Sustainable varroa mite (*Varroa destructor)* control in field conditions

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Abstract

Experiments assessed the comparative efficacy of oxalic acid solution (OA) and combination of formic acid (FA) evaporation and trickling OA to control the honey bee mite, *Varroa destructor,* at two apiary locations. Queen caging, consecutive OA treatments of broodless colonies, or combined treatments using OA and FA in colonies with brood increased mite mortality ($P < 0.05$) in comparison to the pre-treatment period. FA application in colonies with brood in September and October resulted in an average mite mortality of 18% in the first apiary and subsequent FA applications in the same period in the second apiary killed, on average, 73% and 71% of mites; respectively. OA treatment of broodless colonies after queen caging at two apiaries resulted in 18% and 47% mite mortality. Caging the queens and OA treatments in broodless colonies or subsequently use consecutive OA or FA treatments ensure adequate mite reduction before wintering the colonies. Synergistic control methods of *Varroa* mites using OA and FA along with queen caging is discussed.

Beekeeping, mite control, oxalic acid, formic acid, queen caging

Organic acids with high acaricidal activity include formic acid (FA) that is effective against adult and reproductive mites (Rosenkranz et al. 2010), and oxalic acid (OA) effective against adult mites on honey bees (Morrison and Savage 2003). Both FA and OA are used to control varroa mites (*Varroa destructor*). They can be applied alone or in combination with biotechnical measures including drone brood removing and queen caging (Giacomelli et al. 2016; Gregorc and Sampson 2019). Artificial brood interruption by caging the queen is a biotechnical method to expose varroa mites on adult bees that are not able to reproduce in brood cells. After total brood removal, mites on adult bees are exposed to OA treatments (Büchler et al. 2020; Underwood and López-Uribe 2021). It was also established that total brood removal decreased varroa control costs, but on the other hand, increased labour time per colony, and some loss of honey production was recorded (Mancuso et al. 2020). In Central Europe and other countries, FA is used extensively to reduce mite populations in honey bee colonies (Satta et al. 2005). In one study, FA treatment alone killed 43% of mites on adult workers, but was less effective in autumn when colonies received double fumigation with 65% FA (Wilson and Collins 1993). Absorbent cardboard plates (Illertissen mite plates) soaked with 20 ml of 65% FA induced 94% mite mortality in Schwarzwald (Black Forest) bee colonies (Hoppe et al. 1989). Varroa control at the levels of 95–97% can be achieved with longer mite exposures to 85% concentrations of FA impregnating soft fibre plates (Pavatex) inserted to the hive's bottom (Imdorf et al. 1996). Comparative experiments with repeated short term applications of FA or OA (sublimation) during the spring colonies development demonstrate that each of these acaricides killed, on average, 10–20% of mites in whole colonies containing brood (Brødsgaard et al. 1999). The effectiveness of these organic acids to control mites can vary with climate, but generally decreased when capped brood

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were present (Nanetti et al. 1995; Brødsgaard et al. 1999; Büchler et al. 2020; Jack et al. 2020). In fact, the efficacy of three consecutive OA treatments ranged from lows between 39% and 52% in brood-right colonies to a high of 99% in broodless colonies (Gregorc and Planinc 2001, 2004).

Due to a high efficacy and low risk of hive product contamination, OA has been extensively used by itself and in combination with other mite controls including temporarily caging queens and treating colonies when broodless (Giacomelli et al. 2016; Gregorc et al. 2016, 2017). When queens are caged for approximately 25 days there is no brood in the colonies and therefore, the condition is optimal for controlling mites on bees using OA. It is imperative that we determine the method and time schedule using organic acids for mite control. Queen caging and brood reduction, together with application of organic acids, a practice used by many beekeepers, can contribute to effective mite control in the colonies. In previous experiments, procedures for repeated, sustainable and effective mite control during the beekeeping season were not established. This paper presents data assessing natural mite mortality through periodic hive bottom-board counts before and after FA and OA treatments at two beekeeping sites throughout 2019. Our chief objective was to assess the effectiveness of queen caging with subsequent OA and FA treatment for controlling mites in broodless honey bee colonies.

