Assessment of Different Lithium chloride Doses for *Varroa destructor* Control in *Apis mellifera* bees

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Abstract

Ensuring high efficiency in the control of *Varroa destructor* through the use of non-chemical products is critical for the survival of bees globally. In the Andean region of Ecuador, beekeeping activity has become relevant, due to the exuberant melliferous wealth. However, no scientific information has been generated at the country level. Therefore, the present study aimed to evaluate lithium chloride (LiCl) as a potential alternative for the control of *Varroa destructor* mite. To do this, accordingto a completely randomized design, different concentrations of LiCl, 94 mM, 14 mM and 189 mM were evaluated on the mortality of *Varroa destructor*, infestation rates and their corresponding relative efficacy. Data analyzed under a general linear model showed that mortality, infestation as well as relative efficacy was superior against *Varroa destructor* when LiCl was administered at concentrations of 141 and 189 mM, respectively. Consequently, these findings should be corroborated with more studies in

which the edaphoclimatic conditions are considered within the analyses as well as the different methods for their applicability in practice.

Keywords: Mites; Ecuador, small-scale Farming

Introduction

One-third of the food destined for humanity comes directly or indirectly from plants that are pollinated by insects (E. Kolics, Sajtos, et al., 2021). Within this broad group, Dietemann et al. (2015) and Jong et al. (2020) consider honey bees to be the most important pollinating insects in the world, so they represent an indispensable element for ecosystems, also optimizing agricultural yields (Ziegelmann et al., 2018). In addition to this, bees are now being included in studies as a One Health model organism, and thus try to explain the different interactions between climate change and antimicrobial resistance (AMR). Attribute that has been thanks to the symbiosis they form with most of the determinants of environmental health, whether physical, chemical or biological (Smart et al., 2016; Prodělalová et al., 2017).

Globally, beekeeping has progressively been threatened by a Varroa mite,which is characterized by reproducing in the cells of developing bees (Anderson & Trueman, 2000). The mechanism by which this ectoparasite acts, according to Dietemann et al. (2015) consists of feeding on hemolymph, which predisposes to the entry of other external infectious agents, ostensibly reducing the life expectancy of infested individuals (Ziegelmann et al., 2018). To address this problem, multiple chemical derivatives based mainly on amitraz, coumaphos, flumethrin and fluvalinate have been widely used (E. Kolics et al., 2020; E. Kolics, Sajtos, et al., 2021). However, under a scenario of combating AMR and restrictions mainly in developed countries, the use of chemicals in products for human consumption are currently lagging behind (De-Jongh et al., 2022).

Considering these immense challenges for beekeeping globally, it is essential to search or develop new safe and effective components for the control of *Varroa spp.* In view of this, an interesting study developed by Ziegelmann et al. (2018),revealed that lithium chloride (LiCl) could have interesting properties for use as an acaricidal agent. Study that was later contrasted with those carried out by Kolics et al. (2020, 2021, 2022) who applied this salt by different routes decreased the presence of *Varroa* mites to non-harmful levels, also observing a high degree of tolerance of adult bees.

In Ecuador, beekeeping is developed on a small scale, which is basically characterized by being family-type. In addition, according to the latest National Cadastre of Beekeeping Farms published by the Ecuadorian Agency for Agricultural Quality Assurance (Agrocalidad), by the end of 2014, 70% of beekeepers were grouped mainly in the mountain region, thanks to its wide melliferous flora (Agrocalidad, 2016). Despite this, very little importance and productive approach has been given to scientific research as a tool to try to provide solutions regarding beekeeping pathology. With this objective, this study carried out in Ecuadorian high Andean conditions evaluated the use of different doses of lithium chloride as an alternative for the control of *Varroa destructor*.

Materials and Methods

Study site and Experimental conditions

The present research work was carried out in the Riobamba Canton, Calpi parish coordinates (46◦45?55.6"N, 17◦14?52.6" E) whose height is 3250 meters above sea level. The study area is also characterized by an average temperature of 13.8 ºC, relative humidity 63% as well as an average annual rainfall of approximately 486 mm (ESPOCH weather station).

For the development of the present research, a total of 24 Langstroth type hives (505–515 mm long × 376–508 mm wide) of a single complete elevation were used (Mitchell, 2019). To those who were applied under a completely random design, one of the four established treatments; **1.) Control**, if I use LiCl, **2.) Low dose** LiCl04, 94 mM, **3.) Half dose** LiCl06, 141 mM and finally **4.) High dose** LiCl08, 189 mM. The concentrations were previously prepared in a ratio of 1 L of water, 1 kg of sugar and either 4, 6 and 8 mL of 10% LiCl, respectively (lithium chloride hydrate, Szkarabeusz Kft., Pécs, Hungary).4 applications were made according to each treatment, in feeders located in the piquera of each hive. The experiment lasted a total of 60 days. Defining the relative efficiency of the treatments based on the difference between the number of mites at the beginning and at the end of the different LiCl concentrations.

