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Comparative Performance of Organic Acids against Ectoparasitic Mites, *Varroa destructor* and *Tropilaelaps clareae* in *Apis mellifera* Colonies

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Abstract

The present study evaluated the efficacy of formic, oxalic, and acetic acids against *Varroa destructor* and *Tropilaelaps clareae* in *Apis mellifera* colonies. Dislodged mites were recorded at 7, 14, and 21 days after treatment. A significant increase in mite dislodgement was observed in all treatments, with the highest values in formic acid, while control colonies showed no significant change. Brood infestation declined after the first treatment, with a greater reduction under formic acid. Worker bee infestation was reduced to zero immediately after treatment and remained unchanged. Overall, formic acid showed the highest efficacy (96.99%), followed by acetic (92.47%) and oxalic acid (86.29%). Differences between mite species and their interaction with treatments were non-significant. Principal Component Analysis clearly separated treated colonies from the control, indicating effective suppression of mite infestation parameters. The study highlights these acids as effective options for managing mite infestations in honey bee colonies.

Keywords: Ectoparasitic mites, Honey bees, Organic acids, *Tropilaelaps clareae, Varroa destructor*

The European bee, *Apis mellifera* L. (Hymenoptera: Apidae), is one of the most important pollinators, which provides essential pollination services to agricultural and natural ecosystems[1,2]. However, the productivity and survival of *A. mellifera* colonies are severely affected by ecto-parasitic mites, which weaken colonies directly through feeding and indirectly by transmitting pathogens[3]. Among these, *Varroa destructor* Anderson and Trueman (Acari: Varroidae) and *Tropilaelaps clareae* Delfinado and Baker (Acari: Laelapidae) are considered the most destructive, affecting honey bee colonies[4,5]. *Varroa destructor* is a globally recognised parasite of *A. mellifera* that feeds on the fat body and haemolymph of both brood and adult bees and leads to reduced longevity, reduced immunity, and in severe cases, complete colony collapse[6]. In addition to direct damage, *V. destructor* acts as a vector of several honey bee viruses, thereby amplifying disease severity within colonies[7]. Similarly, *T. clareae* has successfully adapted to *A. mellifera* colonies and is characterised by fast reproduction and severe brood damage. Compared to Varroa, *Tropilaelaps* mites complete their life cycle more quickly and depend more specifically on bee brood, making infestations difficult to manage once established[8].

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The management of these ectoparasitic mites has traditionally relied on synthetic acaricides due to their rapid action and ease of availability[9]. However, prolonged and indiscriminate use of chemical acaricides has resulted in several challenges, including the development of acaricide resistance in mite populations, contamination of hive products such as honey and beeswax, and adverse effects on honey bee health[10,11]. These concerns have led to an increase in the restrictions on the use of certain synthetic compounds and have encouraged the exploration of alternative, safer mite management strategies. Organic acids have emerged as promising alternatives for the control of parasitic mites in honey bee colonies. Compounds such as formic acid, oxalic acid, lactic acid, and acetic acid are naturally occurring substances that are biodegradable and generally leave minimal residues in hive products when applied correctly[12,13]. Several organic acids are already incorporated into integrated pest management (IPM) programmes for honey bees due to their relatively low risk to the environment and the consumers[14].

Previous studies have demonstrated the efficacy of individual organic acids against *V. destructor* under laboratory and field conditions, with variable success depending on concentration, method of application, colony strength, and environmental conditions. However, information on the performance of different organic acids under *A. mellifera* colonies co-infested with both ectoparasitic mites is limited. The present study was undertaken to evaluate and compare the performance of selected organic acids against *V. destructor* and *T. clareae* in *A. mellifera* colonies co-infested with both mite species.

