Original Article

FIELD EVALUATION OF OXALIC ACID VAPORISATION FOR VARROA MITE (*VARROA DESTRUCTOR*) CONTROL IN EUROPEAN HONEY BEES (*APIS MELLIFERA*) COLONIES

Pierre Enock Philémon[1] ORCID: 0009-0005-1773-4785
Lekhnath Kafle[1]* ORCID: 0000-0002-5817-9483
Ravindra Chandra Joshi[2] ORCID: 0000-0002-0531-1276

[1]Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Taiwan
[2]Entomology, Philippine Rice Research Institute, Philippines

*corresponding author: kafleln@gmail.com
Received: 17 June 2025; accepted: 02 December 2025

Abstract

Varroa mites (*Varroa destructor*) are a major threat to honey bee health globally. Oxalic acid has gained popularity among beekeepers as a potential varroa mite control agent. This study evaluated the efficacy of oxalic acid vaporisation for managing varroa mites in field conditions in Taiwan. Twenty-four colonies of Italian honey bees (*Apis mellifera ligustica*) were divided into six groups, each consisting of four colonies: a control (no treatment), four treatment groups receiving two, four, six or eight oxalic acid treatments bi-weekly, and a group treated with Flumethrin strips (40 mg flumethrin/strip). Oxalic acid was vaporized at a rate of approximately 2 g per beehive. After thirty-five days, colonies receiving six and eight oxalic acid vaporisations showed significant reductions in varroa mite infestations, by 86% and 97%, respectively. Moreover, significantly higher worker bee mortality was observed in colonies receiving four, six, or eight oxalic acid vaporisations compared to the control. These findings suggest that six or eight oxalic acid (2 g/hive) vaporisations can effectively control varroa mite infestations in honey bees under field conditions, however beekeepers should be aware of potential impacts on worker bee mortality.

Key words: bee mortality, honey bee, mite control, oxalic acid, vaporisation, varroa mite

INTRODUCTION

The European honey bee (*Apis mellifera*) holds immense huge economic and ecological value globally, serving as the most prevalent pollinator for crops worldwide (Hung et al., 2018). In the United States, honey bees are the primary commercial pollinators for over one hundred crops, contributing over $18 billion annually to crop production. Furthermore, honey bee products generate an additional $700 million in value each year within the United States (Anonymous, 2021, 2022; Keel, 2022). Similar economic contributions are seen in Canada and the United Kingdom, with honey bee pollination contributing $7 billion and $640 million, respectively, annually to their economies. In Taiwan, approximately 167,000 European honey bee colonies are managed (Kafle & Chinkangsadarn, 2022), providing not only pollination services but also numerous consumer products, including honey, royal jelly, pollen, propolis, venom, and wax, which all have significant value in pharmaceuticals, food production, and various industrial applications (Mahmood et al., 2012). In 2021, Taiwan harvested 575 tons of royal jelly, 13,260 tons of honey, and 394 tons of honey bee wax (Kafle & Chinkangsadarn, 2022; Anonymous, 2023).

Despite their importance to ecosystems and economies, honey bees face numerous challenges related to management, transportation, nutrition, pesticides, diseases, and parasites (Hristov et al., 2020; Kafle & Chinkangsadarn, 2022). Among all factors, the ectoparasitic varroa mites (*Varroa destructor*), which feed on the bees' fat bodies and weaken their immune systems, represents one of the most serious parasites, causing significant colony losses and economic damage worldwide (Martin et al., 2012; Nazzi et al., 2012). Varroa mite infestations can have severe negative impacts on bees, including shortened lifespans, compromised metabolisms, suppressed reproductive capacities, and increased susceptibility to viral infections (Martin et al., 2012; Mondet et al., 2020; Warner et al., 2024). Varroa mites has been strongly linked to several honey bee viruses, including the Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Sacbrood Virus (SBV), Kashmir Bee Virus (KBV), and Israeli Acute Paralysis Virus (IAPV) (Boecking & Genersch, 2008; Mondet et al., 2014; Noël et al., 2020). Furthermore, varroa mites can indirectly harm honey bee colonies by reducing colony density, which in turn diminishes foraging and pollinating capability, reduces honey production, and weakens the bees, making them more susceptible to other environmental stressors (Warner et al., 2024). Untreated varroa mite infestations can lead to a steady decline and eventual collapse of honey bee colonies within a short period (Spivak & Reuter,2005; Barlow & Fell, 2006).

Varroa mite control can be achieved through the application of such synthetic chemicals as flumethrin, coumaphos, fluvalinate, and amitraz, as well as soft chemicals or organic acids including lactic acid, formic acid, and oxalic acid. Essential oils like thymol, menthol, camphor, and eucalyptol can also be used (Rosenkranz et al., 2010; Noël et al., 2020; Qadir et al., 2021). However, varroa mite control remains a significant challenge for beekeepers worldwide (Beyer et al., 2018; Haber et al., 2019; Oberreiter & Brodschneider, 2020), and a major one facing beekeepers globally is the development of resistance to some acaricides (Elzen & Westervelt, 2002; Rosenkranz et al., 2010; González-Cabrera et al., 2016).

In recent years, oxalic acid (OA) has been increasingly used for varroa mite control (Charriére & Imdorf, 2002; Jack et al., 2021). Oxalic acid ($C_2H_2O_4$) is a white crystalline solid with a molecular weight of 90.03 g/mol, a relative density of 1.90 g/cm³, a melting point of 101.5°C, and sublimates at 150°C (Adjlane et al., 2016). The OA is considered safe for use, leaves no residue, and honey bees can tolerate a 5% dosage (Bogdanov et al., 2002; Rademacher & Harz, 2006). It is easy to apply, inexpensive, no cases of honey bee poisoning have been documented, and it is extremely effective, particularly on broodless colonies (Mutinelli et al., 1997; Rademacher & Harz, 2006). In laboratory conditions, Milani (2001) found OA to be nearly 100% effective against varroa mites. However, Imdorf et al. (2003); Donders and Cornelissen (2005) advise against using OA during the honey production season, as it has limited impact on varroa mites within brood cells and may have minimal efficacy on mites overall.

