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Impacts of oxalic acid, thymol, and potassium citrate as *Varroa* control materials on some parameters of honey bees

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Abstract: Various treatments are currently available for the control of *Varroa* mites. Some of these treatments depend on using natural and nonchemical compounds. In this study, the impacts of some materials (oxalic acid, thymol, and potassium citrate) on the survival of honey bees and some quality parameters were investigated under laboratory conditions. The potential impacts of different feeding types (sugar syrup, sugar candy, honey candy, honey jelly, and creamed honey) mixed with the tested *Varroa* control materials on honey bees were also studied. The study showed that using high percentages of oxalic, thymol, or potassium citrate can passively impact honey bees while using 0.5% is more preferable. The study also proved that feeding type, either as a liquid or solid, except for honey jelly, has no undesirable impacts on the survival or studied parameters of honey bee workers and drones. Drones fed on potassium regardless of feeding type were able to survive significantly at day 4 more so than drones fed on oxalic acid or thymol. Investigations into the role of potassium in enhancing the survival of bee drones and in controlling *Varroa* mites are recommended.

Key words: Honey bees, *Varroa*, thymol, oxalic acid, potassium

1. Introduction

Most of the problems related to bee health are caused by the parasitic mite *Varroa destructor*. This mite can cause severe damage to honey bee colonies including bee infection with viruses, weakness, and loss of bee colonies (1,2). As reviewed by Abou-Shaara (3), there are different materials available to control this mite, including the use of mechanical methods, essential oils, or oxalic acid. The use of oxalic acid or thymol has shown efficacy against *Varroa* mites. The efficacy of oxalic acid ranges from 70.12% to 93.4% (4,5). Oxalic acid solutions of 0.5%, 2.9%, 3%, and 3.2% have shown efficacy against *Varroa* mites (6–9). Rashid et al. (10) found that the efficacy of 3.2% oxalic acid was higher (mean: 95.81%) than concentrations of 4.2% (mean: 81%) or 2.1% (mean: 46%). Additionally, 3% oxalic acid showed higher efficacy against *Varroa* than formic acid (11). Concerning thymol, it has high efficacy against *Varroa* populations with reduction rates of 61.7% (12) and 96.77% for powdered thymol (13), while thymol oil or thymol blended with essential oils has mite mortality of more than 90% (14). According to the American Bee Journal, the Environmental Protection Agency registered potassium salts as a treatment against *Varroa* in 2015 (http://americanbeejournal.com/epa-registers-new-biochemical-miticide-to-combat-varroa-mites-in-beehives/). It could be said that oxalic acid, thymol, and potassium salts can be used as safe and effective *Varroa* control materials.

Oxalic acid, thymol, and potassium can be applied as mixes with sugar syrup or in other ways. For example, Marinelli et al. (15) compared the use of oxalic acid by trickling or vaporization and as cellulose strips soaked in a solution of oxalic acid. They found that the first two methods were better than cellulose strips. Mutinelli et al. (16) found the efficacy of sprayed 5% (w/v) oxalic acid to be higher than that of a topical application. Rashid et al. (10) applied oxalic acid in sugar syrup at 4.2%, 3.2%, and 2.1%, and similar work was done using 2.9% oxalic acid (17). Thymol has been used in powdered form (13) or as oil (14), and it can be mixed with candy (18). Potassium salt is generally used mixed with syrup. The impacts of mixing these materials with different feeding types on honey bees need to be investigated. Honey bee colonies can be provided with different feeding types, including sugar syrup, creamed honey, honey candy, or sugar candy (19,20). Previous studies have shown no harmful impacts of oxalic acid on honey bees (5,10,21), while other studies showed the presence of negative impacts, especially in

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the case of high concentrations (6). Thymol has been shown to have a negative impact on bee drones (22), while Charpentier et al. (23) found no harmful impact of thymol on early larval stages. The effects of potassium citrate on honey bees are not well known. More insights into the effects of these materials on honey bees are required. The objective of this study was to present insights into the impacts of oxalic acid, thymol, and potassium citrate on honey bee workers and drones. The impacts of different concentrations as well as feeding type mixed with these materials on honey bees were investigated. Based on the results obtained, specific recommendations about the future application of these materials are suggested.

2. Materials and methods

The study was performed during the spring of 2016 at the laboratory of the Institute of Apiculture, Animal Production Research Centre (VÚŽV), Slovakia. Carniolan honey bee workers of age approximately less than 21 days were collected from brood combs from the apiary of the Institute of Apiculture and were used in the experiments. Drones were collected from the lateral combs using forceps. Honey bees were anesthetized using CO_2 prior to insertion into the cages. The bees were left at room temperature (about 18 ± 1 °C) until the end of the experiments.

2.1. Cages used in the study

The cages used in the experiments were made using petri dishes and pieces of plastic papers. The dimensions of the cages are shown in Figure 1. The cages were provided with ventilation holes covered with mesh. Perforated Eppendorf tubes were fixed in the holes on the upper cover of the cages. These tubes were used for providing honey bees with sugar syrup only or syrup mixed with *Varroa* control materials as described later.

2.2. Impacts of *Varroa* control materials on honey bee workers

Some common materials used in *Varroa* control were tested, namely oxalic acid, thymol oil, and potassium citrate monohydrate. These materials were mixed with sugar syrup (1:1 sugar and water) at different percentages of 0.5%, 3%, and 6%. Thus, 12 treatments were investigated besides two control groups; the first group contained bees without feeding and the second contained bees provided only with sugar syrup (1:1). For each treatment three cages were used and in each cage 10 workers were placed with a total of 30 workers per treatment. The impacts of these materials on some parameters of honey bees were then measured.

