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THE EFFECTIVENESS AND APPLICATION POSSIBILITIES OF LITHIUM CHLORIDE IN THE PROTECTION AGAINST *VARROA DESTRUCTOR* (ANDERSON AND TRUEMAN, 2000) MITES

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1. INTRODUCTION AND OBJECTIVES

The western honey bee (*Apis mellifera*, Linnaeus, 1758) plays a crucial role in the ecosystem: it not only provides honey and other bee products but also has an indirect impact on food production through its pollination activity. Apiaries face numerous challenges that threaten the future of the industry and its positive impact on pollination. Extreme weather caused by climate change negatively impacts not only bees but also bee pastures. In addition, uncertainties in the honey market, pesticide problems, and parasites and diseases such as the Asian yellow-legged hornet (*Vespa velutina nigrithorax*, du Buysson, 1905), the *Varroa* mite (*Varroa destructor*, Anderson and Trueman, 2000), American foulbrood, or the *Microsporidia* pathogen *Vairimorpha* (formerly *Nosema*), etc., pose serious challenges to the beekeeping sector. Of these, the *Varroa* mite is the most significant global problem, as controlling it is a continuous challenge for beekeepers.

As the original parasite of the eastern honey bee (*Apis cerana*, Fabricius, 1793), *Varroa destructor* spread rapidly worldwide following host switching. If left untreated, it can destroy bee colonies within one to two years, or even within a single beekeeping season in areas with high bee density. Control is made more difficult because the mite reproduces in the capped brood, where most varroacide active ingredients cannot penetrate. Formic acid is somewhat of an exception, but its effectiveness is highly dependent on ambient temperature, and its efficacy can be inconsistent, so its use is limited in beekeeping practice. Beekeepers, therefore, often supplement mite control with beekeeping technologies, such as creating a brood-free state. This method allows the mites to migrate from the cells to the adult bees, where they can be more effectively killed with targeted treatment. The effectiveness of currently used agents is often unsatisfactory and is also burdened with resistance and residue problems. Therefore, there is a continuous need to develop new active ingredients. Ziegelmann et al. discovered the acaricidal effect of lithium chloride in 2018 during their RNA interference (RNAi) research, which they originally used as a carrier in their experiments. Lithium chloride is a relatively inexpensive and readily available agent, which is why it is a promising alternative for beekeepers.

There were no experimental data available on the beekeeping application of lithium chloride, except for the results of Ziegelmann and colleagues (2018), so we started our research based on their results in this study. The aim of our experiments was to comprehensively investigate lithium chloride to obtain as much information as possible about this potential active ingredient to decide at what risk, in what way of application, and at what dose Li+ can be used as a varroacide in beekeeping, enabling beekeepers to safely and effectively control mites if lithium chloride becomes an approved anti-mite product.

OBJECTIVES

1. To investigate the contact effect of lithium chloride in an *in vitro* laboratory model experiment (*ex situ*) and under *in situ* conditions to identify possible new application methods.

2. To compare the efficacy of lithium chloride and oxalic acid in the *Varroa destructor* control strategy.

3. To compare the efficacy of alternative application methods relevant to beekeeping practice, such as hot fogging, cold fogging, long-lasting carrier, and trickling methods.

2. MATERIAL AND METHOD

In line with the objectives, the following chapters will present the investigations in the order of contact effect, oxalic acid-lithium comparison, and the comparison of relevant application methods as interpreted in beekeeping practice.

2.1 Demonstrating the contact effect of lithium against *Varroa*

Ziegelmann et al. (2018) described the systemic mode of action of lithium in their experiments (Ziegelmann et al., 2018). We aimed to investigate whether, in addition to the absorption effect, the lithium ion also has a contact effect. This was investigated in November 2019, both in laboratory model experiments (*ex situ*) and *in situ* experiments.

- In the laboratory model experiments (*ex situ*), we examined mites placed on cardboard sheets saturated with lithium chloride solutions of different concentrations (10.78 mM - 11.04 M). We observed the behavior of the mites (trembling, uncoordinated movement), mortality, and recorded the time of the appearance of the first symptoms, as well as the time of the mites falling off. In the control group, cardboard sheets saturated with deionized water were used.
  The results were analyzed using analysis of variance (ANOVA) and Tukey HSD *post hoc* test.