The effectiveness of OA treatment can be influenced by various environmental conditions, including colony conditions, time of year, presence or absence of brood, and application methods (Rademacher & Harz, 2006). Optimal results are typically achieved when the weather is dry and cool, and colonies have few or no sealed broods (Rademacher & Harz, 2006). Several studies have indicated that OA's toxic effect is primarily on phoretic varroa mites, and therefore, it is most effective when administered during broodless periods (Gregorc & Planinc, 2001; Charriére & Imdorf, 2002; Gregorc & Planinc, 2002). During broodless periods, reported OA efficacy has ranged from 89.6% to 99.5% (Imdorf et al., 1997; Gregorc & Planinc, 2001, 2002, 2004; Rademacher & Harz, 2006; Akyol & Yeninar, 2009). Similarly, Gregorc and Planinc (2001, 2002, 2004) reported OA efficacy in brooded colonies of 52% in August and 39% in September, compared to 88.7%, 97%, and 99.4% in broodless colonies in November. Similarly, Gregorc and Poklukar (2003) observed 77.9% efficacy in colonies with sealed brood in September and 98.6% efficacy in broodless colonies in December. However, Mutinelli et al. (1997) reported 95% effectiveness in the presence of sealed brood after the applications of a 5% OA solution.

Despite its regular use in North America and Europe, no cases of varroa mite resistance to OA have been reported to date (Johnson et al., 2010; Maggi et al., 2017). Beekeepers worldwide apply OA through various methods, including trickling, spraying, and vaporisation/sublimation/fumigation (Al Toufailia et al., 2015). In Taiwan, beekeepers have also been using OA vaporisation for varroa mite control for the past several years, and similarly, no resistance has been reported. While most Taiwanese beekeepers vapor their colonies with OA four to ten times per season, the actual number of applications varies among individual beekeepers. Therefore, this study was conducted to determine the optimal frequency of OA vaporisation for controlling varroa mites in beehives under field conditions in Taiwan.

MATERIAL AND METHODS

Study site

This study was conducted at the NPUST Apiary, located at the National Pingtung University of Science and Technology (NPUST) in Neipu Township, Pingtung, Taiwan (22°38’42.9”N, 120°36’19.8”E, 73 meters above sea level), from August to September 2021. During the study period, the average wind speed was 8 km/h, the average temperature was 20°C, the total rainfall was 800 mm, and the average relative humidity was 78 %.

The NPUST Apiary was established in 2017 to support teaching and research activities. Since then, bee colonies have been continuously maintained according to the standard beekeeping practices employed by local beekeepers. These practices include controlling mites twice a year, (June and November), and providing a 50% sugar syrup feed during extended rainy periods and typhoons. Additionally, the queens are replaced annually at the beginning of the autumn season (October). The bees are housed in standard single-chamber Langstroth hives, which can accommodate a maximum of eight frames.

Honey bee colonies and treatments

Italian honey bees (*Apis mellifera ligustica*) naturally infested with varroa mites at varying levels were used in this study. One week prior to the study’s commencement, honey bee colonies were standardized with the visual Liebefeld estimation method to ensure uniformity in the number of worker bees (approximately 2500 worker bees/comb) and the brood area (50%) (Bhusal et al., 2011; Dainat et al., 2020). The Liebefeld Method relies on visually estimating the number of adult workers present on each side of the comb, as well as assessing the surface area occupied by various components, including open brood, capped brood, honey, and pollen stores (Dainat et al., 2020).

According to Dainat et al. (2020), experienced users could achieve an accuracy rate of over 96% in estimating adult worker population sizes, with capped brood estimations reaching 99% accuracy and open brood estimations at 65%.

The honey bees were housed in standard Langstroth hives (470 mm x 410 mm x 250 mm), with each colony maintained at a strength of eight combs (440 mm x 234 mm). A total of twenty-four honey bees’ colonies were divided into six treatment groups, with four colonies per group. The treatments consisted of a control group (no treatment), four groups receiving OA vaporisation at different frequencies (two, four, six and eight vaporisations administered bi-weekly on Sunday and Wednesday afternoons at 3 pm), and a group treated with Flumethrin strips (40 mg flumethrin/strip) (Tab. 1). Varroa mite counts were conducted every Saturday and Tuesday afternoon to assess the infestation level before each subsequent treatment. The OA vaporisation period varied depending on the treatment group and the study design, ranging from seven to 28 days (Tab. 1). During the study, we also monitored the situation of queen bee of the test hives especially checked her presence or absence. The specific method of OA vaporisation is described in the following section.

Oxalic acid vaporisation procedure

Oxalic acid was administered to the bee colonies as a vapor. The vaporisation process utilized OA crystals (99.6% purity, Star Oxochem Pvt. Ltd., Gujarat, India). Vapor was generated by heating the evaporisor (Fig. 1) to approximately 180°C. A locally manufactured vaporisor was used,

Table 1.

Treatments, application durations, and frequencies

SN	Treatments	Application duration (days)	Application frequency	No. of colonies used
1	Control (no treatment)	-	0	4
2	Flumethrin strip	28	4	4
3	Oxalic acid evaporation	7	2	4
4	Oxalic acid evaporation	14	4	4
5	Oxalic acid evaporation	21	6	4
6	Oxalic acid evaporation	28	8	4

