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Asha
Department of Zoology, CCS Haryana Agricultural University, Hisar-125 004, India.

Rachna Gulati
Department of Zoology, CCS Haryana Agricultural University, Hisar-125 004, India.

S. K. Sharma
Department of Entomology CCS Haryana Agricultural University, Hisar-125 004, India.

Correspondence:
Asha
Department of Zoology, CCS Haryana Agricultural University, Hisar-125 004, India.

Comparative evaluation of oxalic acid and formic acid against *Varroa destructor* Anderson and Trueman in *Apis mellifera* L. colonies

Asha, Rachna Gulati and S. K. Sharma

Abstract
The effect of oxalic acid (3% per hive) was examined by dividing the *Apis mellifera* L. colonies to five groups: wick method, trickling method and top bar method and compared with formic acid, untreated colonies (control). The three week study showed that all the treatments significantly increased the mite fall as compared to control. Among the treatments, top bar method was most effective providing 84.4% reduction in *V. destructor* population over untreated hives followed by wick method (75.9%) and trickling method (75%). Formic acid treatment (5 ml of 85% per hive) provided 85.3% reduction in *V. destructor* population over control. Brood area was increased significantly in all the treated colonies however bee strength and colony stores remained statistical comparable with control.

Keywords: formic acid, honeybee mites, trickling method, top bar method, wick method.

1. Introduction
Honey bee species, *Apis mellifera* L., occurs naturally over vast, varied geographical areas and is well adapted to a wide range of climatic conditions. However, loss of colonies has become a major threat to the apicultural industry and ultimately to food production. In temperate regions, the ectoparasitic mite *Varroa destructor* (Mesostigmata: Varroidae), an invasive species from Asia [1, 2] is a dominant factor for such losses. Out of 17-18 mitochondrial haplotypes of *V. destructor*, Korean (K) haplotype is the most virulent [3]. It negatively affects honey bee health and immune system, thus causing physical and physiological deterioration [4]. It also serves as a vector for several harmful positive-strand RNA viruses [5] which translate into severe disease complex leading to higher mortality in bees, lowered productivity, reduced honey production and a decrease in pollination efficiency. Without periodic treatment, most of the honey bee colonies in temperate climates would collapse within a period of 2–3 years [1]. In India, it has ravaged *A. mellifera* colonies from northern states showing infestation in 90% apiaries [6].
Several control methods against *Varroa* have been tried in the recent past with the use of chemicals; most noted ones include synthetic acaricides and organophosphates [7]. Most of these pesticides are easy to apply, economically convenient, and do not require refined knowledge of the mites' biology, however, these have created the problems of high residue levels in honey [8] and development of resistance in *Varroa* [9]. Formic acid has strong acaricidal effect [10] and low price. It is a natural component of honey and has the advantage of killing mites on adult bees as well as in sealed brood [11]. Therefore, attention is diverted for safe and reliable alternatives like organic acids etc. Oxalic acid by trickling method [12, 13] and vapour method [14] has also been widely used for *Varroa* control. In view of above facts, present investigation was carried out to evaluate the comparative efficacy of different methods of application of oxalic acid and formic acid against *V. destructor* and their effect on colony build up and stores. *Ixora Coccinea, Oxalis corniculata.*

2. Materials and Methods
The treatments, oxalic acid (3% per hive) and formic acid (5 ml of 85% per hive) were evaluated against *V. destructor* (Plate I) in *A. mellifera* colonies and compared with untreated colonies which acted as control. Each treatment was replicated thrice. The period of study was from January to March, 2008. All treatments were applied to colonies having natural mite infestation. Before evaluation, *A. mellifera* colonies were equalized in terms of colony strength and stores for

three week study period. Brood, honey and pollen area were quantified in square centimeters on all frames using a wire grid having squares of 2.5 cm on a side [15]. The total honey area was further converted into weight of honey in grams by multiplying it with a factor of 1.25. Weekly sampling from *A. mellifera* colonies of standard Langstroth hive bodies was done to ascertain the number of mites/hive.