2.3. Impacts of feeding type mixed with *Varroa* control materials on honey bee workers and drones

The lower concentration (0.5%) of the tested materials was mixed with different feeding types to investigate the impacts of the feeding types mixed with these materials on honey bees. These feeding types were sugar syrup (1 sugar : 1 water), honey candy (honey saturated with powdered sugar), sugar candy (water saturated with powdered sugar, 1 water : 4 sugar), honey jelly (200 g of liquid honey mixed with 10 g of gelatin dissolved in 50 mL of water), and creamed honey (i.e. fully crystalized honey). Thus, five treatments for each material were used besides two control groups: bees without any feeding and bees feed only on sugar syrup. In this experiment, 10 workers and 5 drones were used with a total of 30 workers and 15 drones per treatment. About 4 g of feeding type was added per cage. Some parameters were then determined and compared.

2.4. Measured parameters

Bee survival was determined by counting the number of surviving bees every day, and dead bees were removed

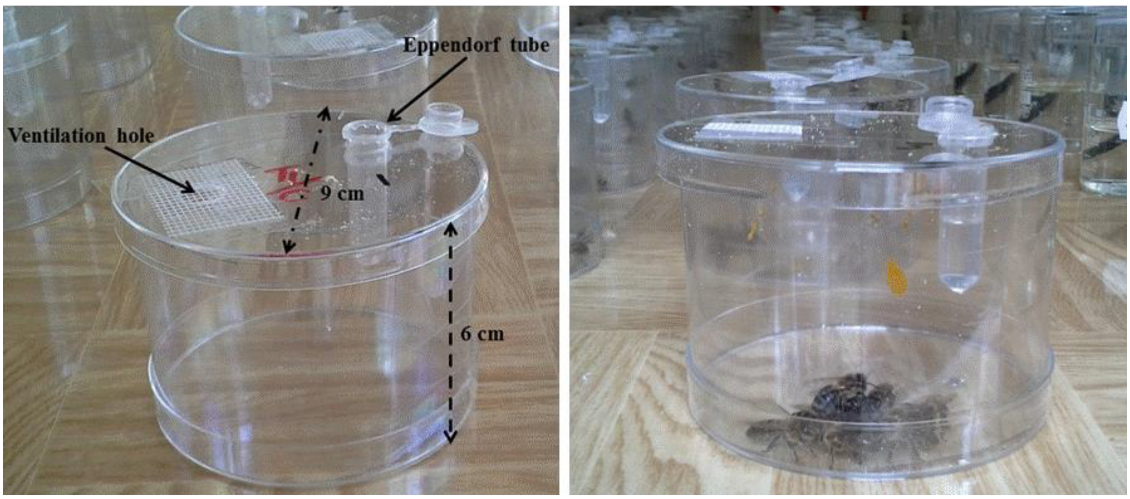

Figure 1. Dimensions and structure of cages used in the study.

daily from the cages. Fresh weight, dry weight, body water content, head weight, and the developmental degree of the hypopharyngeal glands were determined. On day 4, these parameters were determined for the control and test groups. From each cage two freshly dead bees were used to measure body parameters for a total of six bees per group. The fresh weight of the bees (W1) was obtained using a balance (KERN ABJ 220/4NM, Germany) to the nearest 0.01 or 0.001 g. Bees were then placed in an oven at 100 °C for 48 h to obtain their dry weights (W2) as done by Abou-Shaara (24). Body water content was calculated as (W1 – W2/W1) × 100. The heads of some bees were separated and first weighed to the nearest 0.001 g. The developmental degree of the hypopharyngeal glands was then recorded using a scale (25) that contains 4 degrees, ranging from degree I (undeveloped glands) to degree IV (fully developed glands).

2.5. Statistical analysis

For all the measured parameters the means and their standard errors (SEs) were calculated. All mean values were expressed to the nearest 0.01 g, except head weight, which was expressed to the nearest 0.001 g. The percentages were converted to degrees using arcsine transformation prior to the statistical analysis. The means of control and test groups were compared using Duncan's multiple range test$_{0.05}$ after ANOVA. For each group three cages (replicates) were adopted in a completely random design. The SAS 9.1.3 program was used to perform the statistical analysis. Readings for days 6 and 7 for experiment one as well as days 5 and 6 for experiment two were not taken due to specific conditions.

3. Results

3.1. Impacts of *Varroa* control materials on honey bee workers

Figure 2 shows that bees fed only on sugar syrup were able to survive better than other test groups over the 8 days. The impact of the control groups and treatments on the survival of the workers was significant (DF = 10, F = 3.09, P = 0.0012, P < 0.05) over the 8 days. The impact was also significant at each day from day 2 to 8 (DF = 54, F = 16.78, P < 0.0001). All used materials had a passive impact on the survival ability of the honey bees. The mean percentage of surviving bees declined, respectively, by 100% and 33.33% for unfed bees and bees fed on sugar syrup; 95.24%, 100%, and 100% for groups of oxalic acid 0.5%, oxalic acid 3%, and oxalic acid 6%; 75%, 100%, and 100% for groups of thymol 0.5%, thymol 3%, and thymol 6%; and 65.19%, 95.65%, and 100% for groups of potassium 0.5%, potassium 3%, and potassium 6% (Figure 3). The results showed that increasing the percentage of test materials caused a high reduction in the percentage of surviving bees over 8 days. Potassium citrate monohydrate at 0.5% had the lowest impact on the survival of honey bees in comparison with the other treatments, followed by 0.5% thymol and 0.5% oxalic acid. Bees without feeding, bees fed on 3% thymol, and bees fed on 6% thymol died by day 5, while those fed on oxalic 3% acid, 6% oxalic acid, and 6% potassium died by day 8. The remaining groups were able to survive up to day 8.

