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Communication

Evaluation of the Acaricidal Activity of Lithium Chloride against *Tetranychus urticae* (*Acari: Tetranychidae*)

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Abstract: *Tetranychus urticae* is a severe threat and a major source of yield loss in some agricultural and horticultural crops and it is also as a vector for several viruses. The number of active substances used against the pest is limited. Therefore, there is a continuous need for new active substances. Recently, lithium salts have been shown to be one of the most promising potential alternatives to control *Varroa destructor*; an apicultural mite pest. Based on this, we aimed to test whether the efficacy of lithium chloride extends to other agricultural mite pests, such as the two-spotted spider mite. In the present pilot study, we report for the first time that the efficacy of lithium chloride is extended to the two-spotted spider mite. Additionally, this is the first report on the acaricidal effectiveness of lithium on a plant mite pest. In the present study, we report three different concentrations that bear 100% mortality at concentrations of 5.52 M, 2.76 M, and 1.38 M. The symptoms caused were similar and followed the same sequence compared to those observed on animal parasites such as *V. destructor* and *Dermanyssus gallinae*.

Keywords: two-spotted spider mite; lithium; pesticide alternative; horticulture; efficacy; mortality

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1. Introduction

The two-spotted spider mite, *Tetranychus urticae* (C.L. Koch, 1836) (*Arachnida: Tetranychidae*), is a cosmopolitan polyphagous mite species. It can cause significant qualitative damage and yield losses in horticultural and arable crops, as well as in greenhouses and orchards [1,2]. Both larvae, nymphs, and adult developmental stages feed mainly on the lower leaf surface of plants. Their damage can be reflected in reduced photosynthesis and the introduction of phytotoxic substances into the plant through the irreversible destruction of inner plant structures [3]. In addition, web formation, mite excrement, and defoliation can affect the plant's external appearance, thereby reducing its commercial value [3]. Spider mites might be vectors of various plant viruses, e.g., potato Y virus [4]. However, subsequent studies have not confirmed it [5].

Its hidden lifestyle and high reproductive and dispersal capacity impose severe constraints on its defence. The residual pesticides on the market used to control mites are limited [6]. Moreover, the species can quickly develop a resistance to acaricides [6,7]. Chemical control agents also pose a significant environmental burden and can adversely affect humans and even some beneficial organisms. There is an ongoing need for new acaricide compounds to provide for the successful realisation of sustainable and environmentally friendly management against it.

Since synthetic acaricides are not always sufficiently effective to keep mites below the damage threshold, plant extract-based pesticides are emerging as promising options [8]. Using mixtures of biosynthetic compounds (mineral oil, entomopathogen fungi, essential oil, kaolin particle) based on plant extracts and practical application of some natural enemies as biopesticides can restrain the development of resistance [9]. These products are biodegradable with less environmental impact and non-desired side effects [10–14]. Nevertheless, their application may be limited by the problem of manufacturing them in the required quantities and in a sustainable way [9].

Lithium is widely distributed in the environment as the 27th most abundant element of the earth's crust [15]. Lithium-ion is not biodegradable; however, it is not expected to bioaccumulate. Its pollution problems are mostly related to cases where lithium is disposed of with heavy metals [16]. In large quantities, it can be toxic to plants, animals, and humans, with significant differences in susceptibility depending on the organism [17].

The effect of lithium on plants is controversial. Plant species respond differently to environmental lithium exposure while lithium is not an essential element for plant growth and development [18]. In soil, it can be toxic to plants at high concentrations [19] and it can be a limiting element for plant growth [20], biochemistry processes [21], or may inhibit DNA synthesis [22–24]. On the other hand, Li+ at low concentrations stimulates plant growth and increases plant resistance to disease [25] and, in cucumbers, it has been shown to protect against leaf powdery mildew infection when applied through the root system [26]. In maise, Li at low concentration (5 mg dm^{-3}) was shown to effectively increase biomass production by 15% [20]. Regarding plant exposure, it is interesting to note that some plants are not only tolerant but also prone to hyperaccumulate Li [18]. Because of this property, lithium may have interesting implications for the future from an agro-mining perspective [27].

