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Comparative evaluation of sticky paper and hive debris as sampling methods for population assessment of *Varroa destructor* in *Apis mellifera* colonies

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ABSTRACT

In the present investigation, two sampling methods for *V. destructor*; hive debris and sticky paper method was compared year around in *A. mellifera* colonies to know the viable method. Between the two methods, sticky paper method was found significantly better (CD = 0.11; p = 0.05). More mitefall was recorded in sticky paper method (17.58 mites/hive) than in hive debris sampling (11.41 mites/hive). Fortnightly data analysis showed two peaks of *Varroa;* one in second fortnight of May and other in first fortnight of July in both sampling methods. Maximum number of mites (63.27 mites/hive) was recorded in second fortnight of May in both sampling methods which was significantly higher than the mites recorded at other observation periods (CD = 0.38; p = 0.05).

Key words: *Apis mellifera,* Colony strength, Hive debris, Sticky paper method, *Varroa destructor.*

INTRODUCTION

Apiculture is derived from the honeybee's Latin name *Apis mellifera*, meaning 'honey gatherer'. Apiculture is a non-land based income generating activity and an important component of sustainable integrated rural developmental program. As Haryana has immense potential in this field, even free trainings and subsided bee colonies and bee hives are provided by HAIC (HAIC, 2013). It provides free ecosystem services in the form of cross pollination by enhancing the productivity of agricultural crops, conservation of wild flora as well as commercial applications in the form of honey, wax, pollen and royal jelly etc. Despite its great potential, beekeeping industry is facing several constraints, which needs immediate attention. Among these, *Varroa destructor* Anderson and Trueman (Mesostigmata: Varroidae), an ectoparasitic mite of brood and adult bees, is a serious pest of *Apis mellifera* L. which brought "sweet revolution" in India.

Varroa consists of at least four but possibly seven distinct species (Munoz *et al.*, 2008). It has been recently identified as one of the major factor responsible for colony losses worldwide (Martin *et al*., 2012; Nazzi *et al*., 2012). Among the four recognized species *V. jacobsoni*, *V. underwoodi*, *V. rindereri* and *V. destructor*, the most destructive and largest is *V. destructor* (Anderson and Trueman, 2000) which is 1.1 mm long and 1.7 mm broad. Several mitochondrial haplotypes (17-18) of *V. destructor* have been described but only two of them are capable of reproducing on *A. mellifera*. These are the Korean (K) and Japanese (J) haplotypes which vary in their virulence toward *A. mellifera*, with K type assumed to be more virulent (Anderson and Trueman, 2000). In the present scenario, 90 per cent apiaries and 50 per cent colonies of state of Haryana are affected by this mite (Gulati *et al.*, 2009).

V. destructor feeds on the hemolymph of larval and adult bees, inflicting nutritional stress and immune suppression, as well as acting as a major vector for viral pathogen transmission (Rosenkranz *et al.*, 2010). These impacts translate into both lowered productivity and higher mortality at the colony level. At present, hive debris examination is done by beekeepers to know mite infestation level which do not provide accurate estimation of number of mites. In the present investigation, two sampling methods for *V. destructor* were compared year around in *A. mellifera* colonies.

MATERIALS AND METHODS

Comparative effectiveness of sampling methods: To study the effectiveness of sampling methods, the seasonal incidence of *V. destructor* was recorded from May, 2008 to April, 2009 in *Apis mellifera* colonies from University apiary and adjoining apiaries in and around Hisar. All colonies were placed on stands dipped in water filled wooden bowls to

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prevent ants from entering hives. Weekly sampling from six *A. mellifera* colonies of standard Langstroth hive bodies was done to ascertain the number of mites/hive. Two methods of sampling *viz.*, hive debris and sticky paper were adopted. Each sampling method was replicated thrice.

Hive debris sampling method: Mites that die naturally or due to grooming behaviour of bees get dislodged from honey bees and collect on the bottom board of the hive in debris as per standard methods (Dietemann *et al.*, 2013**).** From three infested colonies of 7 to 8 frame strength, hive debris was collected weekly from the bottom board. Debris collected from individual hive was dried for 24 h. It was than flooded with industrial grade alcohol and stirred continuously for 1 minute or up to 10-20 min if debris contained wax or propolis particles. Surface of the alcohol was investigated for the presence of mites (Plate I a and Plate II b).