No significant differences were found among measured parameters for bees belonging to the test groups, as shown in Table 1. This indicates that the measured parameters

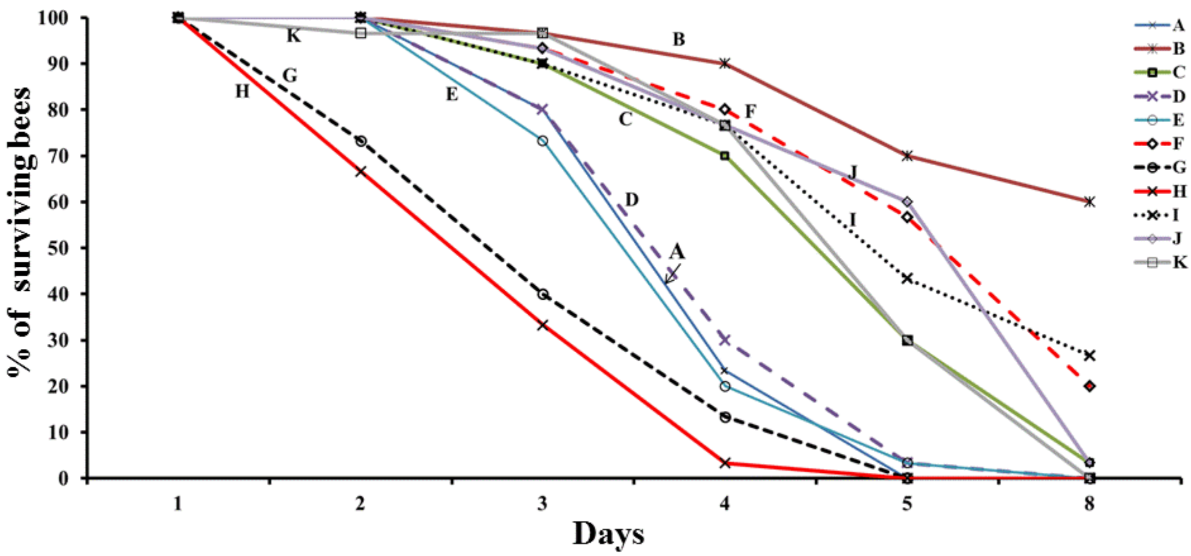

Figure 2. Mean percentage of surviving bees fed on sugar syrup mixed with different treatments over 8 days. A: Bees without any feeding, B: sugar syrup, C: oxalic acid 0.5%, D: oxalic acid 3%, E: oxalic acid 6%, F: thymol 0.5%, G: thymol 3%, H: thymol 6%, I: potassium citrate 0.5%, J: potassium citrate 3%, and K: potassium citrate 6%.

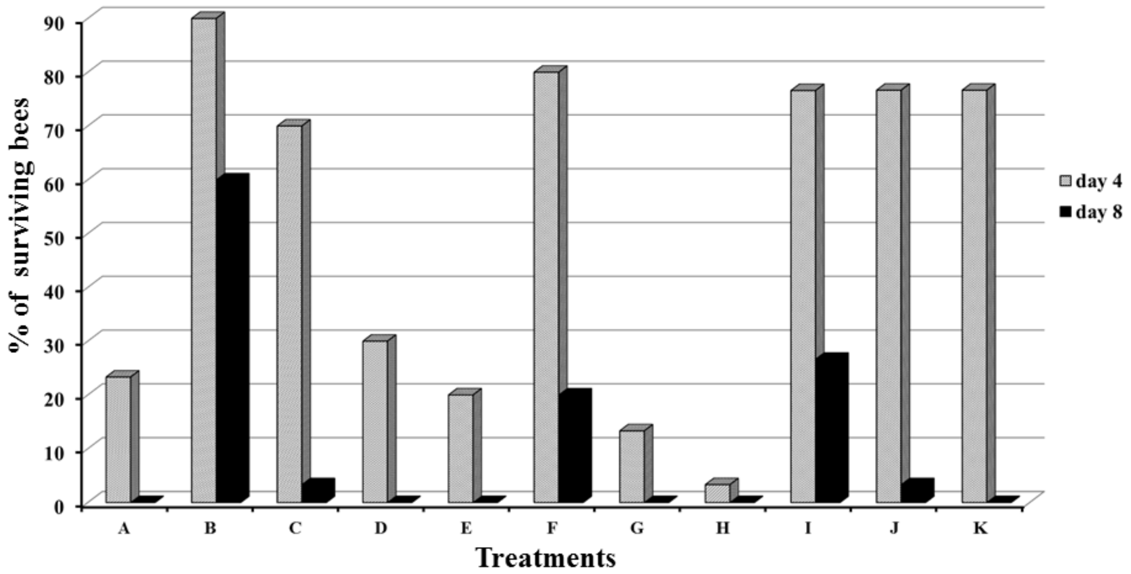

Figure 3. Mean percentage of surviving bees at days 4 and 8 after the treatments. A: Bees without any feeding, B: sugar syrup, C: oxalic acid 0.5%, D: oxalic acid 3%, E: oxalic acid 6%, F: thymol 0.5%, G: thymol 3%, H: thymol 6%, I: potassium citrate 0.5%, J: potassium citrate 3%, and K: potassium citrate 6%.

Table 1. Means ± SEs of measured parameters of honey bee workers after 4 days of feeding on different materials mixed with sugar syrup. *: Means followed by the same letter within the same column are not significantly different according to the Duncan test$_{0.05}$.