- In *in situ* experiments, microfiber cloth strips saturated with lithium chloride were placed in heavily infested, brood-free bee colonies. The mite fall was monitored for 5 days, then after inserting additional strips (5 pieces), the observation was continued for another 10 days. The control bee colony did not receive any treatment during the experiment, only the natural mite fall was recorded. At the end of the experiment, to check for any remaining mites, lithium chloride trickling was performed on all three bee colonies, and a powdered sugar test was used on the bee colonies to check the effectiveness.

2.2 Comparison of the efficacy of oxalic acid and lithium against *Varroa* mites

In the late autumn, brood-free period (November 2019), we compared the efficacy of lithium chloride and oxalic acid. The lithium chloride treatments were carried out by trickling 40 ml of 250 mM lithium sugar syrup. The oxalic acid was applied by sublimation using 2 g of oxalic acid dihydrate to the bee colonies in the hives. Three sub-experiments were set up on a total of 26 bee colonies, applying the treatments in different orders and combinations:

E1): The lithium chloride treatment of nine bee colonies was started on October 26, and then the mite fall was followed for 11 days. This was followed by oxalic acid sublimation on November 6, and the mite fall was observed for 8 days.

E2): The oxalic acid treatment was applied to four bee colonies on November 5, and then the mite fall was followed for 8 days. This was followed by lithium chloride treatment on November 14, and the number of mites that fell onto the hygienic bottom board was followed for 11 days.

E3): Lithium chloride treatment was applied to 13 bee colonies. After the treatment on December 11, the mite fall was followed for 11 days, and then for another 8 days without repeating the treatment.

The efficacy was quantified by the cumulative average of the number of dead mites that fell onto the hygienic bottom board, and the results were analyzed using Kruskal-Wallis ANOVA and Mann-Whitney U *post hoc* tests.

2.3 Comparison of relevant application methods for lithium chloride treatments in beekeeping practice

The efficacy of different application methods of lithium chloride was tested on productive, brood-free bee colonies in "Nagy Boczonádi" hives (2021). Four application methods were examined:

- **Hot fogging (Furetto device):** We tried to apply lithium chloride with different carrier materials (petroleum, paraffin oil, glycerin, alcohol).
- **Cold fogging (VAT-1a device):** 5.5 M concentrated lithium chloride solution was applied.
- **Impregnated paper strip (long-lasting carrier) method:** Paper strips impregnated with 5.5 M concentrated lithium chloride solution were placed in the hives.
- **Trickling method:** Lithium chloride solutions of different concentrations (250 mM, 500 mM, 750 mM) and sugar content (100%, 50%, 0%) were trickled in single and repeated treatments.

The efficacy was evaluated by the number of dead mites and the proportion of surviving mites, and the results were analyzed using a general linear model (GLM) and Tukey HSD *post hoc* test.

3. RESULTS AND DISCUSSION

3.1 Demonstrating the contact effect of lithium against *Varroa*

In the laboratory model experiment (*ex situ*) conducted on paper strips, during the contact tests, the mites on the control strip either remained motionless (n = 58) or moved a maximum of 2 cm on the surface (n = 12). None of them fell off or showed similar signs as the mites on the treated strip ($Z = 11.9$; $p < 0.001$ for both trembling movement and falling between the control and lithium chloride treated groups). The time elapsed until the first appearance of trembling movement of the mites on the paper strips impregnated with different lithium chloride concentrations shortened inversely with the concentration.

At the lowest concentration (10.78 mM), the symptoms appeared on average after 66 minutes, and the mites fell off after 84 minutes. At the highest concentration (11.04 M), the mites started to show trembling symptoms within about 1 minute, and on average fell off in the 3rd minute of the experiment. It is important to note that none of the mites that fell from the treated paper strip at any of the tested lithium concentrations recovered from the symptoms caused by lithium. Their trembling gradually turned into uncontrollable movement, and finally, they became motionless before they died. All mites that were exposed to the lithium treatment first showed trembling symptoms and then fell off the strip. Only the trembling and the time elapsed until the mites fell off showed a difference depending on the concentration.

In the *in situ* study, we investigated whether the contact effect can also be induced under field conditions. During the first treatment, when a single lithium-impregnated strip was placed in hives 2 and 3, 24 and 6 mite falls were recorded during the first five days. However, in the control bee colony (1), only 3 natural mite falls were observed.