MATERIALS AND METHODS

The study was conducted during August-September 2024 in *A. mellifera* colonies kept in the standard Langstroth hives in the Department of Entomology, College of Agriculture, CSK HPKV, Palampur (32° 05′ 59.77′ N, 76° 32′ 50.0′ E; 1250 m amsl). Three organic acids, *viz*. acetic, formic, and oxalic acids, were tested against ectoparasitic mites in *A. mellifera* colonies co-infested with *V. destructor* and *T. clareae*. For each trial, five colonies of similar strength and mite infestation were selected. All colonies were equipped with a mite screening mesh (3 × 3 mm) between the brood chamber and the bottom board. A sticky paper was placed over the bottom board to trap dislodged mites along with hive debris, and it was changed twice a week. Seven days before the first treatment, a new sticky paper was placed in the colonies, and three mite infestation parameters in the colonies, *viz*. weekly dislodged mites, per cent brood and per cent worker bee infestation, were recorded on the zeroth day (on the day of treatment before the acid application), and 7, 14, and 21 days after treatment (DAT).

Mite Infestation Parameters of *Apis mellifera* Colonies

Dislodged mites of both species were observed by counting the number of dislodged mites (*V. destructor* and *T. clareae*) along with hive debris on the sticky paper. To count the number of dislodged mites per week, the mites were separated from debris with a fine brush under the microscope and collected and counted in Petri plates. For per cent brood infestation, the wax capping from cells was first removed with the help of a forceps and 100 pupae were examined after being taken out from the cells. The adult mites attached to the infested bee pupae were visible to the naked eye. For the assessment of per cent worker bee infestation, a jar was filled with 15 grams of sugar powder and 100 worker bees were transferred gently to the jar with the help of a bee brush. The lid of the jar was closed, and the jar was shaken vigorously for one minute to dislodge the mites attached to the bodies of the worker bees. The mites dislodged were counted, and the per cent bee infestation was worked out[15].

Treatment of *Apis mellifera* Colonies

Acetic and formic acid treatments were applied as fumigants using circular sponge discs of 5cm diameter as a slow-release carrier. Each sponge disc was impregnated with the required quantity (5 ml on alternate days). The perforated plastic bag was placed between the mite screening mesh and the bottom board to facilitate uniform volatilisation of the acid within

the hive. The discs were replaced at weekly intervals for three consecutive applications (0, 7th, and 14th day). The oxalic acid was applied after mixing (3.5%, w/v basis) with sugar solution (1: 1). The oxalic acid solution was applied through trickling between the frames (3 ml/ frame covered with bees) with the help of an applicator. A total of three treatments were applied at weekly intervals.

On the 21st day after replacing the sticky paper, all treatment and control hives were kept untreated, and the dislodged mites were counted at 28th day. The treatment efficacy of each organic acid was evaluated using the Henderson–Tilton equation[16]. To calculate efficacy, this formula uses the count of pests in the treated condition before (T_b), control before (C_b), treated after (T_a), and control after (C_a).

$$\text{Efficacy (\%)} = 100 \times \frac{Ta - Cb}{Tb - Ca}$$

Data Analysis

For organic acid-treated and control colonies, a two-way analysis of variance (ANOVA) was performed in randomized block design (RBD) for mite infestation parameters of pre- and post-treatment weeks, as well as for the efficacies of organic acids (following arc-sine transformation), and means were compared based on the critical difference(CD) at $p \leq 0.05$. The combined response of *V. destructor* and *T. clareae* infestation parameters was analysed using principal component analysis (PCA) based on standardised values of mite fall (dislodged mites), brood infestation, and worker bee infestation, and the resulting biplot was used to visualise treatment-wise clustering and relationships among variables.

RESULTS AND DISCUSSION

Effects of Organic Acids on the Infestation Levels of *Varroa destructor* in *Apis mellifera* Colonies

The dislodged mites of *V. destructor* were assessed after 7, 14, and 21 days after the first treatment, while on the 28th day, the dislodged mites due to untreated conditions were counted. After 7 days of the first treatment, a significant increase in the dislodged *Varroa* mites was observed in the acetic, formic and oxalic acid treatment