Materials and Methods

The study was conducted at two apiaries in Slovenia with *Apis mellifera carnica* honey bees. The first apiary with 18 colonies was located at one site near Vipava (45°50'25.0"N 13°56'08.2"E), which has a mild Mediterranean climate. The second apiary consisted of 19 colonies in Bovec (46°12'13.5"N 13°39'12.0"E), which has a temperate climate.

All experimental colonies were queen right, had combs occupied with bees, and were fully developed and productive. In the previous season, all experimental colonies were treated against varroa mites using organic substances: FA, OA, and thymol. All colonies were arranged three weeks before mite treatment and had an average of 8 (± 2 SD) brood combs in each brood chamber. In April 2019, metal sheets were placed on the bottom board of each experimental hive to record mite mortality before, during, and after treatments, for the duration of the entire experiment. All mites fallen on inserted metal sheets during the experimental season were counted and considered for calculations. Wire screens above the sheets prevented adult bees from coming into contact with hive debris containing fallen mites. Both colonies with brood and broodless colonies were monitored regularly for mite mortality every 7 to 10 days, before and during treatments, and inspected regularly for the presence of brood.

An oxalic acid/sucrose solution was formulated using instructions from the original product, ApiBioxal® (Chemicals Laif S. P. A., Vigonca (PD), Italy, serial number A180098). Each OA treated colony received 5 ml of the OA/sucrose solution per occupied comb. The 4.2% OA working solution (w/v) in 60% sucrose solution in water (w/w), was sprinkled onto bees atop and between combs. All colonies received the same OA/sugar-concentration solution. For the FA treatment, a Formivar® device for evaporation of FA was used (Formivar, 85% ad us. vet., Andermatt BioVet AG, Switzerland, serial number 877). One dispenser with 85 ml of 85% FA was inserted in each treated colony above the brood frames for short-term treatment from 4 to 10 days, to evaporate the contest of dispenser. At each time, one FA treatment was performed in order to reduce potential negative side effects in terms of reducing brood rearing.

Treatment design

We conducted two protocols at two locations to control mites in *Apis mellifera carnica* honey bee colonies (Fig. 1). The first protocol was conducted on 18 colonies at the apiary in Vipava. In nine experimental treatment colonies, queens were caged with subsequent OA treatments. Var-Control Cage Mozzato queen cages were used, at dimensions of width: 5 cm, height: 7.8 cm, and depth: 3 cm. The cage made of queen excluder mesh allows the queen bee to be confined while worker bees can pass through. In the second, control group, nine colonies were exposed to periodic OA and FA treatments. The last OA treatment was conducted in colonies without brood. This winter treatment was conducted in colonies with previously caged queens and in control colonies where queens were not caged.

In 19 colonies at the second apiary located in Bovec, we conducted the second treatment protocol. In this experiment, 19 colonies were randomly divided in two groups. Queens in 15 colonies were caged and hives subsequently treated with OA and FA treatments. In the second group, four colonies without queen caging were subsequently treated with OA and FA. A final OA winter treatment was applied to broodless colonies with previously caged or uncaged queens, and that were treated with OA and FA.