However, the number of mite falls increased significantly in the second phase, when five additional strips were placed in the two treated bee colonies and left to act for 10 days (without replacing or refreshing the previous strip). This treatment resulted in 198 and 41 mite falls in hives 2 and 3, respectively, while in the control hive during the same period, there were only 4 mites from natural mite fall. At the end of the 15th day, lithium chloride trickling was applied in all three hives, which resulted in 18, 5, and 120 mite falls after 10 days in hives 2, 3, and 1 (control), respectively. After this, a powdered sugar test (Büchler, 2013) performed on 100 g of bees did not detect any mites in any of the hives. In the case of both the treatments with lithium strips and the trickling, the symptoms of the freshly dead mites (observed on the hygienic bottom board) were the same as the symptoms experienced during the contact test. The paper strip test applied to the bee colonies confirmed that the contact mode of action can exert its effect *in situ* at a given concentration.

Based on our results, it was clearly proven that lithium chloride has a strong contact effect on mites. This broadens the scope of potential application of the compound as a miticide in beekeeping practice. The contact tests with impregnated paper strips also showed the irreversible effect of lithium chloride on mites, even at very low concentrations. It should be noted that we were able to demonstrate and confirm the dual (contact and systemic) effect of lithium applied by the trickling method. The lowest tested concentration of the lithium chloride solution (10.78 mM) was determined based on the fact that the impregnated strip should remain wet throughout the entire duration of the treatment. This criterion was determined because we assumed that the entry of lithium into the mite occurs in an aqueous solution. The lithium-treated strips remained wet for at least 15 days at the highest concentration (11.04 M) both in the laboratory model experiment (*ex situ*) and in the *in situ* studies, which shows that lithium chloride does not need an additional wetting agent during long-term contact treatments if this concentration is used on the long-lasting carrier.

3.2 Comparison of the efficacy of oxalic acid and lithium against *Varroa* mites

Based on our results, lithium chloride proved to be more effective both alone and in combination than oxalic acid. In the different sub-experiments, the efficacy of the acaricides differed significantly depending on the type and mode of treatment (Kruskal-Wallis ANOVA, d.f. = 5, n = 52, H = 40.7, $P < 0.001$). During the first two experiments (E1 and E2), a combination of lithium chloride and subsequent oxalic acid treatment was used. In experiment E1, we first treated with lithium chloride as the main treatment, which was supplemented with oxalic acid treatment, while in E2, the same treatments were applied in reverse order, so their cumulative effect on the mites could be compared.

In experiment E1, a significantly higher percentage of mites were killed during the lithium chloride main treatment (average 95.6%) than in experiment E2 during the oxalic acid main treatment (58.7%). Lithium chloride also outperformed oxalic acid as a supplementary treatment. In experiment E2, 41.3% of the mites were killed during the supplementary lithium chloride treatment, while in experiment E1, only 4.4% of the mites were killed during the supplementary oxalic acid treatment. There was no difference between the percentage of mites killed during the oxalic acid main treatment and the lithium chloride supplementary treatment in E2.

When the lithium treatment was preceded by oxalic acid main treatment (E2), an average of about 200 bee individuals were observed to die, while there was no significant mortality in the other treatment settings.

In the third experiment (E3), it was confirmed that the effect of the lithium chloride main treatment may be prolonged and extended well beyond the predetermined 11-day observation period. 84.2% of the mites were killed during the first 11 days, and a further 15.8% during the following 8 days (from the 11th to the 19th day after the treatment). Moreover, this prolonged effect did not differ from the effect of the supplementary oxalic acid treatment in E1, which also included the residual effect of the preceding lithium chloride main treatment.

All bee colonies participating in the experiment successfully overwintered. No significant bee mortality was observed in any of the bee colonies during the winter.

The results of the sub-experiments showed that lithium chloride trickling, whether applied as a main or supplementary treatment in the winter closing treatment, is more effective in controlling *Varroa* mites than oxalic acid. The better efficacy of lithium chloride is also supported by its long-term effectiveness and alternative mode of action (contact effect). In contrast to oxalic acid, which has an efficacy duration of 7-8 days (Mutinelli et al., 1997), we found that lithium chloride trickling is effective for 15-20 days.