In human medicine, Li^+ is used in human medicine to treat manic-depressive disorders. In uncontrolled therapeutic use, Li carbonate causes toxic symptoms [28]. However, low lithium intake causes behavioural disorders [16]. Moreover, studies indicate lower suicide rates in populations that consume water with higher ionic Li^+ content [29–34]. In recent years, modern psychiatry has proposed the production of foods supplemented with lithium, similar to the model of iodisation of table salt [35,36], in agreement with former findings [37]. Therefore, a beneficial dose of this essential element for humans can be established [38]. The dose has already been proposed with a provisional 1.0 mg daily intake based on findings that lithium can be biologically important for living organisms [37]. Its mode of action or role still remains disputed; intracellular accumulation of Li results in the replacement of Sodium, which reduces intracellular Ca^{2+} concentration, inhibits release, and facilitates the uptake of major transmitters: noradrenaline, serotonin, and dopamine [39].

Neither lithium intake from food and water nor occupational exposure presents a toxicological hazard as long as it remains at a trace element level. It does not appear to pose a considerable threat to flora and fauna on land or water [16]. Lithium can be a potential natural micronutrient component of certain foodstuffs. Many foods are rich in Li, with major Li-containing foods being cereals, vegetables, tea products, and meats [40]. The quantity depends mainly on the soil micro-element lithium content in amounts of the order of a milligram [41,42].

Microelement lithium, which can be regarded as a natural compound of food, has recently been proven effective against *Varroa destructor* (Anderson and Trueman, 2000), the most important parasite of *Apis mellifera* (Linnaeus, 1758). In relation to its potential use against pests, initial studies on the problem of residues have shown that the levels in apiculture products are not alarming and, therefore, merit further investigation. It shows low toxicity to bees under certain field conditions and its efficacy against mites can be superior to that of a significant alternative in organic beekeeping [43]. In addition, a feasible technological solution is already available should lithium become a widely authorised veterinary active substance in the near future [44]. Recently, it was revealed that lithium chloride also exerts effectivity on the poultry red mite [45]. To date, however, research on the acaricidal feature of the biometal has remained limited to its possible veterinary application.

The spider mite, similar to *V. destructor*, is problematic to control because of its hidden lifestyle and the development of resistance. The present study aimed to investigate whether the efficacy of lithium may also be extended to plant pest mite species. By means of these initial results, we are going to enrich the plant protection knowledge of this severe agricultural pest, providing an opportunity for the realisation of a successful control method against it.

2. Materials and Methods

2.1. Experimental Design

T. urticae specimens of mixed age, sex, and gender were collected from non-spraying soybean fields in three places at Kaposvár, Hungary (N: 46.39 E: 17.85) in June 2022. Three subsamples of *T. urticae* were collected from five plant leaves from each location. The sampling areas were separated by at least 1000 m and 300–500 spider mites were collected in each subsample. *T. urticae* mixed and transferred to host soybean plants; these plants were not treated with any acaricide previously. The mites were kept on the plant until they were used in the experiments under laboratory conditions (at 25 ± 1 °C, 60 ± 5% relative humidity (RH), and a photoperiod of L16:D8 h each day). Altogether 60 individuals were investigated in each treatment (20 individuals in 3 replications). In total, 180 individuals were used.

2.2. Acaricidal Bio-Assay

The contact micro-immersion bio-assay was performed according to Dennehy et al. [46] with slight modifications as follows: a batch of mites was immersed in a 1.5 mL Eppendorf tube containing 1 mL lithium chloride solution of the following concentrations: 5.52 M, 2.76 M, and 1.38 M. The tubes were flipped up and down ten times for 10 s (one turn per second) in the bio-assay. These concentrations correspond to those applied in an earlier experiment carried out against the mite parasite of the honey bee [47]. Subsequently, they were placed on a filter disc (Sartorius, d = 150 mm, Grade: 1292) in a Petri dish. To preselect individuals, mites that did not show vigorous movements were discarded, leaving 20 vital individuals on each dish, and their activities were recorded using a binocular microscope. Each treatment was performed in three iterations; the control treatment was carried out with the water used for creating the aqueous solutions.