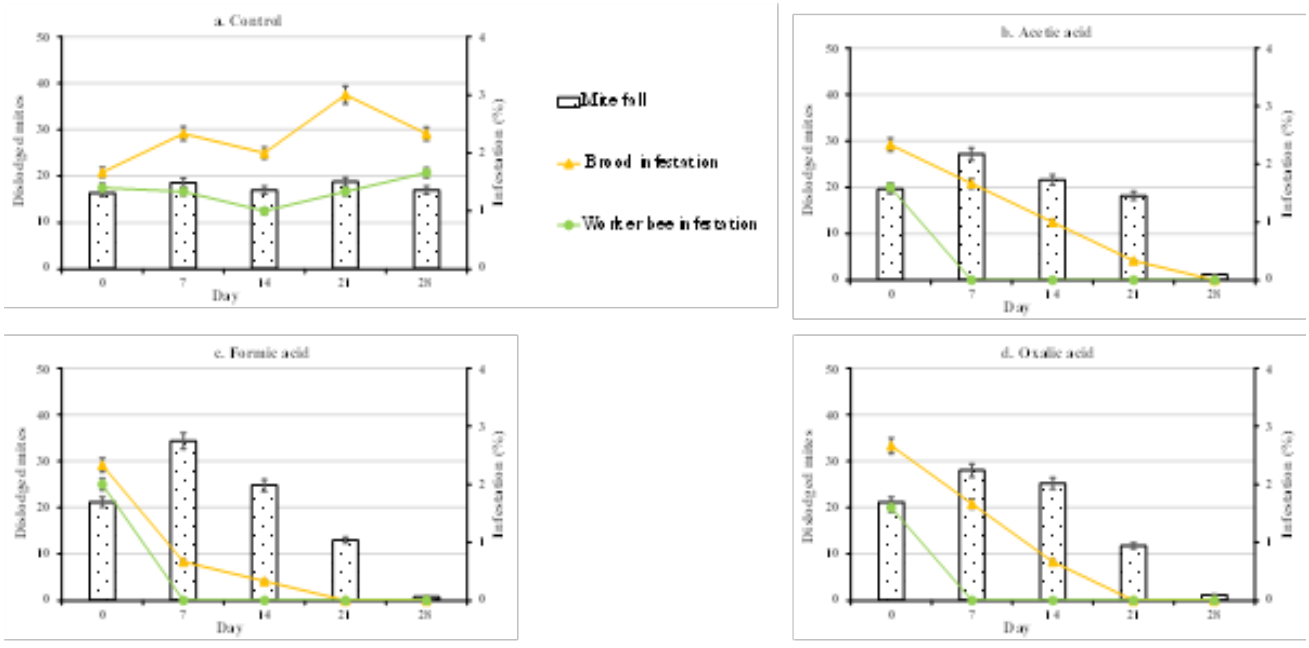


Figure 1. *Varroa destructor* infestation dynamics in *Apis mellifera* colonies subjected to organic acid treatment.

Table 1. Effect of organic acid treatments on the dislodgement of *Varroa destructor* in *Apis mellifera* colonies

Treatment	Dislodged Mites Before Treatment (Day 0)	Dislodged Mites After the indicated Days of First Treatment			Mean
		7	14	21	
Acetic acid	19.60 (4.54)	27.20 (5.31)	21.60 (4.75)	18.20 (4.33)	21.65 (4.73)
Formic acid	21.20 (4.71)	34.40 (5.95)	24.80 (5.07)	13.00 (3.74)	23.35 (4.87)
Oxalic acid	21.20 (4.70)	28.00 (5.38)	25.20 (5.12)	11.80 (3.56)	21.55 (4.69)
Control	19.40 (4.52)	18.60 (4.42)	17.00 (4.23)	18.80 (4.44)	18.45 (4.41)
Mean	20.35 (4.62)	27.05 (5.26)	22.15 (4.79)	15.45 (4.02)	

*Figures in parentheses are square root ($\sqrt{n+1}$) transformed values.