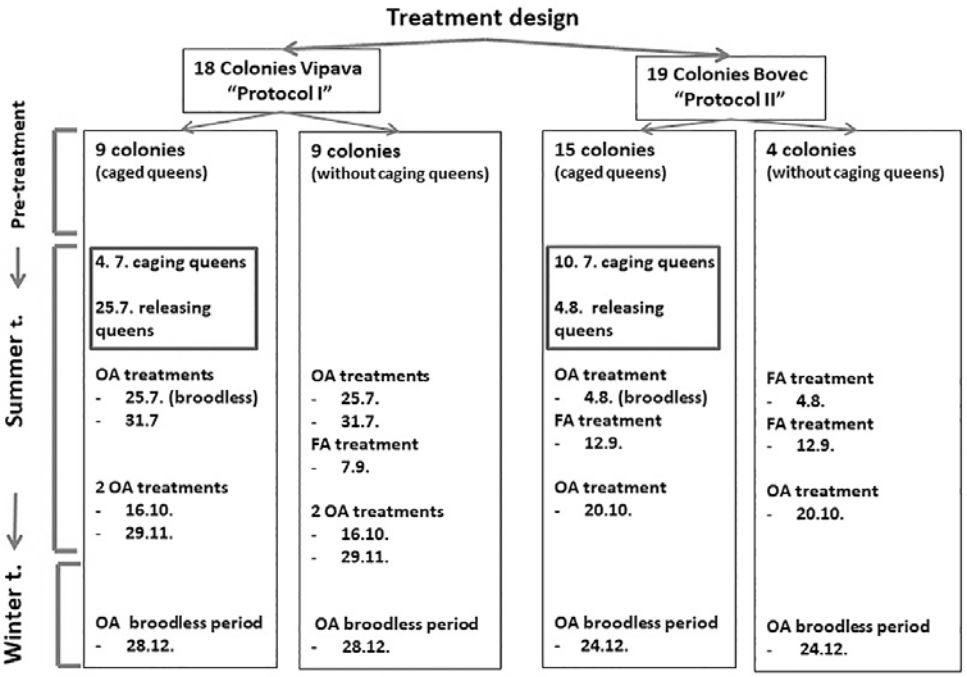

Fig. 1. Field experimental design and the timeline of treatments, including dates of queen caging, oxalic acid (OA) and formic acid (FA) applications. The experiment was performed during the 'Pre-treatment', 'Summer' and 'Winter' treatment periods. The final OA treatments were applied on January 4 (next year) and December 24, respectively, at two locations: Vipava and Bovec. Nine colonies in each experimental group in Vipava and additional 15 colonies and 4 colonies in each experimental group in Bovec were used as replicates. Dates are indicated as day and month: e.g. 4. 7. (July 4); 4. 1. (January 4).

Mite mortality evaluation

The percentage of mites killed (PMK) by OA or FA treatments was estimated after counting dead mites induced by consecutive treatments (T1 – Tn) and after the last OA application in the broodless period, using the formula: PMKT1 = (T1/(T1+ …. + Tn + OA) × 100)% (Gregorc and Planinc 2005; Gregorc et al. 2017). T1– Tn denotes the total number of mites killed after the first and following treatments in each treatment group. OA denotes the number of mites killed after the final OA treatment in broodless colonies. The same formula was modified to calculate the percentage of mites killed after the second and subsequent treatment (PMKTn) by excluding the number of mites killed in the previous treatments. The efficacy of a treatment was estimated by comparing the relative number of mites killed before and after each treatment. Mean mites mortality levels were compared among the treatment groups using one-way ANOVA (analysis of variance) with treatment as a main effect. Statistica 10 StatSoft (Riera 2001) was used for data analyses and in case of significant differences ($P < 0.05$ or $P < 0.001$). Tukey test determined differences among treatments.

Results

Vipava

Mite mortality

During the pre-treatment period of 39 days from April 6 to July 4 (2019), the average natural mite-death per day was 0.35 (± 0.24) and the total mite mortality during the same period was estimated at 13 (± 8.41) mites. Queen caging from July 4 and July 25 significantly increased daily mite mortality when compared with natural mite mortality in control colonies without queen caging ($F = 5.7879$; $df = 16$; $P = 0.0113$). Even higher daily

mite mortality was recorded after the first OA treatment conducted on July 25 in broodless colonies with previously caged queens ($F = 7.9663$; $df = 16$; $P = 0.01226$). Dynamics of mite mortality in colonies treated with FA and OA solutions are shown in Fig. 2 (Plate IV). A final OA treatment applied on January 4 to broodless colonies killed the remaining mites in the hive.

Efficacy

The relative efficacy of killing mites with double OA treatments in colonies with caged queens was significantly higher than the rate of natural mortality in colonies, without caging the queens ($F = 9.4384$; $df = 14$; $P = 0.01189$). FA application on September 7 induced significantly higher mite mortality when compared with colonies that did not receive FA, at the same time period ($F = 6.0025$; $df = 14$; $P = 0.02921$). Relative efficacies of different treatments are shown in Fig. 3 (Plate IV). The OA treatment performed on October 16 to all colonies induced high relative efficacy without differences between two treatment groups. The highest outside temperatures during the experimental treatments were as follows; Vipava apiary: April 6, 2019: 15 °C, July 4: 29 °C, July 25: 33 °C, September 7: 24 °C, October 16: 23 °C, December 28: 12 °C.