Statistical analyses

Using the statistical package SAS v.9.4 (SAS Institute Inc., Cary, NC, USA), the effect of different concentrations of LiCl on the number of *Varroa* killed / day, efficacy of treatments, as well as for the mortality of adult bees and offspring, were analyzed under a general linear model GLM procedure. For this purpose, the data previously transformed logarithmically (Log) and verified with a test of normality and heterogeneity, SAS UNIVARIATE procedure, were subjected to an ANOVA analysis. The means were expressed as least squared, being separated by the PDIFF option in SAS and adjusted by Tukey's test. Statistical differences were declared at a P value <0.05 while trends at a P value < 0.10.

Results

Data on the number of *Varroa destructor* per 200 adult bees are shown in Figure 1.

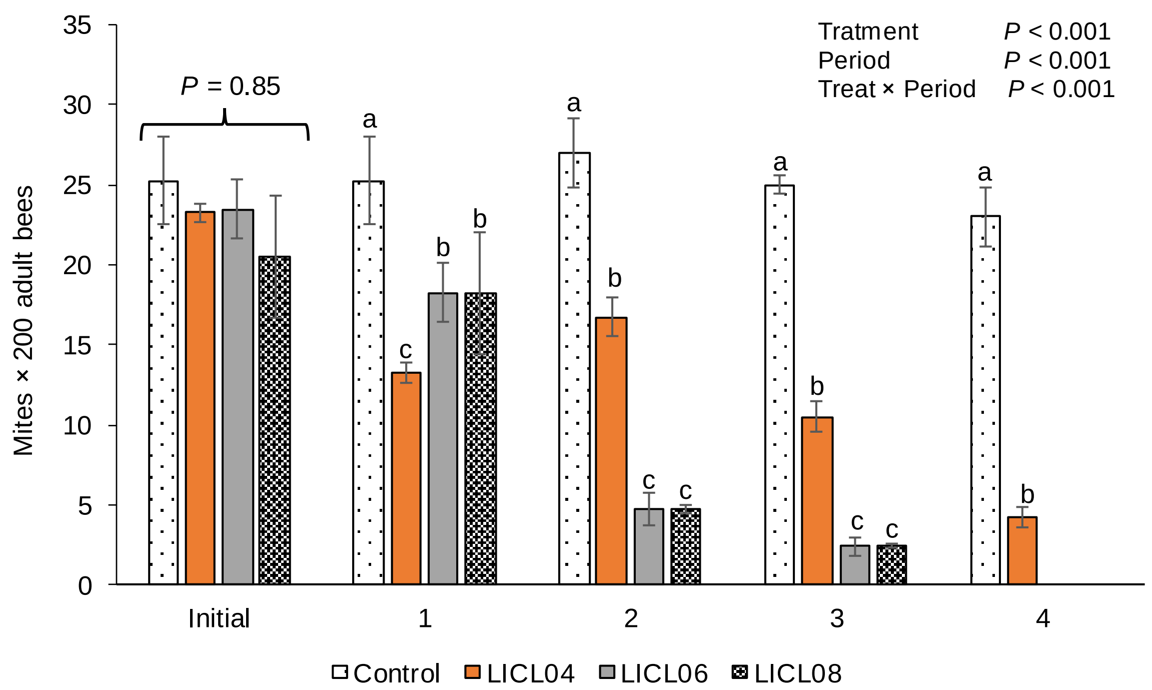


Figure 1. Means adjusted by minimum squares of the number of *Varroa destructor* × 200 adult bees, after applying the different treatments (**Control**, without any treatment; **LiCL04**, 94 mM; **LiCL06**, 141 mM and **LiCL08**, 189 mM).

As can be seen in Figure 1, all treatments based on baseline values showed no statistical differences (23 ± 2.3 *Varroas* × 200 adult bees, on average; $P = 0.85$). However, after applying the different doses of lithium chloride (LiCL), highly significant differences between treatments were observed ($P < 0.001$: Figure 1). Treatments whose LiCl dose was 141 and 189 mM, respectively, showed a significant decrease in the number of *Varroa* (9 ± 1.6; on average; P < 0.001) compared to treatment 94 mM (13.6 ± 0.8, on average) as well as for Control (25 ± 2, on average), which also differed between them ($P = 0.002$).