Treatment	Mean ± SE*				
	Fresh weight (g)	Dry weight (g)	Body water (%)	Head weight (g)	Gland dev. degree (degree)
Without feeding	0.09 ± 0.01 a	0.03 ± 0.003 a	70.33 ± 4.41 a	0.013 ± 0.00 a	3.00 ± 0.00 a
Sugar syrup	0.09 ± 0.01 a	0.03 ± 0.003 a	61.33 ± 5.36 a	0.012 ± 0.001 a	3.00 ± 0.00 a
Oxalic 0.5%	0.12 ± 0.01 a	0.03 ± 0.005 a	74.66 ± 1.85 a	0.012 ± 0.001 a	3.00 ± 0.00 a
Oxalic 3%	0.11 ± 0.01 a	0.03 ± 0.00 a	72.33 ± 3.17 a	0.012 ± 0.001 a	3.00 ± 0.00 a
Oxalic 6%	0.11 ± 0.01 a	0.04 ± 0.01 a	67.33 ± 7.31 a	0.011 ± 0.001 a	3.00 ± 0.00 a
Thymol 0.5%	0.10 ± 0.006 a	0.04 ± 0.003 a	63.33 ± 4.91 a	0.013 ± 0.001 a	3.00 ± 0.00 a
Thymol 3%	0.09 ± 0.01 a	0.03 ± 0.0 a	65.66 ± 4.33 a	0.011 ± 0.001 a	3.00 ± 0.00 a
Thymol 6%	0.11 ± 0.01 a	0.04 ± 0.003 a	65.33 ± 6.74 a	0.01 ± 0.00 a	1.66 ± 0.33 b
Potassium 0.5%	0.10 ± 0.01 a	0.03 ± 0.00 a	70.66 ± 2.9 a	0.011 ± 0.001 a	3.00 ± 0.00 a
Potassium 3%	0.09 ± 0.01 a	0.03 ± 0.00 a	65.00 ± 4.35 a	0.01 ± 0.001 a	3.00 ± 0.00 a
Potassium 6%	0.09 ± 0.008 a	0.03 ± 0.003 a	71.33 ± 5.2 a	0.01 ± 0.001 a	2.33 ± 0.66 a

were not impacted by materials added to the bee feeding (i.e. sugar syrup) except for the developmental degree of hypopharyngeal glands. The fresh weight ranged from 0.09 to 0.12 g, the dry weight ranged from 0.03 to 0.04 g, body water percentage ranged from 61.33% to 74.66%, and head weight ranged from 0.010 to 0.013 g. Bees fed on 6% thymol had significantly less developed glands (about degree I) than glands of bees belong to all the other treatments (degree III).

3.2. Impacts of feeding type mixed with *Varroa* control materials on honey bee workers

The impact of the feeding types on survival of the bees was not significant (DF = 13, F = 1.35, P =0.1873, P > 0.05) over 7 days. However, at each day from day 2 to 7, the impact was significant (DF = 56, F = 23.50, P < 0.0001). As shown in Figure 4, bees of all the treatments were able to survive up to day 7, except bees without any feeding, which survived only up to day 4. The percentage of surviving bees declined from day 4 to day 7 by 100%, 100%, 100%, 91.31%, 100%, 87.5%, 68.18%, 100%, 94.45%, 84.62%, 82.14%, 100%, and 81.82% for groups fed on sugar syrup, honey candy with oxalic acid, sugar candy with oxalic acid, honey jelly with oxalic acid, creamed honey with oxalic acid, honey candy with thymol, sugar candy with thymol, honey jelly with thymol, creamed honey with thymol, honey candy with potassium, sugar candy with potassium, honey jelly with potassium, and creamed honey with potassium, respectively (Figure 5). Therefore, the least decline in percentage of bee survival was recorded for the group of bees fed on sugar candy with thymol, followed by creamed honey with potassium, honey candy with potassium, and honey candy with potassium. It could be said that potassium mixed with different feeding forms, except honey jelly, did not show a passive impact on bee survival ability compared with the other treatments, except thymol mixed with sugar candy.

Measured parameters differed among test groups, as shown in Table 2. For fresh weight, the highest and the lowest means were for sugar candy and honey jelly, creamed honey and honey jelly, and honey candy and honey jelly for the oxalic acid, thymol, and potassium groups, respectively. It is clear that honey jelly groups had the lowest body weights among the test groups. The means of the control groups were higher than the means recorded for bees fed on honey jelly. Concerning dry weight, bees fed on honey jelly also had lower means in the oxalic acid and potassium groups, but bees fed on honey candy did in the case of the thymol group. High similarity was detected for head weights for bees fed with different feeding types, but lower means were recorded for honey candy in the thymol group and sugar candy and honey jelly for the potassium group. In general, it could be said that, apart from honey jelly, all other feeding types showed high similarities without significant differences from the control groups.

3.3. Impacts of feeding type mixed with *Varroa* control materials on honey bee drones

The control treatments and feeding types had insignificant impact on the survival of bee drones (DF = 13, F = 0.38, P