The first recorded sign of lithium poisoning was regarded at the onset of uncontrolled tremorous locomotion performed by the first mite in the block of 20 individuals. Subsequently, the occurrence of the first dead mite in the block, the occurrence of 50% dead mites, the occurrence of 90% dead mites, and the occurrence of all dead mites in the block was recorded.

2.3. Statistical Analysis

The elapsed times from the beginning of the treatments were analysed separately for each treatment for the recorded events detailed above. Abbott's formula [48] was applied to compute the mortality rates. The data were transformed by the ln transformation (natural logarithm). The Kolmogorov–Smirnov and the Shapiro–Wilk tests carried out normality testing for the above events. All the events were found to be normally distributed ($p > 0.05$). The statistical differences were analysed with one-way ANOVA for each event separately. The Levene test justified the homogeneity of variances ($p > 0.05$). The pairwise differences were analysed with Tukey's post hoc test. The mortality rates computed in the statistical tests were calculated by SPSS 22.0 software.

3. Results

Abbott's corrected mortalities of *T. urticae* experimental population triggered by lithium chloride are shown in Figure 1 and Table 1. Depending on the time elapsed after treatment in minutes, the mortality character of some doses were logarithmic types in any case. The mortalities were unequivocally increased by the elapsed times. Besides, their tendencies were approached to the total eradication degree by a steeper curve and relatively faster. The symptoms induced by the treatment were similar to those observed in *V. destructor*, as uncontrolled tremorous insect movement was followed by death, except that the immobility phase was not observed in the case of the two-spotted spider mite. The recovery from paralysis was observed on some mites in the 0.69 M concentration. Therefore, the observation of LT90 was discontinued.

