

Supplementary information for the article:

Stanimirovic, Z.; Glavinic, U.; Jovanovic, N. M.; Ristanic, M.; Milojković-Opsenica, D.; Mutic, J.; Stevanovic, J. Preliminary Trials on Effects of Lithium Salts on Varroa Destructor, Honey and Wax Matrices. *Journal of Apicultural Research* **2022**, *61* (3), 375–391. <https://doi.org/10.1080/00218839.2021.1988277>.



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Supplementary Table S1: *Varroa destructor* mortality (varoacidal efficacy) induced by LiCl and Li-cit in various concentrations (laboratory experiment).

Tested chemicals	Groups ¹	Cages (n)	Significance of differences											
			Lithium chloride (LiCl)						Lithium citrate (Li-cit)					
			Lab-LiCl-1	Lab-LiCl-2	Lab-LiCl-4	Lab-LiCl-7.5	Lab-LiCl-10	Lab-LiCl-25	Lab-Li-cit-1	Lab-Li-cit-2	Lab-Li-cit-4	Lab-Li-cit-7.5	Lab-Li-cit-10	Lab-Li-cit-25
None	Lab-Control	7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Lithium chloride (LiCl)	Lab-LiCl-1	7		<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	Lab-LiCl-2	7			<0.001***	0.001***	<0.001***	<0.001***	<0.001***	0.223	<0.001***	<0.001***	<0.001***	<0.001***
	Lab-LiCl-4	7				0.011***	<0.001***	<0.001***	0.660	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	Lab-LiCl-7.5	7					<0.001***	<0.001***	0.004**	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	Lab-LiCl-10	7						<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	Lab-LiCl-25	7							<0.001***	<0.001***	<0.001***	0.002**	<0.001***	<0.001***
Lithium citrate (Li-cit)	Lab-Li-cit-1	7								<0.001***	<0.001***	<0.001***	0.001**	<0.001***
	Lab-Li-cit-2	7									<0.001***	<0.001***	<0.001***	<0.001***
	Lab-Li-cit-4	7										0.597	0.370	0.001**
	Lab-Li-cit-7.5	7											0.152	<0.001***
	Lab-Li-cit-10	7												0.002**
	Lab-Li-cit-25	7												

¹Group names are indicated in Table 1.

P values of pairwise comparison of bee survivorship (Log-rank test followed by a Bonferroni correction) *P<0.05, **P<0.01 and ***P < 0.001.

Supplementary Table S2: Mortality of bees treated with LiCl and Li-cit in various concentrations (laboratory experiment). Group names are indicated in Table 1.

Tested chemicals	Groups ¹	Cages (n)	Significance of differences											
			Lithium chloride (LiCl)						Lithium citrate (Li-cit)					
			Lab-LiCl-1	Lab-LiCl-2	Lab-LiCl-4	Lab-LiCl-7.5	Lab-LiCl-10	Lab-LiCl-25	Lab-Li-cit-1	Lab-Li-cit-2	Lab-Li-cit-4	Lab-Li-cit-7.5	Lab-Li-cit-10	Lab-Li-cit-25
None	Lab-Control	7	<0.001***	0.007**	0.222	0.007**	0.623	0.987	<0.001***	<0.001***	<0.001***	<0.001***	0.303	0.878
Lithium chloride (LiCl)	Lab-LiCl-1	7		0.361	0.017*	0.362	0.002**	<0.001***	0.045*	0.045*	0.045*	0.045*	0.010*	<0.001***
	Lab-LiCl-2	7			0.122	0.997	0.025*	0.007**	0.008**	0.008**	0.008**	0.008**	0.083	0.011*
	Lab-LiCl-4	7				0.120	0.464	0.218	<0.001***	<0.001***	<0.001***	<0.001***	0.847	0.2861
	Lab-LiCl-7.5	7					0.025*	0.007**	0.008**	0.008**	0.008**	0.008**	0.082	0.010*
	Lab-LiCl-10	7						0.613	<0.001***	<0.001***	<0.001***	<0.001***	0.589	0.736
	Lab-LiCl-25	7							<0.001***	<0.001***	<0.001***	<0.001***	0.297	0.866
Lithium citrate (Li-cit)	Lab-Li-cit-1	7								>0.999	>0.999	>0.999	<0.001***	<0.001***
	Lab-Li-cit-2	7									>0.999	>0.999	<0.001***	<0.001***
	Lab-Li-cit-4	7										>0.999	<0.001***	<0.001***
	Lab-Li-cit-7.5	7											<0.001***	<0.001***
	Lab-Li-cit-10	7												0.381
	Lab-Li-cit-25	7												

¹Group names are indicated in Table 1.