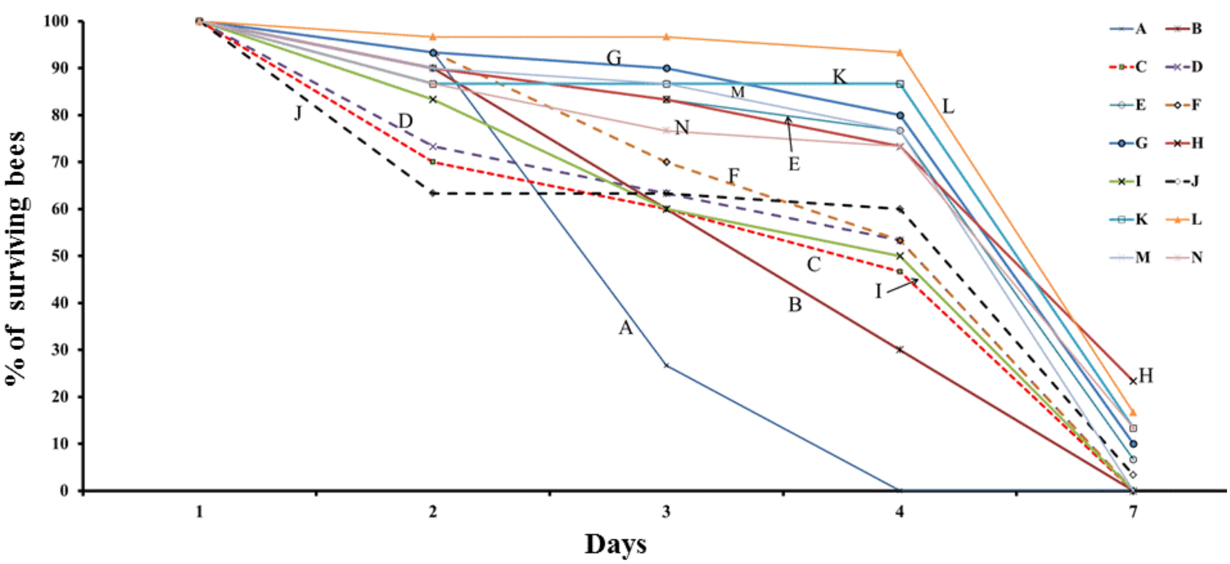

Figure 4. Mean percentage of surviving bees fed on various feeding types over 7 days. A: Bees without feeding, B: sugar syrup, C: honey candy with oxalic acid, D: sugar candy with oxalic acid, E: honey jelly with oxalic acid, F: creamed honey with oxalic acid, G: honey candy with thymol, H: sugar candy with thymol, I: honey jelly with thymol, J: creamed honey with thymol, K: honey candy with potassium citrate, L: sugar candy with potassium citrate, M: honey jelly with potassium citrate, and N: creamed honey with potassium citrate.

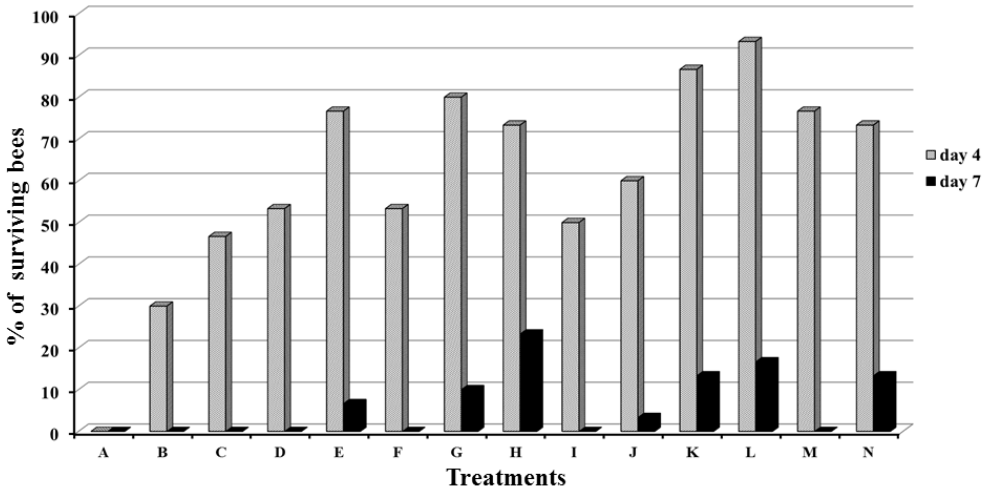

Figure 5. Mean percentage of surviving bees at days 4 and 7 after the treatments. A: Bees without feeding, B: sugar syrup, C: honey candy with oxalic acid, D: sugar candy with oxalic acid, E: honey jelly with oxalic acid, F: creamed honey with oxalic acid, G: honey candy with thymol, H: sugar candy with thymol, I: honey jelly with thymol, J: creamed honey with thymol, K: honey candy with potassium citrate, L: sugar candy with potassium citrate, M: honey jelly with potassium citrate, and N: creamed honey with potassium citrate.

Table 2. Means ± SEs of measured parameters at day 4 of bee workers fed on different feeding types. *: Means followed by the same letter within the same column are not significantly different according to the Duncan test$_{0.05}$.

Treatment		Mean ± SE*			
		Fresh weight (g)	Dry weight (g)	Body water (%)	Head weight (g)
Control	Without feeding	0.10 ± 0.01 abcd	0.03 ± 0.002 abc	70.33 ± 1.45 a	0.012 ± 0.0003 abc
	Sugar syrup	0.10 ± 0.003 abcd	0.02 ± 0.001 c	74.66 ± 0.33 a	0.01 ± 0.002 abc
Oxalic acid	Honey candy	0.10 ± 0.02 abcd	0.03 ± 0.004 abc	68.00 ± 2.51 a	0.01 ± 0.0006 abc
	Sugar candy	0.13 ± 0.01 a	0.04 ± 0.003 a	71.33 ± 1.45 a	0.01 ± 0.003 abc
	Honey jelly	0.08 ± 0.002 cd	0.02 ± 0.0006 c	67.33 ± 0.33 a	0.01 ± 0.0005 abc
	Creamed honey	0.12 ± 0.002 ab	0.03 ± 0.002 abc	74.33 ± 0.88 a	0.013 ± 0.0003 a
Thymol	Honey candy	0.09 ± 0.01 bcd	0.02 ± 0.002 bc	68.66 ± 0.88 a	0.009 ± 0.0005 bc
	Sugar candy	0.10 ± 0.008 abcd	0.029 ± 0.001 abc	71.00 ± 1.15 a	0.01 ± 0.001 abc
	Honey jelly	0.09 ± 0.01 abcd	0.03 ± 0.004 abc	63.00 ± 8.50 a	0.01 ± 0.001 abc
	Creamed honey	0.11 ± 0.008 abc	0.03 ± 0.0005 ab	69.00 ± 2.30 a	0.01 ± 0.00 ab
Potassium citrate	Honey candy	0.11 ± 0.01 abcd	0.03 ± 0.001 abc	69.00 ± 6.50 a	0.01 ± 0.0003 abc
	Sugar candy	0.08 ± 0.002 cd	0.02 ± 0.0001 c	67.33 ± 1.76 a	0.008 ± 0.002 b
	Honey jelly	0.07 ± 0.004 d	0.02 ± 0.001 c	64.33 ± 1.76 a	0.009 ± 0.001 bc
	Creamed honey	0.09 ± 0.02 abcd	0.03 ± 0.002 abc	61.33 ± 13.71 a	0.01 ± 0.003 abc