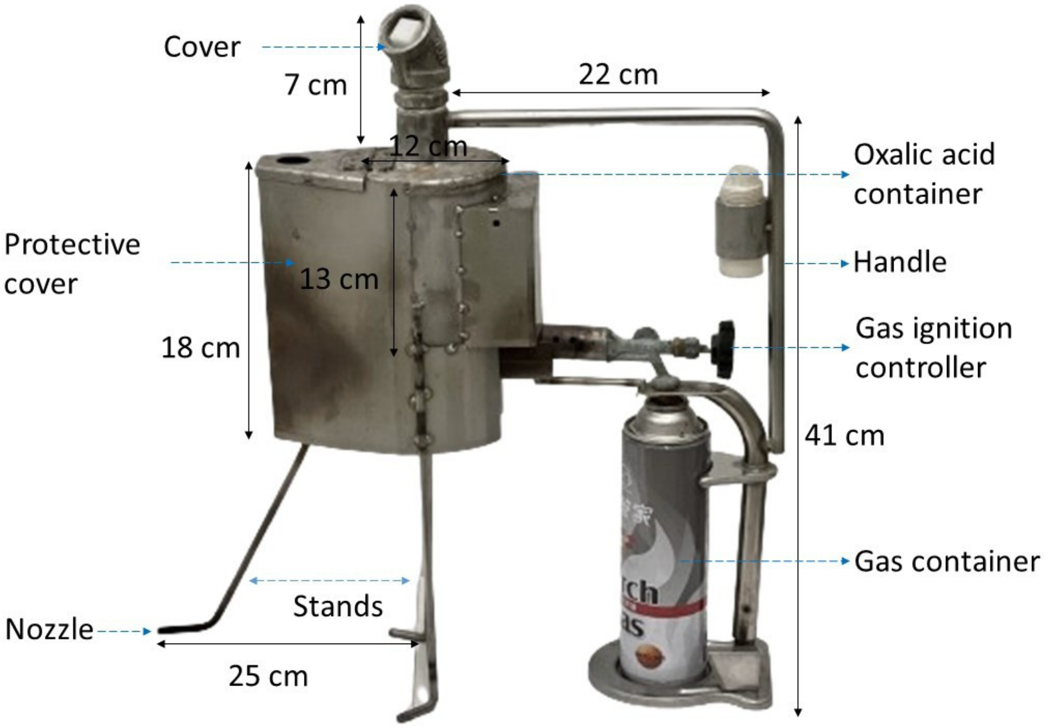

Fig. 1. Evaporisor used during the study with its major parts and measurements.

consisting of a steel container (12 cm diameter x 14 cm height) supported by three 20 cm legs. The entire apparatus was encased in a protective steel layer for operator safety. A tube (3.5 cm diameter) with grooves was securely attached to the container, designed to hold the OA. This tube was fitted with a tightly sealed metallic cover to prevent the escape of OA vapor. A gas container was connected to the vaporisor along with a fire igniter. Activating the igniter heated the bottom of the container, vaporising the OA within. The resulting hot vapor was then expelled through a nozzle located at the top of the container (Fig. 1).

For each treatment, approximately 2 grams of OA per colony was added to the vaporisor and securely capped (Fig. 2a). The specific amounts used each week varied based on the number of colonies being treated: week 1 (32 g for 16 beehives), week 2 (24 g for 12 beehives), week 3 (16 g for 8 beehives), and week 4 (8 g for 4 beehives). The gas supply was then turned on to heat the OA crystals within the vaporisor until vapor was produced and expelled from the nozzle. Prior to the application of the OA vapor, a standard smoker was used to gently smoke the hive entrance, encouraging the bees to remain within the hive. The nozzle of the vaporisor was then placed at the hive entrance for ten seconds, allowing sufficient OA vapor (generated from approximately 2 g of OA) to enter the hive (Fig. 2b, c). Applicators wore appropriate

Fig. 2. Oxalic acid vaporisation steps used during the study, a. Oxalic acid crystals prepared to keep in the vaporisor, b. Beehive prepared for the experiment with a plastic container to collect dead worker bees, c. Vaporing the experimental colonies.

personal protective equipment, including respirators with cartridges (Fig. 2c).

To mitigate potential stress caused by OA vaporisation, we provided 500 ml sugar water syrup (1:1 ratio) to honey bees fifteen minutes after each treatment. This is a common practice by beekeepers in Taiwan feeding 1:1 sugar water syrup after OA vapor exposures. We hypothesized that the sugar syrup would serve as an energy boost, strengthening the worker bees after vaporisation, and thereby increasing their grooming behaviors. This approach could potentially enhance the treatment's efficacy while minimizing any negative impacts on bee health. The sugar syrup may also have provided the bees with a readily available food, energy and water sources, potentially offsetting any stress associated with the treatment. Further research could investigate the specific role of the sugar syrup in mitigating the effects of OA vaporisation and its contribution to overall treatment success. It would also be beneficial to explore other potential stress-reducing strategies in conjunction with OA treatments.

Flumethrin strip treatment

Flumethrin strips (40 mg flumethrin/strip, Sichuan Wangshi Animal Health Co., Ltd., Chengdu, China) were used as a comparative treatment. Each strip was cut into four pieces, which were then placed between the 1st and 2nd, 3rd and 4th, 5th and 6th, and 7th and 8th combs within each hive. Every week, all four pieces were replaced with new ones, maintaining this schedule for four weeks.

Monitoring mite infestation on bee colonies

Varroa mite infestation levels for each colony were assessed twice weekly on Sunday and Wednesday afternoons using the sugar powder shake method as described by Macedo et al. (2002), with assessments conducted on 3, 7, 10, 14, 17, 21, 24, 28, 31 and 35 day after treatment. To calculate the efficacy of OA vaporisation in controlling varroa mites, the following equation (1) modified from (Al Toufailia et al., 2015; Chinkangsadarn et al., 2024) was used:

$$\mathbf{Efficacy\ (\%)}\ = \frac{\mathbf{a-b}}{\mathbf{a}}\ x\ \mathbf{100} \ldots\ldots\ldots\ldots\ldots..(\mathbf{1})$$

a = number of mites observed before first treatment

b = number of mites observed after treatment

Monitoring worker bee mortality

The treatments' impact on honey bee health was evaluated with the use of the method described by Vijaykumar and Shivshankar (2017). For a period of thirty-five days, a tray was placed in front of each hive entrance (Fig. 2b) to collect dead worker bees. Dead bees were collected and counted daily between 6:00 am and 8:00 am.

Statistical analysis

Statistical analysis was performed to evaluate the impact of the treatments on honey bee health using the method described by Vijaykumar and Shivshankar (2017). A one-way ANOVA was used to analyze the data, with Tukey's HSD post-hoc test used to determine statistical significance between treatments ($p<0.05$). To compare the effects of treatments, the percentages of mites observed before and after treatment were analyzed with the use of a paired t-test ($p<0.05$). The percentage data of varroa mites were transformed with the use of the arcsine square root method before the ANOVA was conducted. Lethal time (LT_{50}) of different treatments were calculated through probit analysis with StatPlus (Version 7) (2021).

RESULTS

Effect of oxalic acid on varroa mites

At thirty-five DAT (days after treatment), the percentage reduction in varroa mites was significantly higher in bee colonies treated with eight OA vaporisations compared to all other OA treatments, the Flumethrin strip treatment, and the control group ($F=165.84$; $DF=5$; $P<0.01$). The Flumethrin strip treatment and four OA vaporisation treatments or six OA vaporisation treatments did not significantly influence the percentage reduction of varroa mites (Fig. 3).