Bovec

Mite mortality

Daily mite mortality during the pre-treatment period of 80 days, before the initial OA or FA treatments, and after treatments in colonies used for Protocol II, was significantly different ($F = 0.0097$; $df = 14$; $P = 0.001$) Fig. 4 (Plate V). The average natural mite-death in all experimental colonies per day was 0.76 (± 0.46) and the total average number of mites killed during the experiment in all 19 colonies was 2098.00 (± 1203.20).

In broodless colonies where queens were caged, the OA treatment on August 4 increased the daily mite mortality 15 times from the natural daily mortality rate of 0.8 (± 0.51) to 12.34 (± 5.18). During the same period, FA treatment in colonies with uncaged queens, mite mortality increased ~57 times from an initial rate of 0.59 (± 0.19) to 33.56 (± 11.16) mites per day. Successive FA and OA treatments conducted on September 12 and October 25 increased mite mortality compared to previous mite mortality rates recorded after the final OA treatment on December 24.

Efficacy

Significant differences occurred among mean efficacies of the mite control procedures ($F = 105.2905$; $df = 3$; $P < 0.001$). During the 80-day pre-treatment monitoring period, the relative mite drop during this experiment was 4.87% (± 4.06). Relative mite mortality during the observing periods before and after treatments is shown in Fig. 5 (Plate V). The first OA application on August 4 occurred just after releasing the queens from cages in broodless colonies. This procedure resulted in an average mite mortality of 47.46% (± 22.01). The first FA treatment, which was also conducted on August 4, resulted in a mite mortality of 72.97% (± 21.05). The mite mortality induced by each treatment exceeded the pre-treatment (or natural) levels of mite mortality ($P < 0.01$) in untreated colonies or colonies with caged queens.

Successive FA treatment on September 12 in colonies with both previously caged queens and uncaged queens resulted in mortality rates of 53.66% (± 16.58) and 70.67% (± 3.08), respectively. During the October OA treatment, when there were still broods in the honey bee colonies, efficacy of OA treatments was above 80%. The highest outside temperatures during the experimental treatments were as follows; Bovec apiary: April 21, 2019: 22 °C, August 4: 29 °C, September 12: 29 °C, October 20: 23 °C, December 24: 11 °C.

Discussion

The developmental stage of honey bee colony, their health and use for production during beekeeping season can dramatically affect the efficacy of organic acids to control varroa mites. The two organic acid treatments with OA or FA combined with queen caging in two different apiaries showed improved efficacy against mites during an active field season. Both OA and FA effectively controlled mites in all colonies whether they contained brood or not. However, caging queens to render colonies effectively broodless increased the efficacy of OA and FA mite control. In our study, FA itself accomplished 18% of mite control in colonies with brood and 72% of control at the Vipava and Bovec sites, respectively. In the same period, FA in previously untreated control colonies at the Vipava apiary induced 9% mite mortality. The OA treatment killed 18% mites in the time between July 25 and September 7 in Vipava and 47% mites in Bovec between August 4 and September 12.

The mite mortality rate was significantly improved with queen caging in comparison to the pre-treatment period. Lower natural mite mortality at a rate of 5% was achieved using OA in control colonies with brood and uncaged queens. However, a single OA application combined with queen caging in different experimental settings can reduce summer mite populations by 96% (Marco et al. 2012). Similarly, queen caging coupled with a thymol-based Apiguard treatment performed in experimental colonies in Italy achieved a 97% mite reduction (Giacomelli et al. 2016). We achieved lower mite control than anticipated using FA or OA in both broodless (via caged queens) and brood-right colonies. Late autumn OA applications in October, November or December ensure higher, approximately 98% mite control efficacy in colonies with the last, smaller amount of brood present in the season. This efficacy was recorded using final OA treatment in broodless colonies as winter treatment performed on January 4 and December 24 in Vipava and Bovec apiaries. In fact, a colony's broodless state during the rearing season in the current experiment did not improve levels of desired mite control as achieved in our previous experiments (Gregorc et al. 2016). It is established that mother mites are able to choose nurse bees over foragers and newly emerged bees as their optimal host in the phoretic phase to quickly infest new brood cells and thus contribute in fast mite development (Xie et al. 2016). The production of a larger amount of progeny demonstrates a high degree of adaptation as their population per colony can increase up to ten times in only one beekeeping season (Michalczyk 2019).