CD ($p \leq 0.05$)	
Treatment	0.21
Days	0.21
Treatment × Days	0.41

(27.20, 34.40, and 28.00 mites/hive, respectively), while in the case of control colonies, the number of dislodged Varroa mites remained comparable with the pre-treatment levels across the studied time intervals (Table 1). The interaction effects of organic acids and days after the first treatment were significant, indicating that the number of dislodged mites varied across treated colonies during different days. The per cent brood infestation reduced after the first treatment (on day 7) of all the treatments; however, the decrease was higher in the case of formic acid treatment. The per cent worker bee infestation declined to zero after the first treatment of all acids and remained the same till 28th day (Figure 1).

Organic acids have been widely utilised by beekeepers and researchers for the management of *V. destructor* in *A. mellifera* colonies[17,18]. A method similar to the present study for the application of formic acid was used by Rana *et al.*,[19] to study the effectiveness of formic acid (60%) sponge pads for the control of *V. destructor* in *A. mellifera* colonies. The sponge pads covered with perforated plastic were placed at the top bars, the bottom board and at both top and bottom boards, to evaluate the mite recovery at the 10th and 22nd day. The highest mite recovery was observed at 22nd day in the bottom board treatment (90.11 %), followed by 87.36 per cent on both top bars and bottom board and 83.53 per cent on top bars. Similarly, Toomemaa *et al.*,[20] evaluated oxalic acid for the control of *V. destructor* in *A. mellifera* at various concentrations of aqueous and sucrose solution. Among all the treatment combinations, an aqueous solution of 0.5 per cent oxalic acid (25 ml/comb) was found to be the most effective against mite control (99.01–99.42%) and was least toxic to the bees.

Effects of Organic Acids on the Infestation Levels of *Tropilaelaps clareae* in *Apis mellifera* colonies

The dislodged mites of *T. clareae* increased significantly on the 7th day after treatment with acetic, formic, and

oxalic acid (37.20, 41.40, and 29.80 mites, respectively), while in the case of control colonies, the dislodged mites showed non-significant differences, as shown in Table 2. In the formic acid treatment, the dislodged mites were significantly greater compared to the pre-treatment level after 14 days of the first treatment,