In our experiment, we chose the trickling method of application for lithium chloride dissolved in sugar syrup because, unlike those used in previous studies (feeding application), this method of application may pose a lower risk of potential residue formation and has both systemic and contact effects. Thanks to the social behavior of bees, by licking the lithium sugar syrup off each other or the hive wall, the lithium is spread throughout the entire bee colony. Lithium chloride can be a particularly effective acaricide when used as a closing treatment in bee colonies before wintering, as in this brood-free period, the side effect of the treatment (brood mortality) can be minimized.

3.3 Comparison of relevant application methods for lithium chloride treatments in beekeeping practice

In the I experimental group, where we compared the efficacy of nine lithium chloride treatments, the number of mites that fell due to the treatment ranged from 3 to 327 per hive (mean ± SD: 54.3 ± 73.4). The effect of the number of mites on the efficacy of the treatment varied among the different treatment types. The results of the hot fogging method (Furetto) were excluded from the statistical evaluation because none of the tested carrier materials proved to be suitable for application, as the water evaporated from the lithium chloride aqueous solution and the lithium salt precipitated in the heating coil of the application device. The GLM model analysis revealed significant differences in the efficacy against *Varroa* among the different treatment types. Cold fogging (VAT 1a) did not prove to be a sufficiently effective lithium treatment method, as it killed only 9.9 ± 3.3% of the mites in the hive (mean ± SD). Lithium-impregnated paper strip treatment and 1 x 500 mM lithium chloride solution trickling with a 3-day observation period showed moderate efficacy, killing 55.1 ± 26.2% and 64.8 ± 7.4% of the mites, respectively. The other five trickling treatment methods showed similarly high average efficacy, ranging from 85% to 99.7%.

Our experiments showed that the efficacy of the treatment against *Varroa* is highly dependent on the method of application. Of the four commonly used application methods, hot fogging (Furetto) proved to be unfeasible, cold fogging (VAT1) and application on a long-lasting carrier (impregnated strip) showed weak or moderate efficacy, while trickling proved to be effective.

3.3.1 Hot and cold fogging methods

Although the application of hot fogging for certain acaricides (e.g., amitraz, fluvalinate) is a proven method in beekeeping practice, our experiment showed that hot fogging with any of the carrier materials (petroleum, paraffin oil, glycerin, alcohol, glycerin + alcohol) is not suitable for applying lithium chloride in the hive. The probable reason for this is that lithium chloride does not dissolve in the carriers used in beekeeping practice, the water used as a solvent evaporates during the process, and the lithium salt precipitates in the heating coil of the device.

The application of lithium chloride solution into the hive in the form of cold fogging with the VAT 1a device was successful, but it showed low efficacy against mites. A single treatment with 69.4 mg Li+ removed nearly 10% of the mite population. This efficacy is not comparable to the application of amitraz in a similar way, where the use of the same method can achieve 90% efficacy (Pohorecka et al., 2018). A similar result is obtained when compared to oxalic acid sublimation, which is the most effective application method for this active ingredient; it can result in >90% efficacy in a brood-free state (Rademacher and Harz, 2006).

3.3.2 Application on a long-lasting carrier (impregnated paper strips)

Application on a long-lasting carrier, where the active ingredient is impregnated onto a paper strip or plastic strip, is a frequently used method in beekeeping practice for the treatment of *Varroa* mites. This method, in our experiments, is primarily based on the contact effect of lithium, a mechanism of action that we have confirmed in our previous investigations, both in laboratory model experiments (*ex situ*) and under *in situ* conditions. Although it results in a significant reduction in mites, this efficacy can only be achieved with an increased dose compared to the dose applied by trickling. In our experiment, we used the same dose of lithium chloride as we applied by trickling (500 mM), but this method resulted in only moderate efficacy of 55.1%. Therefore, we believe that the application of lithium through paper strips in the bee colony is not a reliable alternative due to its lower efficacy compared to trickling. In addition, the production of lithium paper strips is more time-consuming, and due to the larger amount of lithium chloride required for impregnation, it is also more expensive than the trickling solution. Moreover, it can be considered a disadvantage that it can lead to the generation of paper waste in the hive (the bees chew the paper strip), and due to its longer presence, it can increase the exposure to residues.