A comparison of varroa mite percentages at before treatment and 35 DAT, revealed a significant reduction in varroa mite percentages at 35 DAT in all bee colonies treated with the various OA vaporisation regimens and Flumethrin strip compared to mite percentages at before treatment (two OA vaporisation: $P<0.01$; four OA vaporisation: $P<0.01$; six OA vaporisation: $P<0.01$; eight OA vaporisation: $P<0.01$; Flumethrin strip: $P<0.01$). However, in in the control group the varroa mite percentages increased significantly at 35 DAT than the 0 DAT ($P<0.01$). The percentage reduction in varroa mites relative to before treatment ranged from 35.42% to 97.65%, depending on the number of OA vaporisations applied. Conversely, the control group (no treatment) experienced a 51.77% increase in varroa mite percentages (Tab. 2).

During the first week of the study, varroa mite percentages increased (14-20%) in all bee colonies treated with OA vaporisation. However, in the Flumethrin strip-treated colonies, mite percentages began to decline from the first week onward. After the second week, mite percentages began to decrease in all treated bee colonies, albeit at varying rates. In contrast, the control group's mite percentages continued to increase throughout the 35-day study period (Fig. 4).

Analysis of the lethal time (LT_{50}) values, which represent the time required to achieve a 50% reduction in mite population, revealed

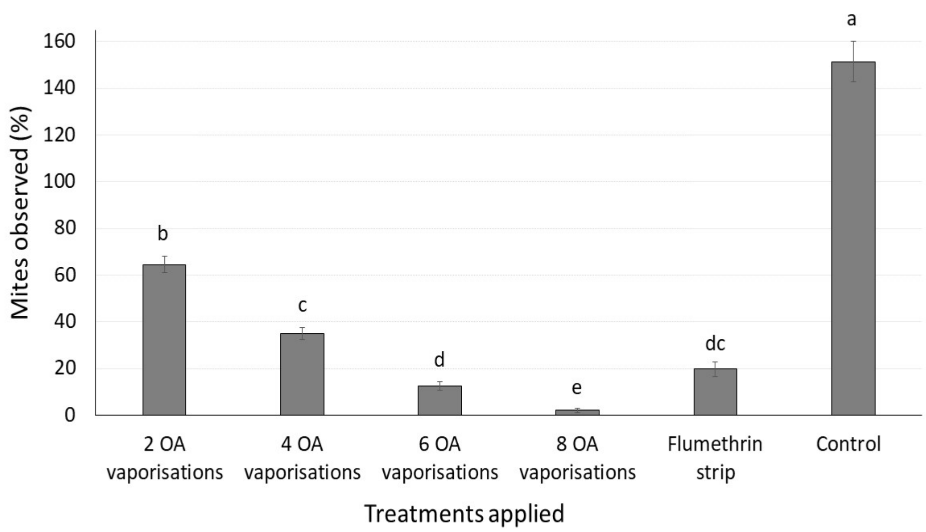

Fig. 3. Cumulative effects of different treatments on the *Varroa destructor* infestation on *Apis mellifera ligustica* under field conditions 35 days after treatment. OA=oxalic acid, Bars (M±SE) with different letters indicate statistically significant differences (n=4, Tukey's HSD, $p<0.05$)

Table 2.

Effects of different treatments on mite infestation before and after treatment

Treatments	Mites observed (%)*		
	Before treatment	After treatment	Increased (+) / Decreased (-)
Control (no treatment)	100 ±0.00a	151.77 ±6.23b	+ 51.77
Flumethrin strip	100 ±0.00a	20.59 ±2.34b	- 79.41
2 Oxalic acid evaporations	100 ±0.00a	64.58 ±2.50b	- 35.42
4 Oxalic acid evaporations	100 ±0.00a	34.90 ±1.87b	- 65.10
6 Oxalic acid evaporations	100 ±0.00a	13.22 ±1.55b	- 86.78
8 Oxalic acid evaporations	100 ±0.00a	2.35 ±0.79b	- 97.65

*Means in the same row followed by the different letters were significantly different (P<0.05) by paired T-test.

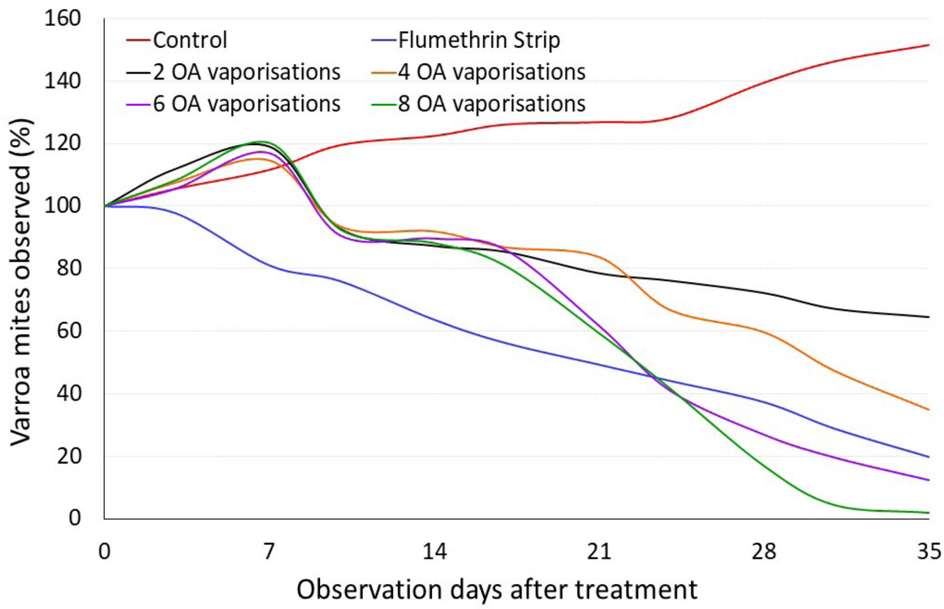

Fig. 4. Effects of different treatments on the *Varroa destructor* infestations at every observation day during 35 days under field conditions. OA=oxalic acid, (n=4)

differences in the speed of mite reduction among the treatments. Among the OA vaporisation treatments, the fastest reduction was observed with eight vaporisations (LT_{50}=20.74 days), followed by six vaporisations (LT_{50}=22.31 days), four vaporisations (LT_{50}=30.724 days), and finally two vaporisations (LT_{50}=52.56 days). Notably, the Flumethrin strip treatment had the lowest LT_{50} value (18.81 days), indicating a faster rate of mite reduction than even the eight OA vaporisation treatment.