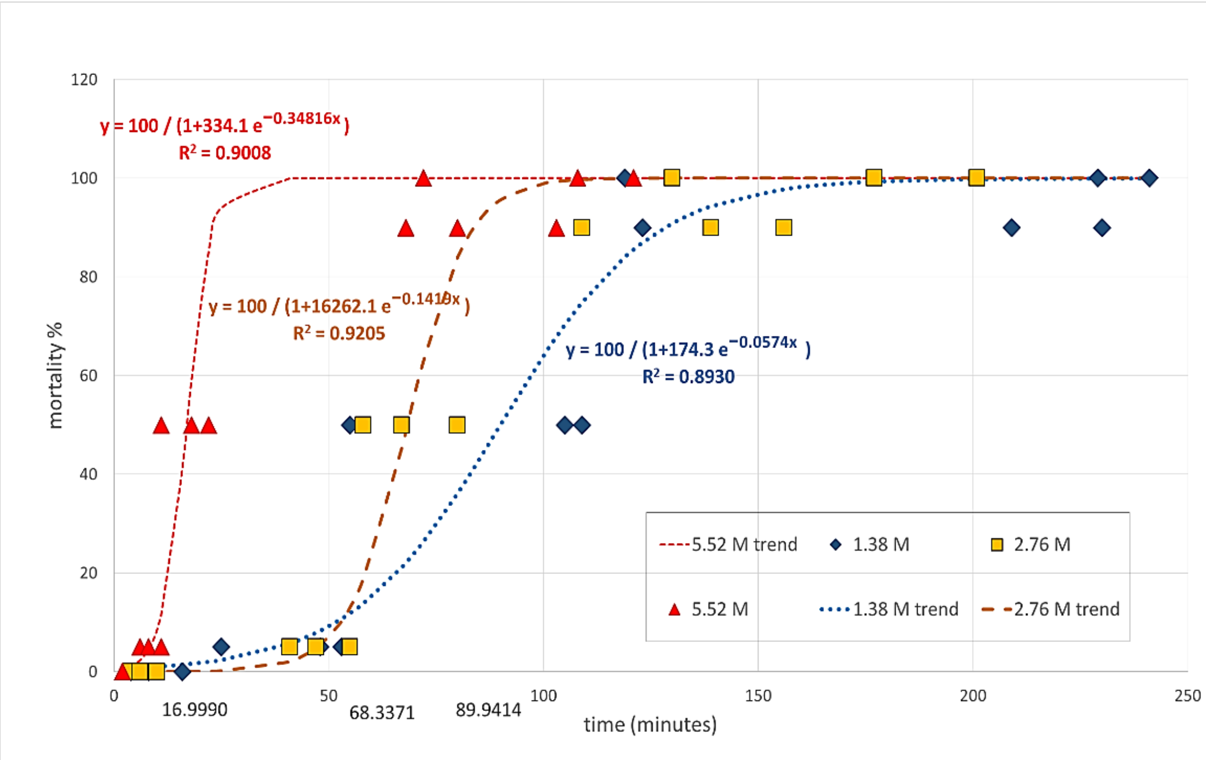

Figure 1. Abbott-corrected mortality rates for the three treatments.

Table 1. Mean ± SE (standard error) of time (in minutes) elapsed after treatment till the occurrence of events.

Treatment	First Symptom *	First Dead *	LD50 *	LD90 *	LD100
1.38 M	9.33 ± 3.528 [a]	42.00 ± 8.622 [a]	89.6 7± 17.372 [a]	187.33 ± 32.733 [a]	196.33 [a] ± 38.822
2.76 M	6.67 ± 1.764 [a]	47.67 ± 4.055 [a]	68.33 ± 6.386 [a]	134.67 ± 13.740 [a,b]	169.33 [a] ± 20.851
5.52 M	1.67 ± 0.333 [b]	8.33 ± 1.453 [b]	17.00 ± 3.215 [b]	83.67 ± 10.269 [b]	100.33 [a] ± 14.655
The trend of mortality rates (y) by time elapsed after treatment in minutes (x)					
1.38 M	$y = 100/(1 + 16262.1 \pm e^{-0.1419x})$; $R^2 = 0.9205$				
2.76 M	$y = 100/(1 + 174.3 \pm e^{-0.0574x})$; $R^2 = 0.8930$				
5.52 M	$y = 100/(1 + 334.1 \pm e^{-0.34816x})$; $R^2 = 0.9008$				

*: indicates significant differences between treatments at 0.05 significance level. [a,b]: indicate significantly different treatments by a Tukey post hoc test. Note: LD50, LD90, LD100: time to the occurrence of 50%, 90% and 100% dead mites respectively.

As is shown in Figure 1, a classic logistic growth curve (also known as the Pearl–Reed logistic curve) was fitted to the Abbott corrected mortality rate data (Abbott, 1925) for each treatment, of the form $y = K/(1 + b \times e^{-cx})$ with $K = 100$, and y representing the mortality rate and x the time (in minutes) elapsed after treatment. The fitted equations are shown in Figure 1 and Table 1, with the respective R^2 values (greater than 0.89). As the figure shows, the higher concentrations lead to faster mortality, reaching both LD50 and LD90 or LD100 in significantly shorter times.

Table 1 provides the observed mean time (in minutes) elapsed after treatment needed to reach the given mortality stages for each treatment. As is shown in Table 1 and in Figure 1, the largest dose required only half of the time of the smallest dose for the LD100 stage, while the difference is even greater for LD50, especially LD90. Similar tendencies hold for the other mortality stages, too. The standard errors (SE) in Table 1 and the confidence intervals in Figure 1 also indicate that the largest dosage produced the most stable elapsed times (in minutes), having the smallest SE values for any stage. This is supported by the parameters of the fitted logistic equations for the three treatments, presented in Table 1, too.