3.3.3 Trickling method

When applying the trickling mixture, the active ingredient is applied in a small amount of a more concentrated solution (<50 ml), which may contain sugar and other adjuvants. In this application, after removing the hive lid, the solution is trickled directly onto the bees between the combs, which they lick off each other, and the drops on the frames are absorbed. The active ingredient applied in this way spreads in the bee colony thanks to the social behavior of bees. Among the investigated alternatives, the trickling method proved to be the most effective way of applying lithium. The different trickling treatments reduced the number of mites by an average of 65-99.7%, depending on the applied dose and the number of treatments. However, the efficacy measured on the 8th day of the experiment was significantly increased by the treatment repeated on the 3rd day in the case of all concentrations, which indicates the need for repeated treatments. Although lithium is decontaminated relatively quickly from bees and their products, the investigated 750 mM concentration may pose an increased risk of residues without showing a statistically verifiable increase in efficacy compared to the 500 mM concentration, especially in the case of repeated treatment. Therefore, we believe that the optimal choice may be the 2 x 500 mM concentrated lithium treatment with trickling, which provides near maximum efficacy regardless of the number of mites present in the bee colony, and has a lower risk of residues than higher concentration treatments. Based on the obtained results, the 2 x 250 mM treatment may also be a suitable alternative in case of lower mite infestation. However, it should be noted that the number of mites in the colonies can only be known approximately based on the mite fall of previous periods. In practice, the actual mite infestation can only be determined in the period before wintering, in a brood-free state.

The results of our first experiment show that in the case of trickling application, the repetition of the treatment itself may be more important than the applied concentration. Due to the high hygroscopicity of anhydrous lithium chloride, it is practical to prepare a stock solution from the total amount of lithium chloride powder, from which further dilutions (trickling mixture) can be prepared, e.g., with sugar syrup. Based on these results, due to the long-lasting effect of lithium (less than half of the mites survived the first treatment in all experiments), combining the 500 mM primary treatment with a 250 mM secondary treatment may be a reasonable solution in some cases.

3.3.4 Clarification of the adjuvant effect of sugar

In the II. experiment, we compared the efficacy of trickling treatments with 500 mM lithium chloride solution at three sugar solution concentrations, in single and repeated treatments. The number of mites that fell due to the treatments ranged from 18 to 684 per hive (245.0 ± 164.7). This variance stems from the natural differences in mite infestation of the bee colonies, as the number of *Varroa* mites depends on the extent of brood in the given year, drifting, and possibly the consequences of robbing. In the statistical analysis, the effect of the number of mites on the efficacy of the treatment was homogeneous among the different treatment types. The efficacy of the treatment tended to decrease with the increasing number of mites, but mostly not significantly. The analysis of covariance showed that the sugar concentration of the trickling solution did not affect the efficacy of the treatment. The repeated 500 mM lithium chloride trickling treatments proved to be significantly more effective (97.3% ± 2.3%) than the single treatments (79.7% ± 9.4%).

In previous studies, we used sugar solution in most trickling treatments, with the assumption that it may promote the spread of lithium in the hive, as it may potentially act as an adjuvant, the bees consume it more readily, and it is better spread in the hive through social behavior. However, our results show that the sugar content of the trickling mixture does not affect the efficacy of the treatment. In practice, the preparation of the trickling solution with water may sometimes be more advantageous than diluted sugar solution, as it has a longer shelf life, and its preparation can be carried out even without the preparation of a stock solution (but in this case, a larger amount of lithium solution needs to be stored).

4. CONCLUSIONS AND RECOMMENDATIONS

Previous studies by Ziegelmann et al. (2018) demonstrated the systemic mode of action of lithium chloride. Our present experiments have clearly shown that lithium chloride, in addition to its systemic effect, also has a strong contact effect on *Varroa* mites. We investigated its efficacy and practical applicability both in laboratory model experiments (*ex situ*) and under *in situ* conditions. The application of lithium chloride as a contact treatment (long-lasting carrier) may be an advantageous alternative to feeding bees and may exploit its systemic mode of action in the control of mites. These contact treatments may pose a lower risk of honey contamination compared to previous approaches where lithium was applied through feeding. Based on all these results, the contact effect confirmed by the experiment justified the investigation of possible application alternatives.