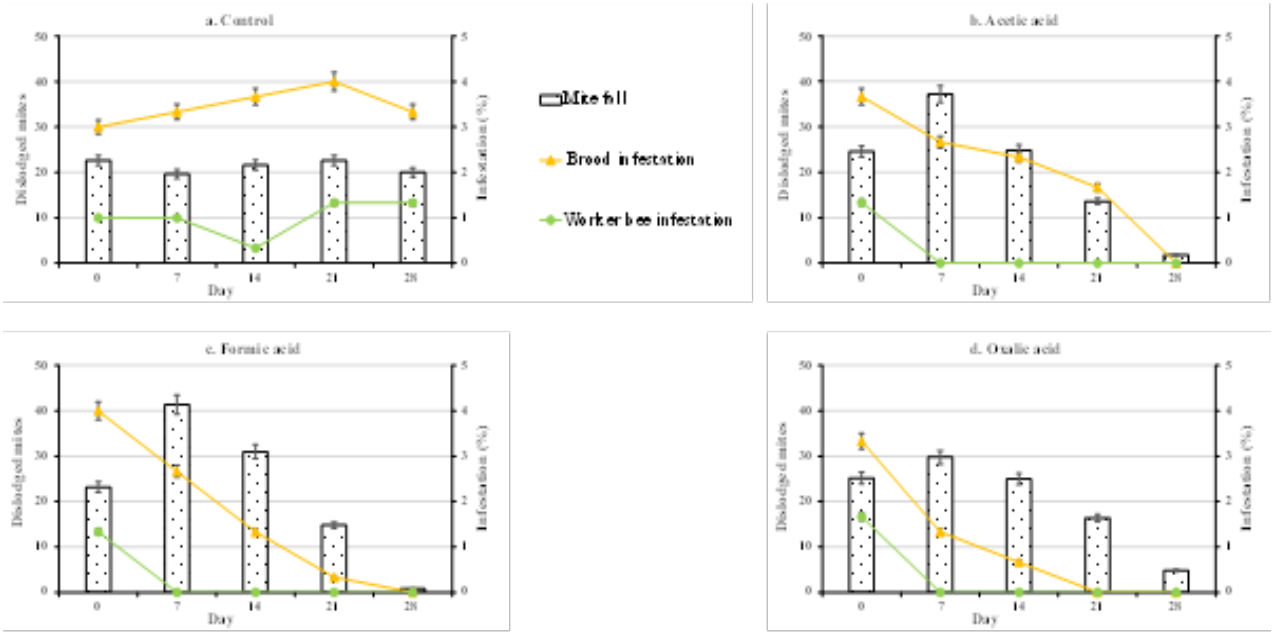


Figure 2. *Tropilaelaps clareae* infestation dynamics in *Apis mellifera* colonies subjected to organic acid treatment.

Table 2. Effect of organic acid treatments on the dislodgement of *Tropilaelaps clareae* in *Apis mellifera* colonies

Treatment	Dislodged Mites Before Treatment (Day 0)	Dislodged Mites After Indicated Days of First Treatment			Mean
		7	14	21	
Acetic acid	24.60 (5.05)	37.20 (6.18)	24.80 (5.08)	13.60 (3.81)	25.05 (5.03)
Formic acid	23.20 (4.91)	41.40 (6.50)	31.00 (5.65)	14.80 (3.92)	27.60 (5.25)
Oxalic acid	25.20 (5.11)	29.80 (5.55)	25.00 (5.09)	16.40 (4.16)	24.10 (4.98)
Control	22.60 (4.86)	19.60 (4.53)	21.60 (4.75)	22.60 (4.85)	21.60 (4.75)
Mean	23.90 (4.98)	32.00 (5.69)	25.60 (5.14)	16.85 (4.18)	

*Figures in parentheses are square root ($\sqrt{n+1}$) transformed values.

CD ($p \leq 0.05$)	
Treatment	0.21
Days	0.21
Treatment × Days	0.41

while other treatments showed non-significant differences. The interaction effects of organic acids and days after the first treatment were significant, indicating that the number of dislodged mites varied across treated colonies during different days. The percentage brood infestation started to decline after the 1st application of all treatments (Figure 2). The per cent worker bee infestation reduced in a similar pattern to that of *V. destructor* infestation and reached 0 per cent immediately after the first treatment. A significant reduction was observed after the first treatment of all acids in the worker bee infestation by *T. clareae*.

The concentrations of organic acids may also determine the extent of mite management in *A. mellifera* colonies. For instance, Raffique *et al.*,[21] tested formic acid (70%) at five concentrations (5, 10, 15, 20, and 25 ml/hive) against *T. clareae* in *A. mellifera* colonies. The efficacy increased with increasing quantities of formic acid, and after five weeks, 782 mites were recorded to have fallen on the bottom board in the case of 25 ml/hive treatment, which proved to be the most effective compared to the control group (99 fallen mites). Similarly, Alam *et al.*,[22] also reported formic acid (65%) to be 95.26 per cent effective compared to the control against *T. clareae* over a period of 3 weeks. Similarly, we reported an efficacy of 95.88 per cent for the formic acid against *T. clareae*.

Treatment Efficacies of Organic Acids

Overall, the formic acid exhibited the highest efficacies against both *V. destructor* and *T. clareae* in *A. mellifera* colonies (Table 3). Against *V. destructor*, an efficacy of 98.10 per cent was observed in the formic acid treatment, while it was 95.88 per cent against *T. clareae*. A significantly greater mean efficacy was observed in the case of formic acid (96.99 %) compared to oxalic acid (86.29%), while acetic acid exhibited an efficacy of 92.47 per cent, which was at par with both the treatments. The differences among species as well as the interaction between treatments and species were reported to be non-significant. The same acid may show different degrees of effectiveness under various methods of application. For instance, formic acid, when applied in the glass vials covered with cotton, showed an efficacy of 66.94 per cent[23], whereas in the present study, we recorded an efficacy of 98.10 per

Table 3. Efficacies of organic acids for the management of *Varroa destructor* and *Tropilaelaps clareae* in *Apis mellifera* colonies

Treatment	*V. destructor*	*T. clareae*	Mean
Acetic acid	93.17 (76.93)	91.77 (77.00)	92.47 (76.97)
Formic acid	98.10 (84.15)	95.88 (80.18)	96.99 (82.16)
Oxalic acid	94.62 (78.94)	77.97 (62.45)	86.29 (70.69)
Mean	95.30 (80.01)	88.54 (73.21)	

*Means in parentheses are angular transformed values.