Sticky paper sampling method: In this method, a sticky white paper sheet was placed on bottom board of each of the three hives with sticky part on upper side so that mites fall down on the paper may not be able to climb again and attach to the bees. At weekly interval, the paper was removed from the hive and number of dead/live mites attached to sticky paper were counted. For easy counting, each sticky paper was divided into squares by marking lines horizontally and vertically at a distance of two inches (Plate II a and Plate II b). The small squares made it easy to count the number of mites in each square which were then added to express as the number of mites per hive. At each observation period, paper was replaced by fresh sticky paper in each hive.

PLATE I a: Hive debris sampling method (floating method)

PLATE I b: Hive debris

Effect of *Varroa destructor* on colony strength and stores: Colonies were equalized in terms of strength and stores at the initiation of the experiment. Number of bees (frames), brood (cm^2), pollen (cm^2) and honey area (cm^2) in each colony and its variation with respect to season were recorded in all the six-test colonies (3 each for two sampling methods). Observations on colony strength (no. of frames and brood area) and colony stores (pollen area and honey) were recorded at 21 days interval till the termination of the experiment. Colony strength and stores have been compared with seasonal abundance of mites by using simple correlation.

The total honey area was further converted into weight of honey in grams by multiplying it with a factor of 1.25 (Chhuneja *et al.*, 1993).

Statistical analysis: The seasonal incidence data was subjected to analysis of variance (ANOVA) Critical difference (CD) was calculated to determine the difference between seasons and sampling methods. Appropriate transformation of data was applied where ever was necessary.

RESULTS AND DISCUSSION

Comparative effectiveness of sampling methods: Sticky paper method was found significantly better (CD = 0.11; p = 0.05) as more number of mites (17.58 mites/hive) were recorded than in hive debris sampling method (11.41 mites/hive) (Table 1). Irrespective of the sampling method, maximum number of mites (63.27 mites/hive) was recorded in second fortnight of May which was significantly higher than the mites recorded at other observation periods (CD = 0.38; p = 0.05). In hive debris sampled colonies, two peaks of *Varroa* population were observed. First peak was witnessed in second fortnight of May (40.05 mites/hive) and second in first fortnight of September (33mites/hive) which showed significant difference with mite numbers at other observation periods. Likewise, in sticky paper method, first peak in second fortnight of May and second in first fortnight of July were observed. The number of mites gradually declined from first fortnight of September to first fortnight of November. Statistically comparable data were recorded in first fortnight of November to first fortnight of December and first fortnight

TABLE 1: Comparative sampling methods to record *Varroa destructor* population in *Apis mellifera* colonies

Observation period	No. of mites/ hive in different sampling methods		
	Hive debris	**Sticky paper**	**Mean (Period)**
1st to 15th, May	36.50 (6.16)	74.00(8.71)	55.25(7.44)
16th to 31st, May	40.05 (6.48)	86.50 (9.40)	63.27(7.94)
1st to 15th, June	16.00(4.23)	37.00 (6.24)	26.50(5.24)[a]
16th to 30th, June	0.50 (1.57)	24.50 (5.13)	12.50(3.35)
1st to 15th, July	22.50 (4.94)	47.00 (6.99)	34.75(5.97)[b]
16th to 31st, July	24.00(5.09)	38.00 (6.32)	31.00(5.71)[b]
1st to 15th, August	29.00 (5.56)	30.00 (5.64)	29.50(5.60)[a,b]
16th to 31st, August	25.00(5.18)	14.50 (4.05)	19.75(4.61)[c]
1st to 15th, September	33.00(5.91)	23.50 (5.14)	28.25(5.53)[a]
16th to 30th, September	17.00(4.35)	21.50 (4.74)	19.25(4.54)[c]
1st to 15th, October	9.50 (3.38)	17.00 (4.35)	13.25(3.87)
16th to 31st, October	9.50 (3.38)	2.00 (1.98)	5.75(2.68)[d]
1st to 15th, November	2.50 (2.11)	0.00 (1.41)	1.25(1.76)[f]
16th to 30th, November	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
1st to 15th, December	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
16th to 31st, December	3.50 (2.33)	2.50 (2.11)	3.75(2.22)[e]
1st to 15th, January	4.50 (2.54)	3.00 (2.22)	3.75(2.38)[d,e]
16th to 31st, January	3.00 (2.20)	2.00 (2.00)	2.50(2.10)[e]
1st to 15th, February	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
16th to 28th, February	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
1st to 15th, March	0.00 (1.41)	0.00 1.41)	0.00(1.41)[f]
16th to 31st, March	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
1st to 15th, April	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
16th to 30th, April	0.00 (1.41)	0.00 (1.41)	0.00(1.41)[f]
Mean (Sampling method)	**11.41(2.21)**	**17.58(2.53)**	**14.50**