= 0.9741, P > 0.05) over 4 days. However, the impact was significant (DF = 41, F = 2.14, P = 0.0017, P < 0.05) at each day from day 2 to 4. Drones of most treatments died by day 4, similar to the control group without feeding, except for the sugar syrup, honey candy with thymol, honey candy with potassium, sugar candy with potassium, honey jelly with potassium, and creamed honey with potassium groups (Figure 6). It is clear that drones fed on potassium regardless of feeding type were able to survive significantly at day 4 more so than drones of the other groups. Only drones in the sugar syrup group and honey candy with thymol group were able to survive until day 4, together with all treatments of potassium. However, feeding types within each treatment (oxalic acid, thymol, or even potassium) had no clear impact on drones' survival ability.

In general, no significant differences were detected among drones fed with different feeding types (Table 3). Only honey candy in the thymol group showed significantly less fresh body weight than the other groups. Drones without feeding or fed on honey candy with thymol had significantly less dry body weight than the other groups. Moreover, the honey candy with potassium group had significantly less body water percentage than the other groups, while no significant differences were detected by test groups in regard to head weight. Unlike worker bees, honey jelly did not show any adverse impact on the body parameters of drones.

4. Discussion

4.1. Impacts of *Varroa* control materials on honey bee workers

The results showed that bees fed on oxalic acid, thymol, or potassium citrate had less survival ability than bees fed only on sugar syrup. Increasing the percentage of tested *Varroa* control materials (from 0.5% to 3% or 6%) caused a higher reduction in the percentage of surviving bees. Therefore, using 0.5% can be considered as less harmful to honey bees, especially with potassium citrate versus the other two materials. In a similar way, Toomemaa et al. (6) found that a low concentration of oxalic acid (0.5%) had high efficacy against *Varroa* mites without negative impacts on bees while higher concentrations (1.0% and 1.5%) had high toxicity for bees. On the contrary, Rashid et al. (10) found that oxalic acid (3.2%) was effective against *Varroa* mites and caused no bee mortality. Concerning thymol, Chiesa (13) found impacts of powdered thymol on bee mortality while another study (26) found no impact of Apiguard (thymol-based material) on the survival of adult bees. Costa et al. (18) found that bees fed on thymol survived significantly longer than those fed on sugar syrup. Thymol oil was used in the present study, unlike previous studies, which used powdered thymol. This could help in explaining the negative impacts of thymol on honey bees found in the present study. It seems that potassium had less negative impacts on the bees than oxalic acid and thymol. The

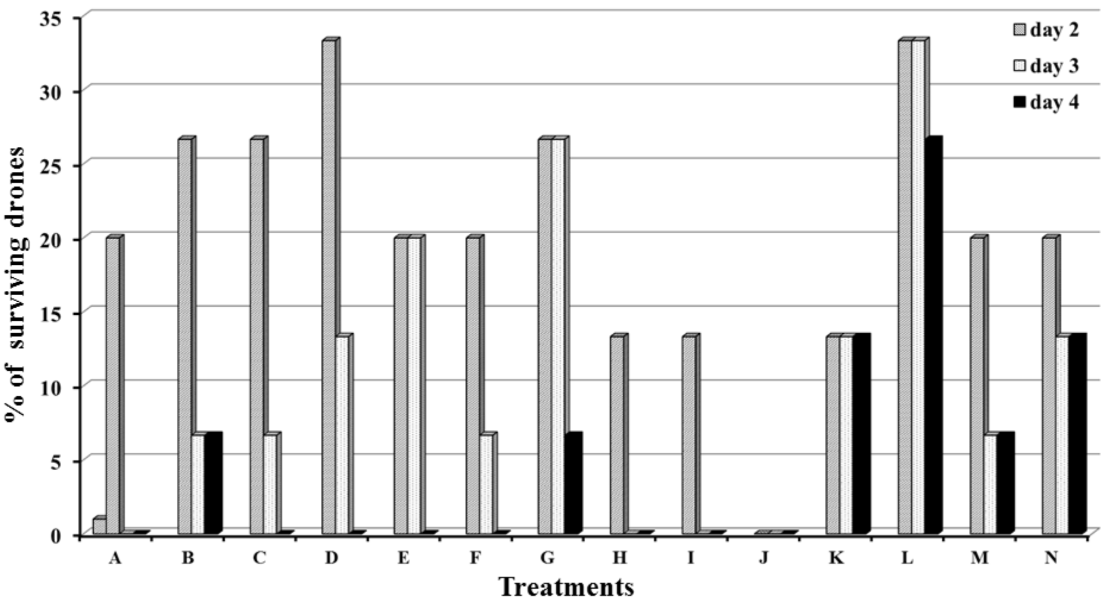

Figure 6. Mean percentage of surviving bee drones at days 2, 3, and 4 after the treatments. A: Bees without feeding, B: sugar syrup, C: honey candy with oxalic acid, D: sugar candy with oxalic acid, E: honey jelly with oxalic acid, F: creamed honey with oxalic acid, G: honey candy with thymol, H: sugar candy with thymol, I: honey jelly with thymol, J: creamed honey with thymol, K: honey candy with potassium citrate, L: sugar candy with potassium citrate, M: honey jelly with potassium citrate, and N: creamed honey with potassium citrate.