Effect of oxalic acid vapors on bee mortality

At 35 DAT, total worker bee mortality was significantly higher in colonies treated with four, six, and eight OA vaporisations compared to the control group (F=14.64; DF=5; P=0.01). However, there were no significant differences in total worker bee mortality at 35 DAT among colonies treated with four, six, and eight OA vaporisations. Similarly, the total number of dead worker bees in the colonies treated with the Flumethrin strips was significantly different from those subjected to the four, six, and eight OA vaporisations. However, there was no significant difference in the total number of dead worker bees compared to the two OA vaporisations and control treatments at 35 DAT (Fig. 5).

Three distinct patterns of worker bee mortality emerged over the course of the study (Fig. 6). A higher rate of mortality was observed in colonies receiving four, six, or eight OA

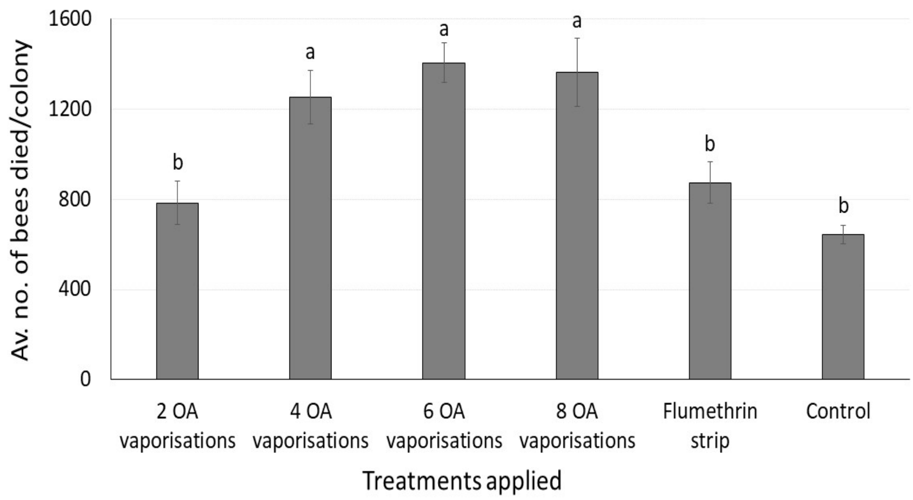

Fig. 5. Cumulative mortality of *Apis melifera ligustica* workers caused by different treatments under field conditions. OA=oxalic acid. Bars (M±SE) with different letters indicate statistically significantly differences (n=4, Tukey's HSD, p-value<0.05)

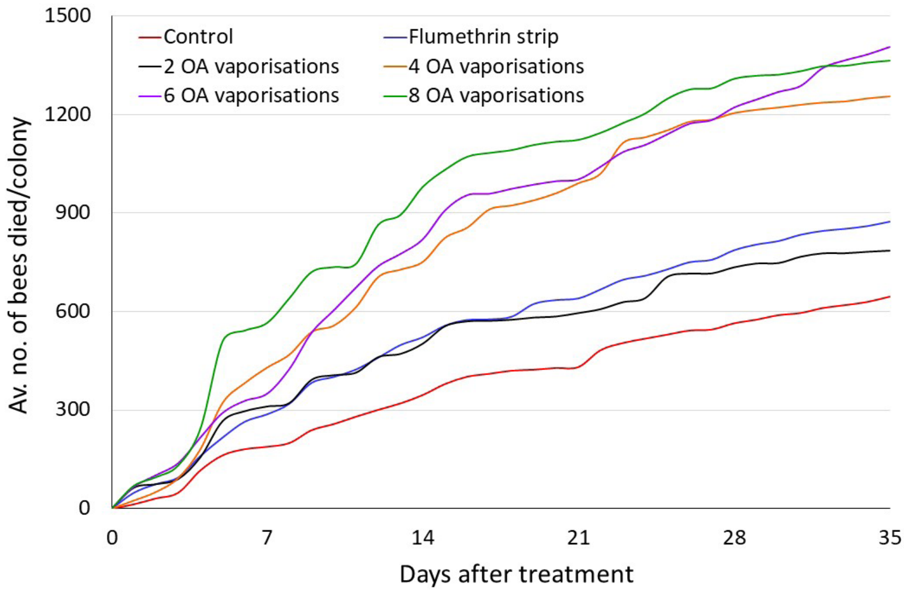

Fig. 6. Effects of different treatments on the mortality of *Apis melifera ligustica* workers at every observation day caused by different treatments under field conditions. OA=oxalic acid (n=4)

vaporisations, followed by colonies treated with Flumethrin strips or two OA vaporisations. The lowest mortality rate was consistently observed in the control colonies. The number of dead worker bees in the control colonies was approximately half the number observed in the four, six, and eight OA vaporisation groups (Fig. 6).

DISCUSSION

This study demonstrated that six and eight OA vaporisations effectively controlled 82% and 98% of varroa mites, respectively, after thirty-five days under field conditions. Several hypotheses exist regarding the precise mode of action of OA against varroa mites, including inhalation of the smoke or direct contact with the crystalline OA. Schneider et al. (2012) suggested that OA application stimulates honey bee grooming behavior, which in turn leads to the dislodgement and removal of varroa mites. It is theorized that as varroa mites fall from their hosts, OA vapors can penetrate the soft pads on their feet, entering their bloodstream and ultimately leading to their death due to the toxic effects of the acid. Additionally, OA vapor may damage the mouthparts of varroa mites (Ramsey et al., 2019), hindering their ability to feed on the honey bees' fat bodies and consequently causing them to starve. Numerous studies have supported the idea that varroa mites are killed through direct contact with the OA vapor (Aliano et al., 2006; Aliano & Ellis, 2008; Papežíková et al., 2017). Wallner and Fries (2003) proposed that the primary acaricidal effect of organic acids like OA is reduction in pH within the colony. This lower pH is thought to be well-tolerated by honey bees but detrimental to varroa mites, contributing to their mortality. This multifactorial mode of action, involving both direct contact toxicity and potential behavioral changes in the bees, may explain the observed effectiveness of OA vaporisation in controlling varroa mite populations. Further research is needed to fully elucidate the complex interactions among OA, honey bees, and varroa mites.