Figures in parentheses are $\sqrt{n+1}$ transformed values
Figures denoted by similar letters do not differ significantly with each other
CD (p=0.05) for Period = (0.38; sampling method = 0.11
CD (p=0.05) for period × sampling method = 0.54

of February to second fortnight of April (Table 1). Interaction between the observation periods and sampling methods was also significant (CD = 0.54; p = 0.05) (Table 1) which indicated that fortnightly observations on number of mites recorded in two sampling methods differed significantly with each other.

In general, maximum population build up of *V. destructor* was noticed in the month of May in both the sampling methods. At each observation period, more number of mites was detected on sticky paper than in hive debris of *A. mellifera* colonies.

Effect of mite infestation on colony build-up and stores: Colony strength was significantly negatively correlated (r= -0.75) with *V. destructor* incidence. It showed the tendency of colony becoming weaker with increased mite infestation in hive debris method (Figure 1). In other words, as colony became weaker, there was increased susceptibility towards mite infestation. The pattern of change in colony strength held true for brood area also, which showed 892.5 cm^2 area/ hive on the first day of study and gradually declined to 338.0

PLATE II a: Sticky paper method (Sticky paper sheet)

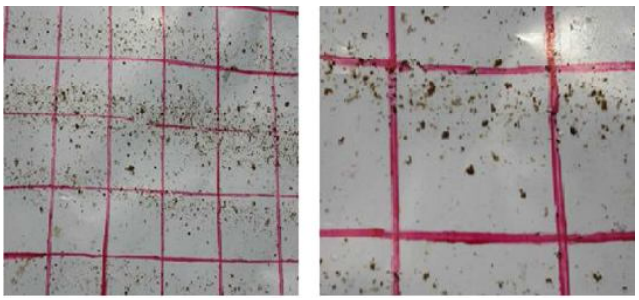

PLATE II b_ c: Squares for counting the number of mites_ close up of squares

cm^2 on 63rd day of observation (Figure 2). A negative correlation (r = -0.78) was obtained between these two parameters. Among colony stores, significant negative correlation was recorded between *V. destructor* infestation and honey (r=- 0.75) (Figure 3) and with pollen area (cm^2) (r=-0.78) (Figure 4). in *A. mellifera* colonies.

The foremost important aspect was aimed at population build up of *V. destructor* in *A. mellifera* colonies in changing weather conditions and to trace the most appropriate sampling method. Mite fall on sticky paper and hive debris was more in warmer months (May to August) than in colder months (November to January). Webster *et al.* (2000) also reported similar observation that proportion of mites that fall in hive debris is more during hot weather. Increased mite fall in hot weather indicates that the elimination of these mites would be most feasible during summer months in this area when nectar and pollen collection is also low.