Table 3. Means ± SEs of measured parameters at day 4 of bee drones fed on different feeding types. *: Means followed by the same letter within the same column are not significantly different according to the Duncan test$_{0.05}$.

Treatment		Mean ± SE*			
		Fresh weight (g)	Dry weight (g)	Body water (%)	Head weight (g)
Control	Without feeding	0.18 ± 0.02 ab	0.05 ± 0.003 bc	68.66 ± 1.76 a	0.01 ± 0.001 a
	Sugar syrup	0.21 ± 0.02 a	0.06 ± 0.001 ab	66.33 ± 2.60 a	0.01 ± 0.001 a
Oxalic acid	Honey candy	0.19 ± 0.03 a	0.06 ± 0.008 abc	67.33 ± 2.02 a	0.01 ± 0.001 a
	Sugar candy	0.21 ± 0.01 a	0.06 ± 0.005 ab	68.33 ± 3.17 a	0.01 ± 0.001 a
	Honey jelly	0.20 ± 0.02 a	0.05 ± 0.0001 bc	70.00 ± 4.04 a	0.015 ± 0.002 a
	Creamed honey	0.17 ± 0.01 ab	0.05 ± 0.002 abc	66.00 ± 1.52 a	0.01 ± 0.0001 a
Thymol	Honey candy	0.13 ± 0.02 b	0.04 ± 0.002 de	62.33 ± 4.66 ab	0.01 ± 0.001 a
	Sugar candy	0.16 ± 0.01 ab	0.06 ± 0.004 abc	62.33 ± 0.66 ab	0.01 ± 0.001 a
	Honey jelly	0.18 ± 0.005 ab	0.07 ± 0.007 a	57.33 ± 4.91 ab	0.01 ± 0.0006 a
	Creamed honey	0.19 ± 0.02 ab	0.06 ± 0.004 abc	66.00 ± 6.50 a	0.01 ± 0.0003 a
Potassium citrate	Honey candy	0.15 ± 0.01 ab	0.07 ± 0.008 ab	52.00 ± 7.02 b	0.01 ± 0.002 a
	Sugar candy	0.16 ± 0.01 ab	0.06 ± 0.001 ab	57.33 ± 6.88 ab	0.01 ± 0.0003 a
	Honey jelly	0.18 ± 0.01 ab	0.06 ± 0.003 abc	65.33 ± 1.45 ab	0.01 ± 0.0003 a
	Creamed honey	0.18 ± 0.008 ab	0.06 ± 0.002 abc	63.66 ± 0.66 ab	0.01 ± 0.001 a

tested materials showed no clear impact on the measured body parameters, suggesting few physiological impacts on the bees. Only bees fed on 6% thymol had significantly less developed glands than those of other treatments. This could be explained by the negative physiological impacts of the high concentration (6%) of thymol on honey bees, a point that needs further investigations.

4.2. Impacts of feeding type mixed with *Varroa* control materials on honey bee workers

Only unfed bees were not able to survive for a long period while all the other groups were able to survive up to day 7. All potassium treatments showed no clear impact on honey bees, except honey jelly. On the contrary, Waller (27) found that bees were not attracted to sugar syrups containing potassium salts. However, this study confirmed that bees can utilize feeding types containing potassium citrate. Thymol and potassium impacted the survival of the bees less than the oxalic acid. Another study (28) showed that using oxalic acid to control *Varroa* mites caused mortality of worker bees at a high percent in the case of using trickling or spraying methods other than a sublimation method. This proves the negative impact of oxalic acid on the survival of bee workers. The impacts of oxalic acid on honey bees can be explained by its negative impact on bee cuticle proteolytic enzymes (29) or due to its high acidity (15). All tested feeding types, in general, showed no impact on bees except honey jelly. Measured body parameters also did not differ among test groups, except the honey jelly group, which had the lowest means. Perhaps honey jelly had a more solid nature, which prevented bees from utilizing nutrients from it. Therefore, this specific feeding had the lowest means for measured body parameters. Apart from honey jelly, this experiment presents evidence that using 0.5% oxalic acid, thymol, or potassium had no negative impact on measured body parameters.

4.3. Impacts of feeding type mixed with *Varroa* control materials on honey bee drones

Drones in the potassium group and in the honey candy with thymol group as well as the sugar syrup group were able to survive more than the other groups (survived only up to day 4). In general, the survival of drones in potassium groups regardless of feeding type was significantly higher at day 4 than drones of the other groups. This indicates a specific role of potassium in enhancing the survivorship of drones, a point worth further investigation. As found with worker bees, oxalic acid impacted the survival of the drones more than thymol and potassium. This can be considered as additional evidence of the negative impacts of oxalic acid on honey bees. Test groups did not show an impact on measured body parameters, except with honey candy with thymol or potassium. Another study (22) showed that oxalic acid and thymol significantly impacted body weight of drones versus a control group. However, the impacts of tested materials in general were not high. Contrary to the results with bee workers, the honey jelly group did not show any adverse impacts on the measured body parameters of the drones. This may be because the drones received their feeding from the attendant bee workers.

4.4. Conclusions

The study showed that using a high percentage of oxalic acid, thymol, or potassium citrate in bee feeding is not recommended, while using 0.5% has few adverse impacts on honey bees. The study also proved that feeding type, either as liquid (sugar syrup) or solid (honey candy, sugar candy, or creamed honey, except honey jelly), has no undesirable impacts on the survival or measured parameters of honey bees. Honey jelly as a solid form of honey is not advisable to be mixed with *Varroa* control materials. It is clear that drones fed on potassium regardless of feeding type were able to survive significantly at day 4 more so than drones fed on oxalic or thymol. Investigations into the role of potassium in enhancing the survival of bee drones and in controlling *Varroa* mites are required.