Continuous monitoring of mite infestation levels associated with effective mite control can contribute to colony health and sustainable bee-keeping management. In the current experiment, we used the commercial OA product ApiBioxal®. Further tests need to be conducted in order to obtain mite control results and comparisons to other commercial or non-commercial OA concentrations. Taken together, this study indicates that combined summer and autumn treatments or a treatment during the winter broodless phase of a colony's lifecycle ensure substantial mite suppression. Our findings are important for the cases in apiculture when the summer queen caging plus OA treatment do not ensure colony survival. One explanation for the seasonal rise in organic acid efficacy could be a greater preponderance of younger and more vulnerable mites on adult bees in the late summer (Wilkinson and Smith 2002). For example, 100% control was reached in artificial swarms later in the year using OA (Büchler 1998). Therefore, for a better mite control in the colonies we recommend that additional OA or FA applications follow the initial summer mite control using OA trickling and queen caging.

Some honey bee populations were found to be naturally surviving mite infestations without any treatment for several years (Fries et al. 2006; Locke 2016; Oddie et al. 2017). Potentially reduced mite reproduction in naturally resistant colonies may be observed in normal beekeeping conditions (Rinderer et al. 2010;

Locke 2016) where less mite control treatments are needed. The timing of organic acid use is particularly important for effective mite management. Later organic acid treatments such as FA in September or OA in October are especially efficacious, killing 60–100% of mites infesting colonies in Bovec and Vipava. Similarly, 97.3–99.5% of mites are killed by late autumn or winter OA treatments in broodless colonies (Nanetti et al. 1995; Imdorf et al. 1997). Moreover, 98% mite control is achievable in colonies with capped brood, but four OA trickling applications are required (Toufailia and Ratnieks 2016). A final OA treatment in broodless colonies is nearly 100% effective for estimating mite control efficacy when applied in late autumn (Spreafico et al. 2001). In this regard our efficacies may be overestimated because final winter OA treatment in broodless colonies is sometimes less than 100% effective (Gregorc and Planinc 2001; Rademacher and Harz 2006) and due to the reproduction of the surviving mites and possible re-invasions during the experiments.

Our results based on counting all fallen mites during the experimental season indicate that consecutive OA trickling or FA fumigation is needed when they are to be used as an effective control option for mites during the brood rearing season, whether or not queens are caged. Therefore, when colonies are broodless in the winter, organic OA is particularly effective. Additional studies are needed to assess mite population dynamics in response to these treatments and to elucidate the mode of action of OA in broodless colonies with caged queens during a temperate summer comparatively with OA acaricidal activity in colonies during a temperate winter.

In conclusion, natural mite mortality was recorded at two different locations and experimental honey bee colonies settings during the pre-treatment period in normally developed colonies with brood. Mite mortality significantly increased in the monitoring period during the queen caging phase and after the OA treatment conducted in broodless colonies. A parallel experiment using FA application in colonies with brood also induced significantly higher mite mortality compared to colonies that did not receive FA, in the same time period. Successive FA and OA treatments in colonies with brood increased mite mortality. It was also found that successive FA treatment in colonies both with previously caged queens or uncaged queens resulted in efficacy rates of approximately 54% and 71%. Later in the season, OA treatment was above 80% effective in colonies with brood. It is therefore evident that in organic varroa control strategies, several repeated treatments are needed to reduce mites to a tolerable level needed for the colonies to enter into winter.