There have been no reported cases of resistance development in varroa mites against Flumethrin in Taiwan. However, the efficacy of Flumethrin strips in controlling varroa mites on bees has been observed to be lower than that of OA vaporization. Several factors may contribute to the superior efficacy of OA vaporisation compared to Flumethrin strips for mite control in beehives. The vaporised OA produces a mist that rapidly coats both bees and hive surfaces, effectively killing mites through direct contact, inhalation of the toxic gas blocking the spiracles and creating barriers for normal respirations. In contrast, Flumethrin strips require physical contact between the bees and the strip for the miticide to be effective. Additionally, OA coat created during fumigation stimulates increased grooming behavior among bees, which aids in dislodging mites from their bodies, a mechanism that differs from Flumethrin's primary mode of action, which involves paralyzing the mites. Furthermore, the vapor can penetrate uncapped brood cells where mites often hide, providing a significant advantage; the likelihood of mites encountering Flumethrin is considerably reduced in these protected environments. Beyond grooming and the effects of contact and inhalation of toxic gas or blocking respiration, OA vaporisation operates through multiple additional modes of action. These factors collectively enhance the efficacy of OA vaporisation against varroa mites compared to Flumethrin strips.

During the study, an initial increase in varroa mite numbers was observed in some colonies after OA vaporisation, but these numbers subsequently decreased, which could be explained by a couple of factors. First, non-genetic factors, such as mite age, may contribute to phenotypic resistance in varroa mites (Kirrane et al., 2012). Second, some varroa mites may either adopt a more protective phoretic position on their honey bee hosts or remain on individual bees that are exposed to lower concentrations of OA vapor. This behavior could reduce their exposure to the acaricide and contribute to their survival. Finally, some varroa mites might have entered the reproduction phase. The OA is known to be less effective against mites within capped brood cells, where they reproduce and develop, attached to their honey bee hosts. This means that mites within the brood cells are lesser exposed to the OA vapor, and some of them could survive and infest the adult bees. This highlights the importance of timing OA treatments to coincide with periods of low capped brood levels to maximize efficacy.

This study revealed significantly higher worker bee mortality in colonies treated with four, six, and eight OA vaporisations compared to the control group. One possible explanation for this increased mortality is that some worker bees, particularly those near the hive entrance, weaker bees, or those directly exposed to the intense heat of the OA vapors, may have succumbed to the treatment. Al Toufailia et al. (2015) also reported minor colony damage associated with OA vaporization. A healthy bee colony typically consists of over 20,000 worker bees. The daily mortality of approximately eighteen bees in the control group, and a maximum of forty bees in the group treated with six applications of OA vapor is unlikely to cause significant negative impacts on the colony's strength. This level of mortality can be easily compensated by the daily emergence of new worker bees within the hive. Furthermore, the practice of inducing broodless conditions to optimize OA vaporization treatment, as suggested by some researchers, may not be feasible for all beekeepers in every location (Jack et al., 2020). Our findings demonstrate that six OA vapor applications, even in the presence of brood, can be an effective treatment against varroa mite infestations on honey bees.

Throughout the study, the situation of queen was also monitored, and no queens' deaths or hive abandonments were observed, suggesting that the OA treatments, at the levels used in this study, did not negatively impact queen survival or colony cohesion.

As beekeepers globally seek improved and alternative methods for varroa mite control, OA vaporisation emerges as a promising and effective approach for reducing varroa mite infestations in honey bee colonies. The results of this study suggest that beekeepers can effectively manage varroa mite infestations by evaporising their colonies with OA (2 g/hive) twice per week for a total of six or eight treatments. This regimen demonstrated significant reductions in mite loads. Given that OA is a readily available, cost-effective, and easy-to-apply chemical, its vaporisation offers a viable and potentially superior alternative for varroa mite management compared to some other chemical treatments. This method allows beekeepers to target the mites effectively while minimizing the risks associated with harsher chemicals; however, it is important to consider the potential for increased bee mortality with higher vaporisation frequencies. Therefore, while six to eight treatments showed high efficacy, beekeepers should carefully monitor their colonies for signs of stress and adjust treatment frequency as needed. Further research is warranted to refine OA vaporisation protocols, including exploring the impact of different application methods, treatment timing in relation to brood cycles, and the potential synergistic effects of combining OA with other integrated pest management strategies. This will help ensure the long-term health and productivity of honey bee colonies while minimizing the development of acaricide resistance.

ACKNOWLEDGEMENT

We would like to thank the NPUST Bee Team members, especially Mr. Jhang Ni and Ms. Ramita Prajapati, for their assistances during this study.

Author contributions

Conceptualization, methodology, resources, L. K.; writing-review and editing L. K. and R. C. J.; experiments, software, validation, analysis, data curation, P. E. P.; writing-original draft preparation, P. E. P and L. K.

Declarations

Conflict of interest: The authors declare no conflict of interest.

Funding

No external funding is involved for this study.

Data Availability declaration

The data that support the findings of this study are available from the corresponding author [L. K.], upon reasonable request.

REFERENCES

Adjlane, N., Tarek, E.-O., Haddad, N. (2016). Evaluation of oxalic acid treatments against the mite *Varroa destructor* and secondary effects on honey bees *Apis mellifera. Journal of Arthropod-Borne Diseases, 10*(4), 501-509.

Akyol, E., & Yeninar, H. (2009). Use of oxalic acid to control *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies. *Turkish Journal of Veterinary and Animal Sciences, 33*(4), 285-288. https://doi.org/10.3906/vet-0712-16

Al Toufailia, H., Scandian, L., Ratnieks, F. L. (2015). Towards integrated control of varroa: 2) comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts. *Journal of Apicultural*

Research, 54(2), 108-120. https://doi.org/10.1080/00218839.2015.1106777

Aliano, N. P., & Ellis, M. D. (2008). Bee-to-bee contact drives oxalic acid distribution in honey bee colonies. *Apidologie, 39*(5), 481-487. https://doi.org/10.1051/apido:2008030

Aliano, N. P., Ellis, M. D., Siegfried, B. D. (2006). Acute contact toxicity of oxalic acid to *Varroa destructor* (Acari: Varroidae) and their *Apis mellifera* (Hymenoptera: Apidae) hosts in laboratory bioassays. *Journal of Economic Entomology, 99*(5), 1579-1582. DOI: 10.1603/0022-0493-99.5.1579