It was important to clarify which application methods used for other acaricides are also effective and efficient in the case of lithium chloride. We investigated the hot fogging method (Furetto), cold fogging (VAT 1a), impregnated paper strips, and trickling application methods to determine which of these could be practically applicable alternatives to the feeding method. In the case of the hot fogging method, we tried to apply lithium chloride with several carrier materials (glycerin, petroleum, alcohol, glycerin + alcohol), but none of them yielded results, as the lithium precipitated in the heating coil of the device. Although the application with the VAT 1a device was successful, its efficacy against mites was low, with only 10% of the mites being removed from the hive with a single treatment. Based on our results with this method, the possibilities for its future application are limited, as it could only be used for mite reduction during the beekeeping season in combination with another method or acaricide, and a successful beekeeping mite control strategy cannot be based on this alone.

The efficacy of the impregnated paper strip method was confirmed under field conditions, not only in laboratory model experiments (*ex situ*), but despite the fact that it resulted in a more significant mite reduction compared to the previous method, as shown by the number of mites that fell onto the hygienic bottom board, its efficacy was only 55.1% even with the application of 500 mM active ingredient. In addition, application on a long-lasting carrier has several disadvantages. On the one hand, proportionally much more lithium needs to be impregnated onto the carrier than is required for the trickling mixture, which significantly increases the cost of this method. On the other hand, the bees chew the strips placed in the hive, which leads to the generation of paper waste, increasing the risk of residues in bee products. Furthermore, the production of the strips is also much more time-consuming, which also hinders the application of the method. Based on all this, it can be stated that these three methods - hot fogging, cold fogging, and impregnated paper strips - did not prove to be effective enough in practice, or are not suitable at all for applying lithium in the hive.

In summary, the contact effect of lithium has been confirmed. However, the experiments carried out on productive bee colonies (*in situ*) highlighted that the methods based on the contact efficacy of lithium, using long-lasting carriers or cold fogging procedures, which were applied in our experiments and are relevant in beekeeping practice, are not sufficiently effective for practical use.

The trickling method proved to be the most effective. We investigated the relative efficacy of 500 mM concentrated lithium chloride trickling treatments at three concentrations of the sugar solution in the trickling mixture (saturated sugar syrup, water and saturated sugar syrup mixed in a 1:1 ratio, and sugar-free water), as well as in single and repeated treatments. The trickling should be adjusted to the size of the bee colony in all cases. The generally applicable volume is considered to be 40 ml of trickling mixture per bee colony. This is the amount where the solution is still distributed on the bees without excess dripping to the bottom of the hive Thus, per bee space, the most widespread Hungarian standard for the "Nagy Boczonádi" frame size (in a horizontal hive) projected surface is 4 ml, which is the optimally applicable amount by trickling. During the protection period, typically from mid-August to mid-October, bees do not cover more than 10-11 "Nagy Boczonádi" bee spaces. The different treatments reduced the number of mites by an average of 65-99.7%, depending on the number of treatments and the applied dose. The most effective dose was the 500 mM concentrated trickling mixture, especially in the case of two repeated treatments, regardless of the number of mites present in the bee colony. The single 250 mM treatment alone did not prove to be effective enough, but in repetition, it can show adequate efficacy at lower mite infestation levels. If the 500 mM treatment is supplemented with a second 250 mM treatment, this may even be an optimal treatment alternative for the end-of-year closing treatment in brood-free bee colonies. The additional efficacy of the 750 mM concentration compared to the 500 mM concentration was not statistically verifiable, but the increased concentration may pose an increased risk of residues in bee products, primarily in honey.

A further advantage of using the trickling method is that it can be easily adapted to any type of hive, as well as to the size of the bee colony. The amount of trickling solution to be applied is easy to calculate, and unlike the feeding method used by previous authors, it can be effectively applied at any time of the year, as the bees readily consume the small amount that we apply by trickling, and the risk of residues may also be lower compared to feeding.

We investigated whether the sugar content of the trickling mixture has an effect on the efficacy of the treatment. Our initial hypothesis was that due to the sugar content, it may be more attractive to the bees if the trickling mixture is prepared with a sugar solution, and it may also act as a potential adjuvant if it induces increased cleaning behavior in the bees. Although the latter effect cannot be ruled out, based on the results, the sugar content did not have an effect on the efficacy of lithium against mites. Therefore, preparing the trickling mixture with water may be more useful in practice, as it can be stored for a longer time, and the preparation of the trickling mixture may even be relevant without the need for a stock solution.