Similarly, highly significant differences by period effect as well as by the interaction Treatment × Period were observed (*P*< 0.001; Figure 1). In addition, in the regression analysis, the number of *Varroa* decreased progressively according to each application of LiCl, as shown in (Figure 2; *P*< 0.001), which was more effective when the LiCl was 141 and 189 mM per hive, respectively.

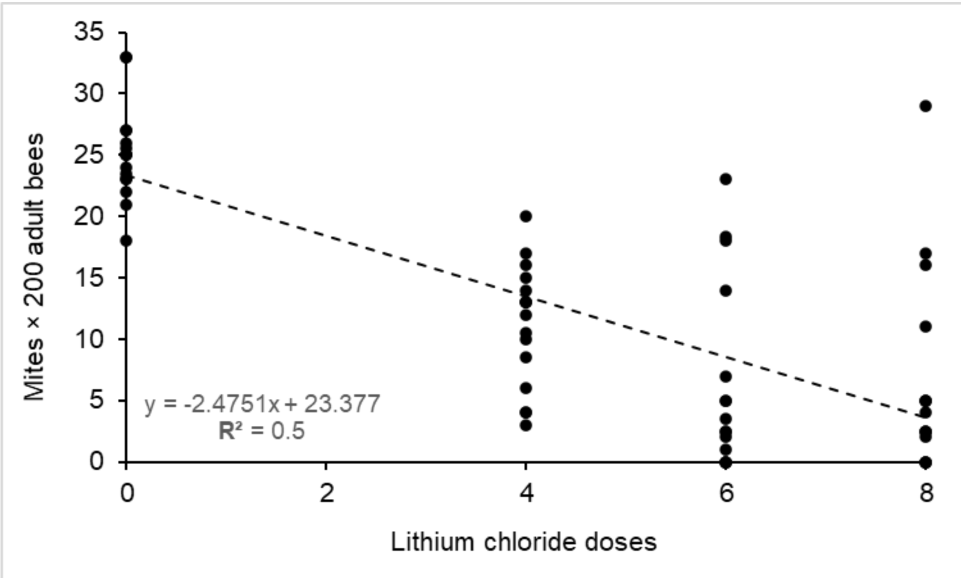
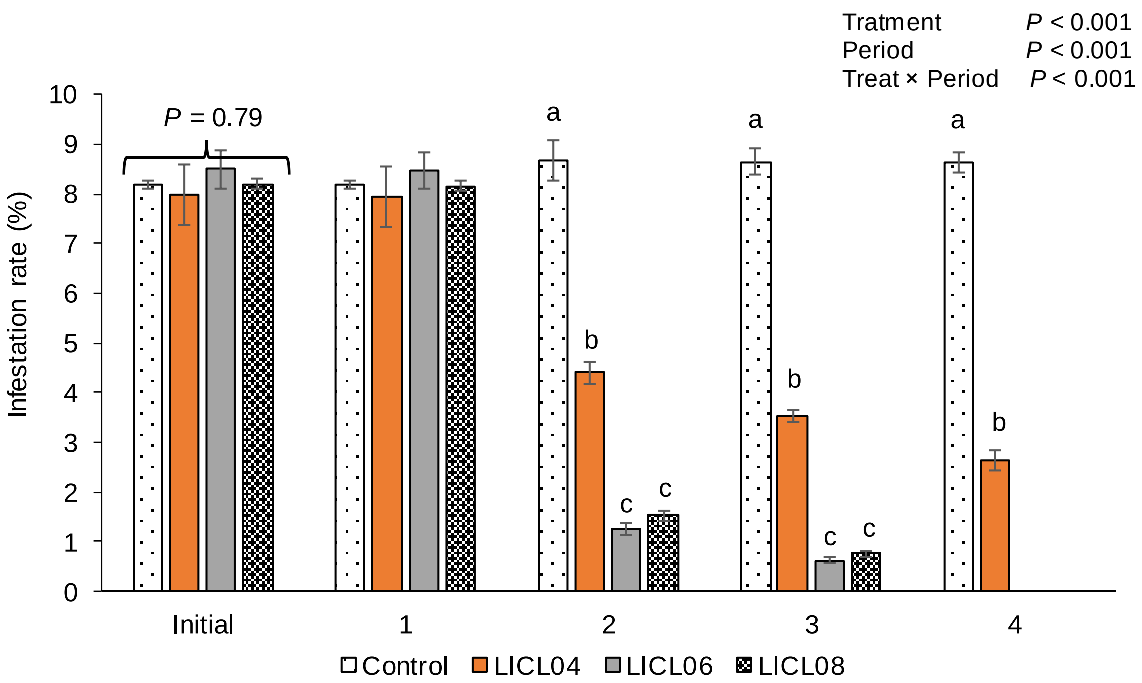


Figure 2. Significant negative linear regression analysis of the number of *Varroa* × 200 adult bees, after the application of Li CL at different doses (**Control**, without any treatment; **LiCL04**, 94 mM; **LiCL06**, 141 mM and **LiCL08**, 189 mM).