For pre-treatment count, sticky paper was inserted on to the bottom board of experimental colonies, removed three days later and mite drop was quantified[16] (Plate II, III). The effect of oxalic acid (3% per hive) was examined by dividing the *A. mellifera* colonies to four groups: wick method, trickling method and top bar method and untreated colonies (control). In wick method (Plate IV), oxalic acid in sugar solution was kept in a Petri plate placed at the bottom board. A cotton pad was placed in Petri plate to allow slow evaporation of oxalic acid. In trickling method (Fig. V), oxalic acid was trickled in between the frames in *A. mellifera* colonies. In top bar method (Plate VI), oxalic acid was released on the top bar of each frame with the help of pipette at concentration of 3% per hive. The number of mites in hive was estimated on sticky paper at each observation period, i.e. 7, 14 and 21 days after treatment. At each observation period, old sticky paper was replaced with new to avoid the confusion in counting the number of earlier dropped mites over latest mite drop per hive. To determine the efficacy of the treatments and to collect all the mites in the treated and untreated *A. mellifera* colonies, formic acid (5 ml of 85%) (wick method) was applied after 21 days. Residual mites were collected after three days from the bottom of hives using sticky paper method in both treated and untreated groups. Efficacy (%) in mite population over control was calculated using the method of Eguaras et al. [17]:

% Efficacy = 100 [I_t / (I_f + I_t)]

Where I_t = Total number of mites on the sticky paper of the hive after treatment – Total number of mites on the sticky paper of the hive before treatment

I_f = Total number of mites on the sticky paper of the hive final treatment

% reduction over untreated hives = [T_s - C_s]/ T_s × 100

Where T_s and C_s are the percentage of mites on sticky paper in treated and untreated hives, respectively

The impact of treatment on bee strength and area of brood, pollen and honey was also studied and compared with the similar data on untreated hives. The statistical significance of data was assessed through analysis of variance (ANOVA). Critical difference (CD) was calculated and means of different treatments were compared to observe the significant difference between the treatments, if any (p=0.05).

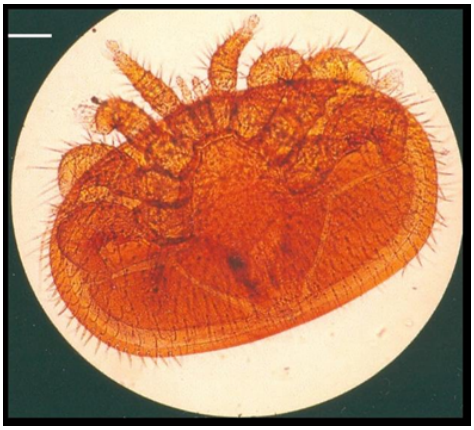

Plate I: ***Varroa destructor***

Plate II: Method of Placing Sticky paper on Bottom board

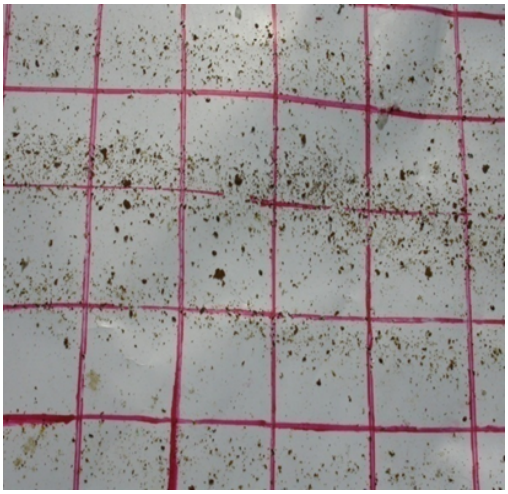

Plate III: Sticky paper divided into Squares for easy counting

Plate IV: Oxalic acid (Wick method)

Plate V: Oxalic acid (Trickling method)

Plate VI: Oxalic acid (Top Bar method)

Table 1: Efficacy of Oxalic acid (wick method) against *Varroa destructor* in *Apis mellifera* colonies

Treatment	Pre Treatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Oxalic acid (Cotton swab method) @ 3%/hive	16.60	17.00	18.00	16.00	51.00	17.00	17.00
Control	13.30	9.60	9.30	11.00	29.90	9.96	135.50
CD (p = 0.05)	N.S.				2.22		8.86
% efficacy	75.00						
% reduction over control	75.90						

DAT = Days after treatment

*Formic acid (5 ml of 85%) was applied to record residual mite count

Table 2: Effect of Oxalic acid (wick method) on colony strength and stores in *Apis mellifera* colonies

Treatments		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	Oxalic acid (Cotton swab method) @ 3%/hive	6.1	402.6	147.3	151.6
	Control	6.0	402.0	158.2	140.0
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
Post treatment	Oxalic acid (Cotton swab method) @ 3%/hive	6.0	666.0	208.2	161.0
	Control	6.5	467.0	330.6	294.0
	CD (p = 0.05)	N.S.	15.175	N.S.	N.S.