In our investigations, we compared the efficacy of lithium chloride with oxalic acid when applied as a winter closing treatment on bee colonies in a brood-free state. Lithium chloride was applied by trickling, while oxalic acid was sublimated. Based on our results, we found that lithium chloride, whether applied as a main or supplementary treatment, outperformed oxalic acid in efficacy against *Varroa* mites. The better efficacy of lithium may also be supported by its prolonged effectiveness. If lithium becomes an approved acaricide in the future, it may be a relevant alternative to oxalic acid-based control in organic beekeeping, as the number of agents that can be used in these apiaries is currently limited (oxalic acid, lactic acid, formic acid).

In the case of acaricides, an important factor is the dosage adjusted to the current strength of the bee colony, as the difference between the toxicity to bees and *Varroa* mites is often small (e.g., formic acid). In previous studies, lithium was mostly administered *ad libitum*. However, the feeding application method is not relevant for any currently approved *Varroa* control agent. There are several possible reasons for this:

I) Sugar syrups, which are intended to replace the natural food of bees, mainly during periods without honey flow, are predestined to be mixed into and even accumulate in the bees' stored food to a significant extent. Since this cannot be easily separated from the honey to be extracted in the hive, either in time or space, the application of the feeding method may involve an increased risk of residues.

II) The dosage of the active ingredient given during the feeding method cannot be carried out with sufficient accuracy either, as during mass treatments, it is not possible, or very difficult, to achieve a dosage appropriate to the strength of the given bee colony.

III) There are critical periods when feeding cannot be applied in beekeeping practice. In addition to the honey flow period, this includes the late autumn or early winter closing treatment, but also certain autumn periods when the bees preparing for wintering do not remove the food placed in the feeding tray. However, this type of treatment is also critical during the mid-autumn period, when the brood is already declining, as it stimulates the queen's egg-laying, thereby - undesirably - inducing a resurgence of brood in the period before wintering, which can lead to a resurgence of *Varroa* mites at a time that is critical for the survival of the bees.

The trickling application method is an effective method that allows for dosage adjusted to the strength of the bee colony, even in the case of other active ingredients (e.g., oxalic acid - Dany's Bienenwohl, thymol - Hive Alive, coumaphos - Perizin).

Based on the available data, lithium chloride may be promising in the control of *Varroa* mites in both conventional and organic apiaries. It is important to emphasize that lithium, as a trace element, may represent a fourth active ingredient family in *Varroa* mite control, in addition to synthetic acaricides, organic acids, and essential oils.

However, further research is needed to fully understand its efficacy, safety, the potential development of resistance, and its effects on bee products. However, several questions arise regarding the application of the active ingredient. On the one hand, although the laboratory results are promising, the mode of action and potential risks are not yet fully understood. On the other hand, environmental and food safety concerns may also arise in connection with the application of lithium chloride, as the active ingredient can potentially enter honey and other bee products. Currently, the approval of the agent is unlikely due to the high costs, the currently incomplete impact assessments, and regulatory challenges. However, in light of all this, lithium chloride may represent a promising opportunity for beekeepers, as previous research results are encouraging, which may contribute to lithium chloride becoming a safe alternative to current acaricides in the future.

5. NEW SCIENTIFIC RESULTS

1. We were the first to describe the contact effect of lithium chloride against *Varroa destructor* mites, which we confirmed in our investigations both in laboratory model experiments (*ex situ*) and under *in situ* conditions.

2. We demonstrated the better efficacy of lithium chloride trickling when applied as a main and supplementary treatment compared to oxalic acid sublimation, on brood-free bee colonies during the winter (November) closing treatment.

3. During the investigation of alternative application methods under *in situ* conditions, we found that hot fogging (with the Furetto device) is not suitable for the application of lithium chloride. In contrast, cold fogging (with the VAT 1a device) and the impregnated paper strip method proved to be effective against mites, but their efficacy was moderate or weak.

4. The trickling method proved to be the most effective application method. Based on the investigation of different doses, we found that 2 x 500 mM lithium chloride trickling is the most effective.

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LIST OF PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

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