With respect to the rate of *Varroa destructor* infestation, all groups in the pre-treatment showed no statistical differences (8.2 ± 0.3%, on average; *P* = 0.79; Figure 3). In contrast, rates of *Varroa destructor* infestation varied significantly according to LiCl dose (*P* < 0.001; Figure 3). As we can see, the application of 141 and 189 mM of LiCl per hive showed a lower percentage of infestation (4 ± 0.1%, on average), compared to the treatments of 94 mM hive (5.3 ± 0.4%, on average) and Control (8.5 ± 0.2%, on average), respectively. Similarly, when comparing the infestation rate between Control and 94 mM hive, highly significant differences were observed (8 vs 5%; $P = 0.002$; Figure 3).

Figure 3. Rate of infestation of *Varroa destructor*, after applying the different



treatments (**Control**, without any treatment; **LiCL04**, 94 mM; **LiCL06**, 141 mM and **LiCL08**, 189 mM).

The infestation rate also decreased dramatically with respect to the period of its application ($P< 0.001$; Figure 3), corresponding to the doses of LiCl between 141 and 189 mM hive. Consequently, highly significant differences were observed for the Treatment × Period interaction ($P< 0.001$; Figure 3).

Additionally, the infestation rate was adjusted to a highly significant negative linear regression (Figure 4; *P*< 0.001), where it can clearly be observed that starting from an intercept of 8%, at the fourth application an average infestation rate below 0.7% was observed, which was more effective when the LiCl is in concentrations of 141 and 189 mM hive, respectively.

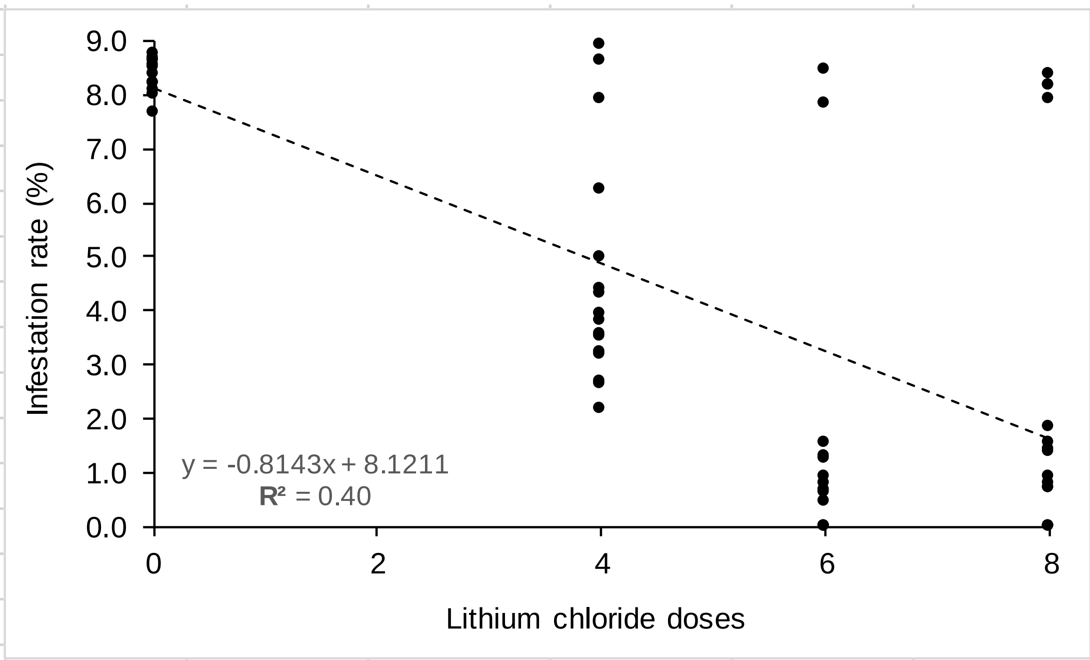


Figure 4. Significant negative linear regression analysis for the rate of infestation (%) of *Varroa destructor* after application of LiCl at different doses (**Control**, without any treatment; **LiCL04**, 94 mM; **LiCL06**, 141 mM and **LiCL08**, 189 mM).