NS = Non- significant

Table 3: Efficacy of Oxalic acid (Trickling method) against *Varroa destructor* in *Apis mellifera* colonies

Treatment	Pre Treatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Oxalic acid (trickling method) @ 3%/hive	16.60	119.00	137.00	110.00	366.00	122.00	102.00
Control	13.30	13.30	16.60	17.00	46.90	15.60	135.50
CD (p = 0.05)	N.S.				22.87		8.86
% efficacy	76.35						
% reduction over control	75.06						

DAT = Days after treatment

*Formic acid (5 ml of 85%) was applied to record residual mite count

Table 4: Effect of Oxalic acid (Trickling method) on colony strength and stores in *Apis mellifera* colonies.

Treatment		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	Oxalic acid (trickling method) @ 3%/hive	6.50	640.00	1034.30	181.00
	Control	6.00	750.00	1037.50	180.00
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
After treatment	Oxalic acid (trickling method) @ 3%/hive	6.50	875.50	405.00	125.00
	Control	4.50	801.00	223.70	8.00
	CD (p = 0.05)	N.S.	41.18	N.S.	N.S.

NS = Non-significant

Table 5: Efficacy of Oxalic acid (Top bar method) against *Varroa destructor* in *Apis mellifera* colonies

Treatment	Pre Treatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Oxalic acid (on top bar) @ 3%/hive	10.50	16.60	18.50	17.00	52.10	17.36	6.50
Control	13.00	13.50	13.30	7.50	34.30	11.40	134.00
CD (p = 0.05)	NS				5.84		6.78
% efficacy	88.20						
% reduction over control	84.45						

DAT = Days after treatment

*Formic acid (5 ml of 85%) was applied to record residual mite count

Table 6: Effect of Oxalic acid (Top bar method) on colony strength and stores in *Apis mellifera* colonies

Treatment		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	Oxalic acid (on top bar) @ 3%/hive	6.50	798.30	202.00	178.00
	Control	6.00	750.00	210.00	166.60
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
After treatment	Oxalic acid (on top bar) @ 3%/hive	5.80	924.00	60.00	43.00
	Control	4.50	710.00	223.70	8.00
	CD (p = 0.05)	N.S.	179.40	33.40	28.10

NS = Non-significant

Table 7: Efficacy of Formic acid against *Varroa destructor* in *Apis mellifera* colonies

Treatment	Pre Treatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Formic acid (5 ml of 85%)	26.00	25.00	21.60	20.00	66.60	22.20	16.0
Control	15.50	11.90	9.00	11.00	31.90	10.60	140.5
CD (p = 0.05)	N.S.				4.86		8.86
% efficacy	71.73						
% reduction over control	85.36						

DAT = Days after treatment

*Formic acid (5 ml of 85%) was applied to record residual mite count

Table 8: Effect of Formic acid on colony strength and stores in *Apis mellifera* colonies

Treatment		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	**Formic acid (5 ml of 85%)**	6.50	734.60	147.30	250.60
	Control	6.00	760.00	158.30	248.30
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
After treatment	**Formic acid (5 ml of 85%)**	6.50	939.00	105.30	263.30
	Control	6.50	736.60	156.60	294.00
	CD (p = 0.05)	N.S.	188.41	N.S.	N.S.

NS = Non-significant

3. Results

In oxalic acid (wick method) treatment, natural V. destructor infestation (pre-treatment count) in treated and untreated hives were comparable with each other (Table 1). Treatment showed a significant effect on mite fall in treated colonies (51 mites/hive) as compared to control (29.9 mites/hive) colonies (CD = 2.22; p = 0.05). The residual treatment showed the efficacy of treatment as number of mites were significantly low in treated hives (17 mites/hive) as compared to 135.5 mites/hive in control hives (CD = 8.86; p = 0.05). The per cent efficacy and per cent reduction in V. destructor population over untreated hives was 75 and 75.9, respectively in oxalic acid (wick method) treatment. Colony strength and colony stores (honey and pollen) showed no significant difference during the treatment. However, significant difference was observed in brood area of treated and untreated *A. mellifera* colonies after twenty one days of study period (Table 2).

In the second method of application of oxalic acid by trickling method the average V. destructor population treated and control hives was statistically comparable with each other before the start of the experiment (Table 3). It showed a significant effect (CD = 22.87; p = 0.05) on total mite drop in treated colonies (366 mites/hive) as compared to 46.9 mites/hive in untreated *A. mellifera* colonies. The per cent efficacy and per cent reduction in V. destructor population over untreated hives was 76.35 and 75.06 respectively (Table 3). No significant differences were observed in colony strength and stores (honey and pollen) in treated and untreated colonies. Brood area showed an increase from 640 cm^2 to 875.5 cm^2 which was statistically significant (Table 4).