In the present study, two peaks in *V. destructor* population are clearly depicted. Earlier study also indicated two peaks of *V. destructor* population (Kokkinis and Liakos, 2004; Delaplane *et al*., 2005). Average mite population per colony showed a period of stabilization or marginal increase characterized by fluctuation from May to August-September in both the sampling methods in the present study. However, Kokkinis and Liakos (2004) and Calatayud and Verdu (1995) showed an increase from end of March to mid August and end of April to mid October, respectively, corresponding to a rate of daily finite increase of 1.0168 and 1.0177 in first and second year of study. The change in number of mites during various months may be due to different seasons in different geographical location of bee colonies around the world. Various workers have also reported that fall of mites continued during winter although at a slow rate, possibly because of phoretic mites are securely positioned between segments of bee's abdomen (Fries, 1992). Martin (2001) noted smaller number of female offspring in winter months due to high level of male offspring mortality. In the cooler ambient temperature, mite stayed outside the cells for a shorter time (Woyke, 1987) which may be another reason for low mite count in winter months.

One colony collapsed during the research (July) after witnessing peak in *V. destructor* population in second week of May. The reason could be that before this period, *A. mellifera* colonies in this apiary were showing *V. destructor* infestation which gradually increased in brood and adult bees and this particular colony was not able to sustain under these circumstances. Furthermore, in May-June pollen and nectar sources were sparsely available in the region which further stressed the colonies. At this stage, sugar feeding and pollen supplement were not enough to sustain the colony which showed highest *V. destructor* infestation. Rest of the test colonies survived *V. destructor* infestation in the present study suggesting a possible host parasite co-adaptation. Earlier, Fries *et al.* (2006) described the survival of mite infested colonies in Nordic climate for up to six years. In the present study, one colony showed the tolerance to *V. destructor* population. It not only survived but more brood area and bee strength was noticed in this hive resulting in more colony stores as compared to other *V. destructor* infested colonies.

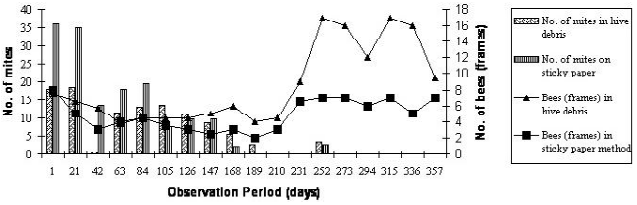

FIG 1: Effect of mite incidence of bee strength in two sampling methods.

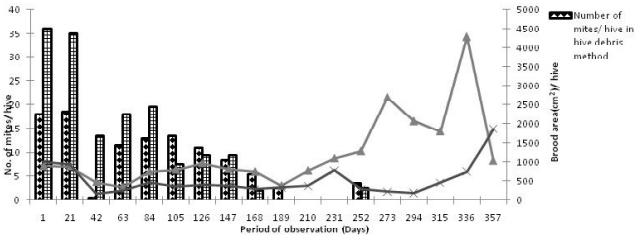

FIG 2: Effect of mite incidence on brood area in two sampling methods.

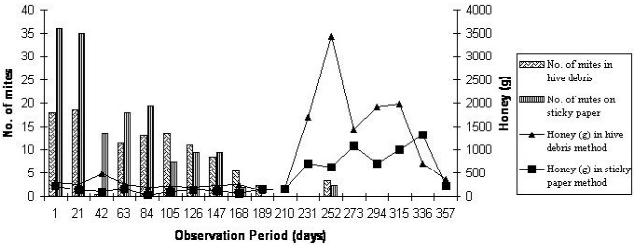

FIG 3: Effect of mite incidence on honey in two sampling methods.

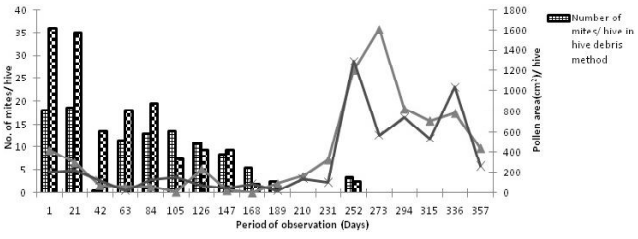

FIG 4: Effect of mite incidence on pollen area in two sampling methods.