Acknowledegments

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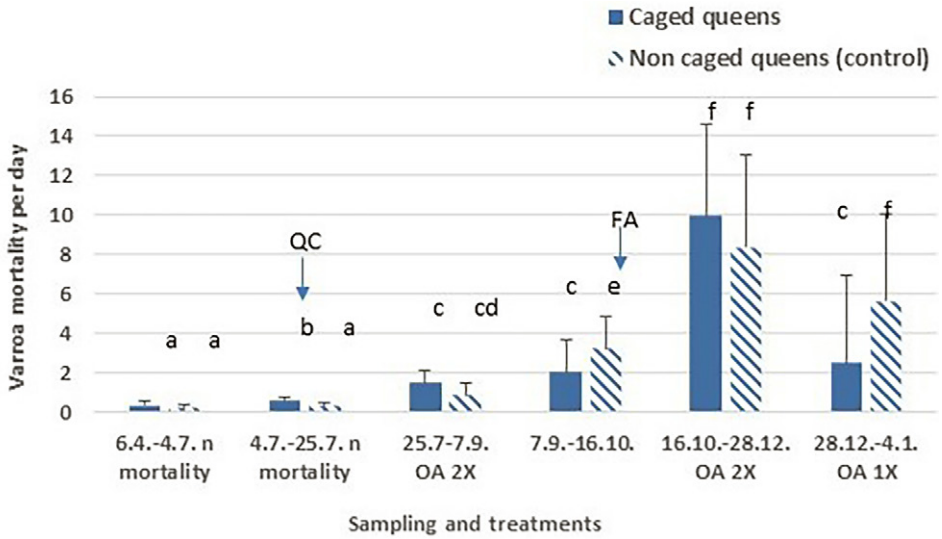

Fig. 2. Comparative daily mite mortality in the pre-treatment period, during the time of queen caging (QC), between July 4 and 25 following oxalic acid (OA) and formic acid (FA) treatments. Numbers under primary horizontal axis present dates. Queens were caged in nine colonies; colonies without queen caging (control colonies) received the FA treatment on September 9. The two OA treatments were conducted in the periods between July 25 and September 7, and between October 16 and December 28. The final OA treatment in broodless colonies was conducted on January 4. The same letters indicate that Tukey tests ($P < 0.05$) established that the daily mite mortality rates were not significantly different within the observed period and between periods. Bars indicate standard deviation.

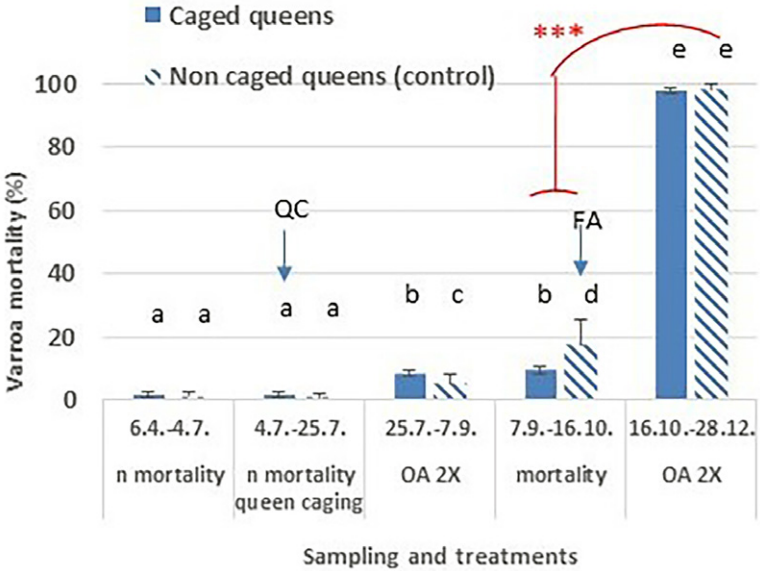

Fig. 3. Relative mite mortality (%) of the oxalic acid, or formic acid treated colonies with queen caging and colonies without queen caging (control) in the pre-treatment period and after queen caging at the Vipava apiary. The same letters indicate that Tukey tests ($P < 0.05$) established that the mite mortality rates were not significantly different within the observed period and between periods. Asterisks show ANOVA significance levels ($P < 0.001$***) for comparisons of the mite mortality in treatment groups between the two times periods (September 7–October 16 and October 16–December 28) under corresponding arch. Bars indicate SD.