CD ($p \leq 0.05$)	
Treatment	8.56
Species	NS
Treatment × Species	NS

cent against *V. destructor* when the acid was applied after impregnating it in a sponge. Similarly, different concentrations of oxalic acid, *viz.* 3.2% (94.84% efficacy) and 4.2% (92.68% efficacy) were found to be the most effective in controlling *Varroa* mite population as compared to the control, while formic acid applied at the rate of 10 ml per hive showed the least efficacy (54.13%) among all treatments[24].

Principal Component Analysis (PCA) for the Response of *V. destructor* and *T.clareae* to Organic Acid Treatments

The PCA biplot summarises the multivariate relationships among infestation parameters of *V. destructor* and *T. clareae* in response to organic acid treatments and the untreated control (Figure 3). The first two principal components together accounted for a major proportion of the total variability, indicating that dislodged mites or the mite fall (MF), brood infestation (BI), and worker bee infestation (WBI) effectively captured treatment-induced differences.

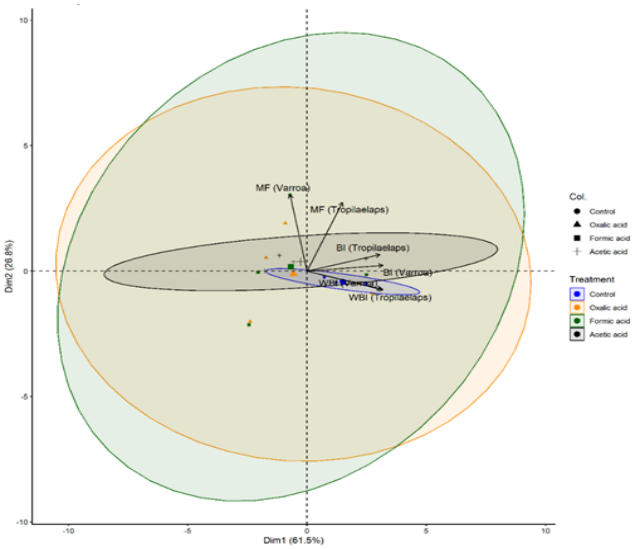


Figure 3. Principal Component Analysis (PCA) biplot for the response of *Varroa destructor* and *Tropilaelaps clareae* to organic acid treatments in *Apis mellifera* colonies

Clear separation was observed between treated colonies and the control, which reflects the influence of organic acids on mite infestation dynamics. Colonies treated with oxalic and formic acids were positioned farther from the control along the principal axes, suggesting a stronger association with increased dislodged mites and reduced brood and worker bee infestation for both mite species. Acetic acid showed an intermediate response, partially overlapping with the treated clusters. In contrast, control colonies were closely associated with vectors representing higher brood and worker bee infestation, indicating persistent mite pressure in the absence of treatment.

CONCLUSION

The study demonstrates that organic acids are effective in reducing infestations of *V. destructor* and *T. clareae* in colonies of *A. mellifera*. Among the tested treatments, formic acid showed superior performance, resulting in the highest mite dislodgement, greater reduction in brood infestation, and complete elimination of worker bee infestation shortly after application. Acetic and oxalic acids also proved effective, though comparatively less than formic acid. The absence of significant differences between mite species indicates that these treatments can be broadly applied for the management of multiple parasitic mites in honey bee colonies. Overall, formic acid emerges as the most reliable and efficient organic acid for mite control, offering a practical option for improving colony health under the tested conditions.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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AUTHORS CONTRIBUTION

PS: conducted the experiment, analyzed the data and wrote the original manuscript. SDS: conceptualized the experiment, methodology, reviewed and edited the manuscript. SKS and SS: reviewed and edited the manuscript.