Comparison of sampling methods: Delaying the chemical application in a hive is a key objective of IPM, so it is paramount that beekeepers have the means of accurate sampling method to monitor *V. destructor* population and criteria to determine when mites have achieved levels that warrant chemical treatment. Under this retrospective, two simple and feasible methods of sampling were tested *viz*. hive debris and sticky paper. Due to grooming/foraging activity of bees mites were dislodged and collected on bottom board/ sticky paper.

The results showed that across the 12 sampling months, the average number of *V. destructor* recorded on sticky paper was significantly higher (17.58 mites/hive) than the colonies with hive debris collection (11.41 mites/hive). Interaction between months and sampling methods was also detected in present study. Hive debris method although is a simplest, economical, least time consuming method in which no additional material is required, but it is shown that the mites that fall from colony into hive debris constituted 50 (Webster *et al.,* 2000) to 60 (Webster and Thacker, 1999) per cent live mites which can climb back to brood frames and cause reinfestation. Webster *et al.* (2000) observed that the proportion of mite fall that was alive was higher at the rear and sides of the hive compared with that falling from centre frames near the hive entrance.

In sticky paper method, mites dislodged due to grooming activity from bees get attached to the paper and are not able to climb to the colony again. In the present study, commercially available sticky paper was used which costs around Rs. 4 and from one paper, two sticker sheets can be made to be placed in two hives. This cost effective, feasible and easy method provides accurate estimation of mites in a colony. It prevents live mites from reentering into the colony. Further, it would be easy to determine economic threshold of mite population on sticky paper. On the basis of 24 h mite count on hive floor sticky sheets, mite threshold levels (12 mites for northwest and 0.7 to 12.2 mites for south east) for April and February are recommended in USA (Delaplane and Hood, 1999). Region specific thresholds for *V. destructor* using sticky paper coupled with known methods of slowing mite growth like screen floors, powdered sugar dusting, use of organic acids etc. will be a viable comprehensive IPM paradigm for managing this mite (Asha *et al*., 2009; Asha *et al*., 2014). Many researchers preferred screen floors instead of hive debris and sticky paper to monitor populations of *V. destructor* (Sanford, 1999). Delaplane and Hood (1997) reported that sticky bottom boards are more reliable sampling method for making treatment decisions. Macedo *et al.* (2002) used inert dusts including powdered sugar to detect and access *Varroa* population in *A. mellifera* colonies in USA. Thus, Sticky boards are better sampling method as compared o hive debris.

Effect of mite infestation on colony build-up and stores: During present investigation, the effect of *V. destructor* incidence was also studied on the *A. mellifera* colony strength and stores. A negative correlation was obtained between *V. destructor* population and colony parameters. During the summer season, when brood area was decreasing, more mites fell on hive debris/sticky paper resulting in decrease in mite population. As brood area started expanding, favouring the entry of *Varroa* into brood cells as well as their reproduction, its population showed an increasing trend. These results corroborated the earlier studies that large number of sealed brood cells contributed to increase in mite population (Donze *et al*., 1996).

Bee strength also reduced in the present study with increase in *V. destructor* population. A significant negative correlation (r= -0.55) was measured between the two parameters. In the absence of *V. destructor* and improving nectar and pollen foraging conditions, bee strength showed a significant increase. Similar observations were made by Kokkinis and Liakos (2004).

A significant negative correlation (r=-0.48) between *V. destructor* population and honey in the present study showed that with lower mite incidence, bee strength of colony improves and they collect more nectar. No earlier information is available on the effect of *V. destructor* incidence on colony stores; however, Hosamani *et al*. (2005) reported that *T. clareae* incidence has no effect on honey and pollen stores of *A. mellifera* colonies.

CONCLUSIONS

Studies on evaluation of sampling methods revealed sticky paper as significantly better method (CD = 0.11; p = 0.05) as more number of mites (17.58 mites/hive) was recorded than in hive debris sampling (11.41 mites/hive). Fortnightly data analysis showed maximum number of mites (63.27 mites/hive) in second fortnight of May. Colony becomes weaker with increased *V. destructor* incidence as shown by significant negative correlation with colony strength (r= -0.85), brood (r = -0.78), honey (r=- 0.75) and pollen (r=-0.78) area.

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