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References

1. Chen Y, Pettis JS, Evans JD, Kramer M, Feldlaufer MF. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. Apidologie 2004; 35: 441-448.
2. Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of *Varroa destructor*. J Invert Pathol 2010; 103: 96-119.
3. Abou-Shaara HF. Continuous management of *Varroa* mite in honey bee, *Apis mellifera*, colonies. Acarina 2014; 22: 149-156.
4. Škerl MIS, Nakrst M, Žvokelj L, Gregorc A. The acaricidal effect of flumethrin, oxalic acid and amitraz against *Varroa destructor* in honey bee (*Apis mellifera carnica*) colonies. Acta Vet Brno 2011; 80: 51-56.
5. Akyol E, Yeninar H. Use of oxalic acid to control *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies. Turk J Vet Anim Sci 2009; 33: 285-288.
6. Toomemaa K, Martin AJ, Mänd M, Williams IH. Using oxalic acid in water solution in control of Varroa mites and its influence on honey bees. Agron Res 2010; 8: 345-350.
7. Gregorc A, Planinc I. Dynamics of falling *Varroa* mites in honeybee (*Apis mellifera*) colonies following oxalic acid treatments. Acta Vet Brno 2004; 73: 385-391.
8. Higes M, Meana A, Suárez M, Llorente J. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. Apidologie 1999; 30: 289-292.
9. Mahmood R, Wagchoure ES, Raja S, Sarwar G. Control of *Varroa destructor* using oxalic acid, formic acid and bayvarol strip in *Apis mellifera* (Hymenoptera: Apidae) colonies. Pak J Zool 2012; 44: 1473-1477.
10. Rashid M, Wagchoure ES, Mohsin AU, Raja S, Sarwar G. Control of ectoparasitic mite *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies by using different concentrations of oxalic acid. J Anim Plant Sci 2012; 22: 72-76.
11. Cornelissen B, Gerritsen L. Swarm prevention and spring treatments against *Varroa destructor* in honey bee colonies (*Apis mellifera*). Proc Neth Entomol Soc Meet 2006; 17: 133-139.
12. Al-Zarog AA, El-Bassiouny AM. Influence of some plant extracts on *Varroa* mite and performance of honey bee *Apis mellifera* colonies. Egypt Acad J Biol Sci 2013; 5: 15-20.
13. Chiesa F. Effective control of varroatosis using powdered thymol. Apidologie 1991; 22: 135-145.

14. Imdorf A, Bogdanov S, Ochoa RI, Calderone NW. Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie 1999; 30: 209-228.

15. Marinelli E, Formato G, Vari G, De Pace FM. Varroa control using cellulose strips soaked in oxalic acid water solution. Apiacta 2006; 41: 54-59.

16. Mutinelli F, Baggio A, Capolongo F, Piro R, Prandin L, Biasion L. A scientific note on oxalic acid by topical application for the control of varroosis. Apidologie 1997; 28: 461-462.

17. Gregorc A, Planinc I. Use of thymol formulations, amitraz, and oxalic acid for the control of the *Varroa* mite in honey bee (*Apis mellifera carnica*) colonies. J Apic Sci 2012; 56: 61-69.

18. Costa C, Lodesani M, Maistrello L. Effect of thymol and resveratrol administered with candy or syrup on the development of *Nosema ceranae* and on the longevity of honeybees (*Apis mellifera* L.) in laboratory conditions. Apidologie 2010; 41: 141-150.

19. Barker RJ. Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees. J Nutr 1977; 107: 1859-1862.

20. Abd Elhamid AM, Abou-Shaara HF. Producing clover and cotton creamed honey under cooling conditions and potential use as feeding to honey bee colonies. J Apic 2016; 31: 135-142.

21. Pileckas V, Švirmickas GJ, Razmaitė V, Paleckaitis M. Efficacy of different ecological methods for honeybee (*Apis mellifera*) Varroa prevention in spring. Vet Med Zoot 2012; 59: 65-70.

22. Shouky RS, Khattaby AM, El-Sheakh AA, Abo-Ghalia AH, Elbanna SM. Effect of some materials for controlling *Varroa* mite on the honeybee drones (*Apis mellifera* L.). Egypt J Agric Res 2013; 91: 825-834.

23. Charpentier G, Vidau C, Ferdy JB, Tabart J, Vetillard A. Lethal and sub-lethal effects of thymol on honeybee (*Apis mellifera*) larvae reared in vitro. Pest Manag Sci 2014; 70: 140-147.

24. Abou-Shaara HF. Thermal tolerance characteristics of two honey bee races. J Agr Urban Entomol 2015; 31: 1-8.

25. Hess G. Über den Einfluß derWeisellosigkeit und des Fruchtbarkeitsvitamins E auf die Ovarien der Bienenarbeiterin. Beihefte zur Schweizerischen Bienen-Zeitung 1942; 1: 33-110 (in German).

26. Mattila HR, Otis GW, Daley J, Schulz T. Trials of Apiguard, a thymol-based miticide part 2. Non-target effects on honey bees. Am Bee J 2000; 140: 68-70.

27. Waller GD. Evaluating responses of honey bees to sugar solutions using an artificial-flower feeder. Ann Entomol Soc Am 1972; 65: 857-862.

28. Al Toufailia H, Scandian L, Ratnieks FL. 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. J Apic Res 2015; 54: 108-120.

29. Strachecka A, Paleolog J, Olszewski K, Borsuk G. Influence of amitraz and oxalic acid on the cuticle proteolytic system of *Apis mellifera* L. workers. Insects 2012; 3: 821-832.