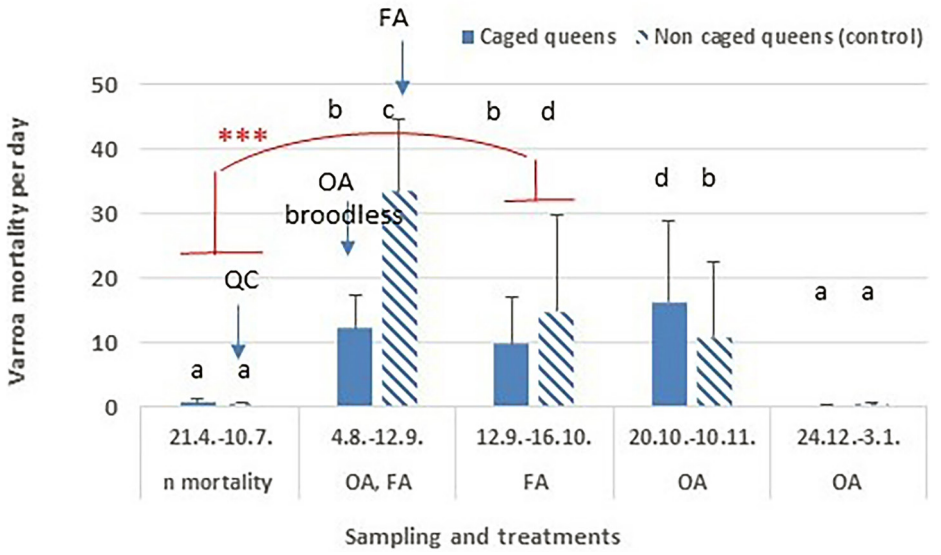

Fig. 4. Daily mite mortality in the pre-treatment period at the Bovec apiary, prior and during queen caging (QC), between July 10 and August 4, followed with OA and FA treatments according to Protocol II. Numbers under primary horizontal axis present dates. In one group of colonies queens were caged and mites counted; in the second group, control colonies received the first FA treatment on August 4. The same letters indicate that Tukey tests ($P < 0.05$) established that the daily mite mortality rates were not significantly different. Bars indicate standard deviation. Asterisks show ANOVA significance levels ($P < 0.001$***) of mite mortality per day comparisons between the pre-treatment natural mite mortality and mite mortality after different treatments under the corresponding arch.

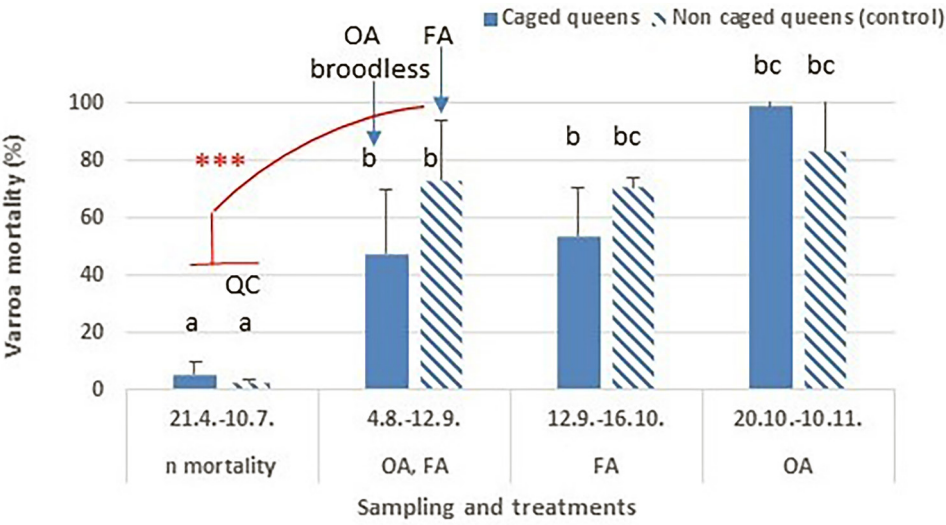

Fig. 5. Relative mite mortality (%) of the OA or FA treated colonies according to Protocol II. Mites were counted in the pre-treatment period and after queen caging and after the control applications of OA or FA at the Bovec station. The same letters indicate that Tukey tests ($P < 0.05$) established that the relative mite mortality rates were not significantly different. Asterisks show ANOVA significance levels ($P < 0.001$***) for comparisons between the relative mite mortality prior and after conducted treatments under corresponding arch. Bars indicate SD.