Anonymous. (2021, 2022.12.22). *Statistical Overview of the Canadian Honey and Bee Industry and the Economic Contribution of Honey Bee Pollination, 2021.* Agriculture and Agri-Food Canada. Retrieved 07.09 from https://agriculture.canada.ca/en/sector/horticulture/reports/statistical-overview-canadian-honey-and-bee-industry-2021

Anonymous. (2022). *National Pollinator Strategy: Pollinator Action Plan, 2021 to 2024* Retrieved from https://assets.publishing.service.gov.uk/media/62878a17d3bf7f1f4469542b/Pollinator_Action_Plan_2021_to_2024.pdf

Anonymous. (2023). *Agricultural Production.* Taipei, Taiwan: Ministry of Agriculture, ROC Retrieved from https://eng.moa.gov.tw/upload/files/eng_web_structure/2505724/2-3%E7%95%9C%E7%89%A7%E7%94%9F%E7%94%A2111(%E9%83%A8).pdf

Barlow, V. M., & Fell, R. D. (2006). Sampling methods for Varroa mites on the domesticated honeybee. *Virginia Cooperative Extention, 444,* 1-3. Retrieved from https://vtechworks.lib.vt.edu/server/api/core/bitstreams/61415c49-93b2-49e1-90a0-118f2be4ebea/content

Beyer, M., Junk, J., Eickermann, M., Clermont, A., Kraus, F., Georges, C., … Hoffmann, L. (2018). Winter honey bee colony losses, *Varroa destructor* control strategies, and the role of weather conditions: Results from a survey among beekeepers. *Research in Veterinary Science, 118,* 52-60. https://doi.org/10.1016/j.rvsc.2018.01.012

Bhusal, S. J., Kafle, L., Thapa, R. B., Shih, C.-J. (2011). Effect of colony strength on the performance of honeybees (*Apis mellifera*) in Nepal (Hymenoptera: Apidae). *Sociobiology, 58*(2), 435-447.

Boecking, O., & Genersch, E. (2008). Varroosis-the ongoing crisis in bee keeping. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 3*(2), 221-228. DOI: 10.1007/s00003-008-0331-y

Bogdanov, S., Charrière, J.-D., Imdorf, A., Kilchenmann, V., Fluri, P. (2002). Determination of residues in honey after treatments with formic and oxalic acid under field conditions. *Apidologie, 33*(4), 399-409. https://doi.org/10.1051/apido:2002029

Charriére, J.-D., & Imdorf, A. (2002). Oxalic acid treatment by trickling against *Varroa destructor*: recommendations for use in central Europe and under temperate climate conditions. *Bee World, 83*(2), 51-60. DOI:10.1080/0005772X.2002.11099541

Chinkangsadarn, S., Mendez, C. N., Clerger, F., Kafle, L. (2024). An assessment of the effectiveness of the thymol-based acaricide (Apiguard) gel against the Varroa mites on European honeybees in Pingtung, Taiwan. *Journal of Entomological Research, 48*(4), 436-442. DOI: 10.5958/0974-4576.2024.00087.9

Dainat, B., Dietemann, V., Imdorf, A., Charrière, J.-D. (2020). A scientific note on the 'Liebefeld Method' to estimate honey bee colony strength: its history, use, and translation. *Apidologie, 51*(3), 422-427. DOI: 10.1007/s13592-019-00728-2

Donders, J., & Cornelissen, B. (2005). Residue determination in honey after a spring treatment with thymovar and formic acid. *Apiacta, 40*(2005), 1-4.

Elzen, P., & Westervelt, D. (2002). Detection of coumaphos resistance in *Varroa destructor* in Florida. *American Bee Journal, 142*(4), 291-292.

González-Cabrera, J., Rodríguez-Vargas, S., Davies, T. E., Field, L. M., Schmehl, D., Ellis, J. D., … Williamson, M. S. (2016). Novel mutations in the voltage-gated sodium channel of pyrethroid-resistant *Varroa destructor* populations from the Southeastern USA. *PLoS ONE, 11*(5), 1-10. https://doi.org/10.1371/journal.pone.0155332

Gregorc, A., & Planinc, I. (2001). Acaricidal effect of oxalic acid in honeybee (*Apis mellifera*) colonies. *Apidologie, 32*(4), 333-340. https://doi.org/10.1051/apido:2001133

Gregorc, A., & Planinc, I. (2002). The control of *Varroa destructor* using oxalic acid. *The Veterinary Journal, 163*(3), 306-310. DOI: 10.1053/tvjl.2001.0675

Gregorc, A., & Planinc, I. (2004). Using oxalic acid for Varroa mite control in honeybee colonies during the beekeeping season. *Slovenian Veterinary Research, 41*(1), 35-39.

Gregorc, A., & Poklukar, J. (2003). Rotenone and oxalic acid as alternative acaricidal treatments for *Varroa destructor* in honeybee colonies. *Veterinary Parasitology, 111*(4), 351-360. DOI: 10.1016/s0304-4017(02)00408-9

Haber, A. I., Steinhauer, N. A., vanEngelsdorp, D. (2019). Use of chemical and nonchemical methods for the control of *Varroa destructor* (Acari: Varroidae) and associated winter colony losses in US beekeeping operations. *Journal of Economic Entomology, 112*(4), 1509-1525. DOI: 10.1093/jee/toz088

Hristov, P., Shumkova, R., Palova, N., Neov, B. (2020). Factors associated with honey bee colony losses: A mini-review. *Veterinary Sciences, 7*(4), 166. DOI: 10.3390/vetsci7040166

Hung, K.-L. J., Kingston, J. M., Albrecht, M., Holway, D. A., Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences, 285*(1879), 1-8. DOI: 10.1098/rspb.2017.2140

Imdorf, A., Charrière, J.-D., Kilchenmann, V., Bogdanov, S., Fluri, P. (2003). Alternative strategy in central Europe for the control of *Varroa destructor* in honey bee colonies. *Apiacta, 38*(2003), 258-278.

Imdorf, A., Charriere, J., Bachofen, B. (1997). Efficiency checking of the *Varroa jacobsoni* control methods by means of oxalic acid. *Apiacta, 32*(1997), 89-91.