One-way ANOVA results identified significant differences between the mean ln-transformed times elapsed after treatment (in minutes) of the three treatments with three replications for each treatment. The treatments significantly differ at $p = 0.05$ in the occurrence times of each mortality stage, except the last one (LD100). A post hoc test (Tukey HSD) was applied to compare the treatments pairwise with regard to elapsed times (in minutes) after treatment to the mortality stages. According to Table 1, the largest dosage significantly differs from the other two treatments up to the occurrence of 50% dead mites (LD50) and it significantly differs from the smallest concentration for the occurrence of 90% dead mites (LD90). However, the treatments do not differ significantly in the occurrence of 100% dead mites.

4. Discussion

Our results highlight the potential of lithium salts as a putative control alternative for different mite species. The control of *T. urticae* is complicated in pest management due to the species' polyphagous and cosmopolitan nature. Regarding synthetic acaricides, nonsynonymous mutations in the pesticide target site of these agents are often reported [49], implying an inevitable risk of long-term resistance. In recent years, the interest in nature-identical pesticides has increased significantly due to environmental concerns and the resistance of the two-spotted mites to conventional pesticides.

The veterinary potential of lithium appeared as the most recent feature of the trace element proposed first for *Varroa destructor*, a detrimental pest of the honey bee [50], with promising results in its potential practical application [43,44], yet with a single example of registration as a veterinary medicine (Serbia).

Another study [47] tested the impact of various lithium concentrations on *Varroa destructor*, applying 11 concentration levels of lithium chloride solution ranging from 10.78 mM to 11.04 M, measuring the exposure time needed until the occurrence of the tremorous movement of the animals and then their fall. In this study, every mite exposed to lithium was first affected by tremors and subsequently dropped and the exposure times decreased with increasing concentrations. The exposure time until the mite fell down showed significant differences according to the concentration. Lithium was also found to be effective for poultry red mite. Lithium concentrations of 5.52 M, 2.76 M, and 1.38 M were also tested on poultry red mite (*Dermanyssus gallinae*) recently [45]. The time needed for the death of 50% of the animals—i.e., reaching LD50—was 25, 45, and 134 min for the 5.52 M, the 2.76 M, and the 1.38 M concentrations, respectively, and similarly significant differences occurred for reaching the LD90 stage (64, 87, and 312.5 min for the 5.52 M, the 2.76 M, and the 1.38 M concentrations, respectively). Of all the species tested so far, lithium was found to be most effective on *V. destructor* and least effective on *T. urticae*.

The use of elements of the earth's crust has already been an example in plant protection, such as copper compounds, for instance, with several publications discussing how their long-term use alters the mineral composition of the soil [51]. Supposing vegetative parts remain in the area, the potential use of lithium may increase its quantity in soil compared to its original presence. It may possess different levels of risk as lithium is not a heavy metal but a geochemically mobile element [16], but further studies may comprehensively evaluate these aspects. As regards the risks for soil, lithium may be a concern for contamination in the case of repeated exposures. Possibly, mass outbreaks of spider mites (*Tetranychus* spp., *Panonicus* spp., *Bryobia* spp.) caused by extremely arid climatic conditions might justify furhter research on its occasional emergency use in field crops. Concerning horticultural production and in closed growing facilities due to the involvement of beneficial mites occurring there [52,53], it is not considered feasible at this stage. It is interesting that there seems to be some mites species, e.g., the *Thyrophagus putescentiae* (Schrank, 1781) species we studied, on which lithium does not show efficacy. It is also essential to investigate the effect of lithium on predatory mite species (e.g., *Phytoseiulus* sp., *Ambliseius* sp.) used in certain crops to reveal the potential hazards in this aspect as well.

5. Conclusions

Our studies were limited to in vitro experiments but revealed for the first time that lithium exerts effectiveness on a crop pest, the two-spotted spider mite, with the aim to extend the knowledge about lithium biometal. The study remained restricted to concentrations at which lithium chloride effectiveness is demonstrated with full mortality. At this stage, it is too early to conclude any practical relevance for plant protection. Nevertheless, our results may contribute to ecotoxicological studies, but it seems that the targeted efficacy of the compound remains primarily for apicultural purposes.

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