected, counted and stored in 80 % alcohol.

Total number of mites in the colony was calculated by adding the number of mites in the sealed brood and mites collected from the adult bees. Data obtained from each apiary was recorded and expressed as mean number of mites per sealed brood cell and mean number of mites on adult bees.

3.6 SEASONAL VARIATION

Studies on the seasonal abundance of the mite were conducted between 2008 and 2011. Mites were collected and counted from the colonies once in a week using sugar shake method, sticky board or sticky paper trap method. Data obtained from each apiary was recorded and expressed as mean
number of mites per sealed brood cell and mean number of mites in bottom board.

3.7 CHEMICAL CONTROL OF VARROA DESTRUCTOR

Among chemical methods, treatment with formic acid (55%, 65% and 75%) and a mixture of formic acid and commercially available eucalyptus oil mixture (40:5) was carried out in 04 isolated, naturally infested colonies in Javagal apiary. The lid of the hives was removed and smoke was applied to disperse the bees. A small surgical pad was placed on top bars and formic acid-eucalyptus oil mixture was poured. The pad was left undisturbed for about 18–24 hours. Care was taken to prevent dripping of the acid. Mites were counted the very next day from 100 capped brood cells and from the bottom board.

Three to four such treatments were provided at an interval of four to seven days. Data obtained from each apiary was recorded and expressed as mean number of mites per sealed brood cell and mean number of mites in bottom board.
3.8 COMB TRAPPING

The queen was temporarily confined to a single brood frame or portion thereof and she was transferred to different combs at an interval of nine days and finally released. This method is labour intensive, slows down colony development and suitable only for a dedicated small time bee keeper.

3.9 DRONE BROOD REMOVAL

Drone brood removal is considered as a very effective method to suppress the mite population during the brood rearing season. After the cells of the drone pupae are capped, the mite infested drone brood frames were removed from the colony and the developing brood (with the mites inside the cells) were killed by freezing at 4°C for 1–3 days. After 3 days, the drone pupae were removed and the frame was returned to the colony to trap additional mites.

3.10 ANALYSIS OF DATA

All statistical analysis were made using Microsoft Excel. The results were expressed in terms of means and standard deviation for all categorical variables.
Figure 4: Map of Karnataka showing the different survey areas

Note: Map not to scale
A-Chikkegowdanapalya; B- Javagal; C- Arasikere; D- Hiriyur; E- Devanuru; F- Bijjahalli; G- Ujjanahalli
### Materials and Methods

**Table 1: Number of colonies analyzed for mite infestation at different study sites**

<table>
<thead>
<tr>
<th>District</th>
<th>Location</th>
<th>Number of colonies selected</th>
<th>Number of colonies analyzed for mite infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangalore</td>
<td>Chikkegowdanapalya</td>
<td>135</td>
<td>40</td>
</tr>
<tr>
<td>Bangalore</td>
<td>Kantanakunte</td>
<td>14</td>
<td>00</td>
</tr>
<tr>
<td>Hassan</td>
<td>Javagal</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>Hassan</td>
<td>Arasikere</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Chitradurga</td>
<td>Hiriyur</td>
<td>81</td>
<td>40</td>
</tr>
<tr>
<td>Chikkamagaloor</td>
<td>Devanuru</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Ramanagaram</td>
<td>Bijjahalli</td>
<td>140</td>
<td>45</td>
</tr>
<tr>
<td>Ramanagaram</td>
<td>Ujjanahalli</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Ramanagaram</td>
<td>Shivanahalli</td>
<td>21</td>
<td>00</td>
</tr>
<tr>
<td>Ramanagaram</td>
<td>Kanakapura</td>
<td>18</td>
<td>00</td>
</tr>
</tbody>
</table>
Plate 4: A model Apis mellifera apiary at Chikkegowdanapalya of Bangalore district
Plate 5: A model *Apis mellifera* apiary at Javagal of Hassan district
Plate 6: A model *Apis mellifera* apiary at Arasikere of Hassan district
Plate 7: A model *Apis mellifera* apiary at Hiriyur of Chitradurga district
Plate 8: A model *Apis mellifera* apiary at Devanuru of Chikkamagaloor district
Plate 9: A model *Apis mellifera* apiary at Bijjahalli of Ramanagaram district
Plate 10: A model *Apis mellifera* apiary at Ujjanahalli of Ramanagaram district
Plate 11: Determination of colony weight of *Apis mellifera*
Plate 12: Measurement of brood area in *Apis mellifera*