Jack, C. J., van Santen, E., Ellis, J. D. (2020). Evaluating the efficacy of oxalic acid vaporization and brood interruption in controlling the honey bee pest *Varroa destructor* (Acari: Varroidae). *Journal of Economic Entomology, 113*(2), 582-588. DOI: 10.1093/jee/toz358

Jack, C. J., van Santen, E., Ellis, J. D. (2021). Determining the dose of oxalic acid applied via vaporization needed for the control of the honey bee (*Apis mellifera*) pest *Varroa destructor*. *Journal of Apicultural Research, 60*(3), 414-420. DOI: 10.1080/00218839.2021.1877447

Johnson, R. M., Ellis, M. D., Mullin, C. A., Frazier, M. (2010). Pesticides and honey bee toxicity-USA. *Apidolo*gie, 41(3), 312-331. DOI: 10.1051/apido/2010018

Kafle, L., & Chinkangsadarn, S. (2022). Beekeeping and bee product quality control in Taiwan. *Journal of Entomological Research, 46*(suppl), 1069-1077. DOI: 10.5958/0974-4576.2022.00182.7

Keel, C. C. (2022, 2022.06.22). *The Buzz About Pollinators.* U.S. Department of Agriculture. Retrieved 07.09 from https://www.usda.gov/media/blog/2022/06/22/buzz-about-pollinators

Kirrane, M. J., de Guzman, L. I., Rinderer, T. E., Frake, A. M., Wagnitz, J., Whelan, P. M. (2012). A method for rapidly marking adult varroa mites for use in brood inoculation experiments. *Journal of Apicultural Research, 51*(2), 212-213. DOI: 10.3896/IBRA.1.51.2.10

Macedo, P. A., Wu, J., Ellis, M. D. (2002). Using inert dusts to detect and assess varroa infestations in honey bee colonies. *Journal of Apicultural Research, 41*(1-2), 3-7. https://doi.org/10.1080/00218839.2002.11101062

Maggi, M. D., Damiani, N., Ruffinengo, S. R., Brasesco, M. C., Szawarski, N., Mitton, G., … Eguaras, M. J. (2017). The susceptibility of *Varroa destructor* against oxalic acid: a study case. *Bulletin of Insectology, 70*(1), 39-44.

Mahmood, R., Wagchoure, E. S., Raja, S., Sarwar, G. (2012). Control of *Varroa destructor* using oxalic acid, formic acid and bayvarol strip in *Apis mellifera* (Hymenoptera: Apidae) colonies. *Pakistan Journal of Zoology, 44*(6). 1473-1477.

Martin, S. J., Highfield, A. C., Brettell, L., Villalobos, E. M., Budge, G. E., Powell, M., … Schroeder, D. C. (2012). Global honey bee viral landscape altered by a parasitic mite. *Science, 336*(6086), 1304-1306. DOI: 10.1126/science.1220941

Milani, N. (2001). Activity of oxalic and citric acids on the mite *Varroa destructor* in laboratory assays. *Apidologie, 32*(2), 127-138. DOI: 10.1051/apido:2001118

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., … Le Conte, Y. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. *International Journal for Parasitology, 50*(6-7), 433-447. DOI: 10.1016/j.ijpara.2020.03.005

Mondet, F., de Miranda, J. R., Kretzschmar, A., Le Conte, Y., Mercer, A. R. (2014). On the front line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *Varroa destructor. PLoS Pathogens, 10*(8), e1004323. https://doi.org/10.1371/journal.ppat.1004323

Mutinelli, F., Baggio, A., Capolongo, F., Piro, R., Prandin, L., Biasion, L. (1997). A scientific note on oxalic acid by topical application for the control of varroosis. *Apidologie, 28*(6), 461-462. DOI:10.1051/apido:19970612

Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., … Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens, 8(6)*, 1-16. DOI: 10.1371/journal.ppat.1002735

Noël, A., Le Conte, Y., Mondet, F. (2020). *Varroa destructor*: how does it harm *Apis mellifera* honey bees and what can be done about it? *Emerging Topics in Life Sciences, 4*(1), 45-57. https://doi.org/10.1042/ETLS20190125

Oberreiter, H., & Brodschneider, R. (2020). Austrian COLOSS survey of honey bee colony winter losses 2018/19 and analysis of hive management practices. *Diversity, 12*(3), 1-27. DOI: 10.3390/d12030099

Papežíková, I., Palíková, M., Kremserová, S., Zachová, A., Peterová, H., Babák, V., Navrátil, S. (2017). Effect of oxalic acid on the mite *Varroa destructor* and its host the honey bee *Apis mellifera. Journal of Apicultural Research, 56(4)*, 400-408. DOI: 10.1080/00218839.2017.1327937

Qadir, Z. A., Idrees, A., Mahmood, R., Sarwar, G., Bakar, M. A., Ahmad, S., … Li, J. (2021). Effectiveness of different soft acaricides against honey bee ectoparasitic mite *Varroa destructor* (Acari: Varroidae). *Insects, 12*(11), 1032. DOI:10.3390/insects12111032

Rademacher, E., & Harz, M. (2006). Oxalic acid for the control of varroosis in honey bee colonies - a review. *Apidologie, 37*(1), 98-120. DOI: 10.1051/apido:2005063

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., … Ellis, J. D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences, 116*(5), 1792-1801.

Rosenkranz, P., Aumeier, P., Ziegelmann, B. (2010). Biology and control of *Varroa destructor. Journal of Invertebrate Pathology, 103*(Suppl. 1), S96-S119. DOI: 10.1016/j.jip.2009.07.016

Spivak, M., & Reuter, G. (2005). A sustainable approach to controlling honey bee diseases and Varroa mites. *Sustainable Agriculture Research and Education, 2005*, 1-6. Retrieved from https://hdl.handle.net/11299/182082

Vijaykumar, P. V., & Shivshankar, P. C. (2017). Monitoring on impact of insecticides on mortality of honey bees (*Apis mellifera* L.) in front of beehives. *Journal of Applied and Natural Science, 9*(2), 905-911. DOI: 10.31018/jans.v9i2.1296

Wallner, K., & Fries, I. (2003). Control of the mite *Varroa destructor* in honey bee colonies. *Pesticide Outlook, 14*(2), 80-84. https://doi.org/10.1039/b301510f

Warner, S., Pokhrel, L. R., Akula, S. M., Ubah, C. S., Richards, S. L., Jensen, H., Kearney, G. D. (2024). A scoping review on the effects of Varroa mite (*Varroa destructor*) on global honey bee decline. *Science of the Total Environment, 906*(3), 167492. DOI: 10.1016/j.scitotenv.2023.167492