Table 1 shows the relative efficacy for the control of *Varroa destructor according to* the different concentrations of LiCl. As we can see, the treatments whose LiCl concentrations of 141 and 189 mM were applied, demonstrated a high efficacy (100%, on average; *P*< 0.001) compared to Control (-5%, on average; Table 1) and when the LiCl was at a concentration of 94 mM (67%, on average).

Table 1. Relative efficacy of the different treatments with LiCl for the control of Varroa destructor in Ecuadorian high Andean conditions.

Treatments	Initial infestation (%)	Final infestation (%)	Difference (%)	Relative efficacy (%)
Control	8.2[a]	8.6[a]	-0.4	-5[c]
LiCl04 (94 mM)	8[a]	2.65[b]	5.4	67[b]
LiCl06 (141 mM)	8.5[a]	0[b]	8.5	100[a]
LiCl08 (189 mM)	8.2[a]	0[b]	8.2	100[a]
WITHOUT	0.3	0.1	2.6	-

SEM, standard error of the mean, [a,b,c] Means with different letters in the same line differ at $P < 0.05$.

Discussion

Global climate changes, environmental pollution and "chemization" in all human activities, especially in agriculture, have caused alterations in the ecosystem, plant production and the production of high-quality food for bees (Stanimirović et al., 2019).On the other hand, the efficacy of the drugs usually used (coumaphos, amitraz and synthetic pyrethroids) for the control of *Varroa destructor* mites has been questioned for their deliberate use (Gregorc & Planinc, 2012),which has contributed to an increase in multiresistant microorganisms (De-Jongh et al., 2022). . Under this scenario of fight against AMR as well as climate change product of the indiscriminate use of chemicals in beekeeping production. It is necessary to search for non-chemical alternatives as an alternative for the control of *Varroa*, thus guaranteeing the future of the most economically viable pollinator species in a context of global pollinator decline (Dietemann et al., 2015). According to Ziegelmann et al. (2018) and Stanimirović et al.

(2019) one of the recent varroicides discovered in laboratory tests and somewhat by chance has been lithium chloride (LiCl) that acting at a systemic level in a wide range of concentrations has been able to effectively eliminate Varroa *destructor* , although with little evidence of its use in terms of toxicity to adult bees. One of the first works developed by Ziegelmann et al. (2018) revealed a high efficacy (100%, mortality of *Varroa destructor*) when LiCl was applied at a dose of 25 mM. Contrasting these results, Stanimirović et al. (2019) and Kolics et al. (2021) using low concentrations of LiCl (10 to 25 mM) reported 100% mortality. Results that could be supported by the observed Kolics et al. (2020),who using a dose of 10.78 mM was sufficient to achieve a total mortality of 100% of *Varroa destructor*. However, the latter researchers used another method of application (strips impregnated with LiCl) revealing the irreversible effect of LiCl on mites even at a very low concentration. Consequently, these findings open new fronts of research on the effect of the application of LiCl to control *Varroa* by different routes. Because contact treatments could imply a possible decrease in the risk of honey contamination (Faichnie et al., 2021). However, in light of our results, this work showed that using LiCl at higher concentrations (between 141 and 189 mM, respectively) of those mentioned above had a mortality of 100% of *Varroa destructor* under Ecuadorian high Andean conditions. In addition, our results could be comparable to those observed by Kolics et al. (2021, 2022) who state that concentrations greater than 250 mM were more effective than low concentrations. Similarly, due to the high hygroscopicity of lithium chloride powder, it is practical to make a stock solution to prepare other dilutions, for example, with sugar syrup, as was used for our experiment. In addition, this solubility prevents it from accumulating in beeswax, which is a crucial problem for long-term treatment concepts using synthetic varrocides with lipophilic properties (Wallner, 1999; Sandra et al., 2012). But as discussed above, given a growing global concern for safe food, more work should be done to elucidate possible contamination by LiCl. Considering that the veterinary use of LiCl has not yet been authorized (Kolics et al., 2021), this salt should be used with caution, as recommended by Prešern et al. (2020).

Conclusions

Based on our results, LiCl at moderate concentrations (between 141 to 198 mM × hive) in high Andean conditions of Ecuador proved to be an effective alternative for the control of *Varroa destructor*. But despite that, more research work should be proposed and developed in order to determine the optimal method and concentration for its application in practice, considering the conditions of temperature and relative humidity.

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