In the third method (top bar method) of application of oxalic acid (3%), pre-treatment count recorded as natural mite fall on sticky paper in treated and untreated colonies was at par with each other (Table 5). Significantly more mites dropped on sticky paper after

treatment (52.1 mites/hive) than in untreated *A. mellifera* colonies (34.3 mites/hive) (CD = 5.84; p = 0.05). Residual treatment with formic acid (5 ml of 85%) showed significantly higher natural mite fall in untreated hives than in treated hives(CD = 6.78; p = 0.05). The per cent efficacy and per cent control over untreated hives was 88.2 and 84.45, respectively in oxalic acid (top bar method) treatment. During the initiation of experiment, colony strength and stores showed comparable data (Table 6) in treated and untreated *A. mellifera* colonies. After treatment, brood area increased and showed significant difference (CD = 179.4; p = 0.05) in treated colonies as compared to untreated colonies. Colony strength and stores data was however at par in treated and untreated colonies (Table 6).

The total number of mites collected on sticky paper at the bottom board before, during and after the formic acid treatment has been presented in Table 7. The pre-treatment count in treated and untreated *A. mellifera* colonies showed no significant difference with each other. More mites were collected during first week of treatment (25 mites/hive) which gradually declined to 21.6 and 20.0 mites/hive during the second and third week, respectively. The per cent efficacy and per cent control over untreated hives was 71.73 and 85.36, respectively. During the course of study, bee strength and colony stores (pollen, honey area) remained statistically comparable; however, significant increase in brood area was witnessed in formic acid treated colonies (Table 8).

4. Discussion

The current study demonstrated a significantly (p = 0.05) higher numerical count on sticky paper in oxalic and formic acid groups as compared to control. However, efficacy of oxalic acid varied depending on the method of application. Top bar method proved to be significantly better followed by wick method and trickling method in reducing V. destructor population in hives. Trickling method has also been employed by other workers to reduce Varroa population [18, 19]. Marinelli et al. [20] reported that under brood less condition, oxalic acid in sugar solution (4.2%) gave 80 per cent control whereas Charriere & Imdorf [19] reported it as 90 per cent effective. However, when oxalic acid (2-3g; 2 treatments in 4 days) was applied through evaporator, it reduced the mite level from initial 20 per cent infestation to 5 per cent. They further reported that oxalic acid mixed in 2:1 sugar water exhibits greater miticidal effectiveness than solutions with half as much sugar, but increasing the sugar content beyond this increased its toxicity to bees. Sammataro et al [9] reported that three applications of oxalic acid 7 days apart in brood less colonies caused significant Varroa mortality. Studies are available in which oxalic acid crystals are heated in hives, creating a vapour or evaporating it by using appropriate electrical device [14]. In the vapour phase, it is effective but has low volatility [21], a condition similar to wick method in present study. In addition, vapour created near the hive by heating could be hazardous to beekeeper because it can cause severe irritation and burning [9].

During the present study, no adverse effect of oxalic acid was observed on colony strength and stores. Brood area was significantly more at the end of three week study period in all the three methods of application as compared to control. These results are in agreement with earlier studies [18]. However, long term spray applications of oxalic acid (3%) in autumn and spring showed significantly negative effects on brood development, queen survival (and increased adult bee mortality[19]. Anonymous [22] reported that in trickling method of application, bees become quite acidified at the first application and die early without showing on the floor board. Although present study was of three week duration only but effectiveness is depicted by significantly lower mite count after formic acid treatment to collect remaining mite population in oxalic acid treated colonies as compared to higher residual population in untreated colonies with no significant changes in bee strength. Its inclusion in any beekeeping management system is warranted as low cost, ease of application, low risk of honey contamination and no resistance in Varroa [23].

The efficacy reported in literature ranges from 29.6 per cent [24] to more than 90 per cent [25] depending on dose, modalities of application, and experimental or environmental conditions. In the present study, formic acid 85% (5ml) applied by wick method provided 85.3 per cent control over untreated hives. Three to four applications of formic acid (65%) @ 300ml provided significant reduction of Varroa infestation [10]. Formic acid (15ml) caused 55-60 percent mite mortality in brood cells [10] which was increased to 87-89 percent in trapped worker brood [26].

Earlier studies indicated that treatment with formic acid can increase queen mortality, damage to hatching bees [27] and have detrimental effect on brood production, but this is likely to be due to a direct effect of the acid on brood survival and can affect the physiology of the immature and young workers [28]. However, in the present study of three weeks, no adverse effect on colony strength (bees, brood) and colony stores (pollen, honey) was observed. On the contrary, the brood area showed a significant increase in formic acid treated *A. mellifera* colonies as compared to untreated colonies. The results are in conformity with some studies which showed that long term formic acid treatment did not damage brood and young bees and did not limit colony development (Underwood and Currie, 2005). It has been speculated that combination of high temperatures and high concentrations of formic acid may contribute to queen loss [29].

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