P values of pairwise comparison of honey bee survivorship (Log-rank test followed by a Bonferroni correction) *P<0.05, **P<0.01 and ***P < 0.001.

Supplementary material

Supplementary Table S3: Varroacidal efficacy (%) of different Li-cit concentrations vs Amitraz and control (Field-1 experiment)

Group ¹	No. of colonies	Mean	Std. Dev.	Minimum	Maximum	Coef. of Var.
F1-Control	6	8.30	1.23	6.25	9.86	14.85
F1-Amitraz	6	89.76	1.13	88.26	91.54	1.26
F1-Li-cit-5	6	93.23	1.36	91.87	95.61	1.46
F1-Li-cit-10	6	94.20	0.68	93.30	94.94	0.73
F1-Li-cit-15	6	94.65	1.10	92.90	95.70	1.16
F1-Li-cit-20	6	95.09	1.26	93.45	96.63	1.33
F1-Li-cit-25	6	95.46	0.74	94.34	96.39	0.77

¹Group names are indicated in Table 1.

Supplementary Table S4: Lithium residues ($\mu\text{g}/\text{kg}$) in brood chamber honey in groups treated with Li-cit vs control (Field-1 experiment)

Group ¹	No. of colonies	Median	Min.	Max.	Lower quartil	Upper quartil	Coef. of Var.
F1-Control	6	3.16	2.53	3.52	2.89	3.29	10.53
F1-Li-cit-5	6	169.30	85.67	1805.00	126.30	1084.00	127.63
F1-Li-cit-10	6	214.10	62.18	1140.00	115.40	754.20	104.21
F1-Li-cit-15	6	1294.00	613.20	3850.00	908.60	2614.00	68.78
F1-Li-cit-20	6	1756.00	7.16	4240.00	24.85	4190.00	106.81
F1-Li-cit-25	6	205.00	14.38	2420.00	53.45	1273.00	147.17

¹Group names are indicated in Table 1.

Supplementary Table S5: Lithium residues ($\mu\text{g}/\text{kg}$) in brood chamber wax combs in groups treated with Li-cit vs control (Field-1 experiment)

Group	No. of colonies	Median	Min.	Max.	Lower quartil	Upper quartil	Coef. of Var.
F1-Control	6	1431.00	307.80	6996.00	578.30	4134.00	107.67
F1-Li-cit-5	6	483.60	311.10	1647.00	397.70	905.30	73.89
F1-Li-cit-10	6	410.50	52.07	724.40	81.39	657.90	78.74
F1-Li-cit-15	6	2314.00	506.60	12796.00	671.40	7614.00	114.63
F1-Li-cit-20	6	421.80	66.65	18187.00	93.44	7528.00	185.78
F1-Li-cit-25	6	970.30	174.00	7919.00	432.70	5336.00	122.78

¹Group names are indicated in Table 1.

Supplementary Table S6: Li residues ($\mu\text{g}/\text{kg}$) in honey (H) from brood chamber (Bch) and honey chamber (Hch) – hives treated with 5 mM and 20 mM Li-cit and control (Field-2 experiment)

Sample	n	Mean	Std. Dev.	Minimum	Maximum	Coef. of Var.
H-Control	10	17.62	1.68	15.38	20.62	9.51
H-Bch-5	10	19.16	1.11	17.55	21.00	5.77
H-Bch-20	10	27.81	4.07	22.68	37.59	14.63
H-Hch-5	10	26.91	6.36	18.52	36.21	23.62
H-Hch-20	10	33.71	9.45	21.98	47.92	28.04

Supplementary Table S7: Li residues ($\mu\text{g}/\text{kg}$) in wax combs (Wcomb) and wax cappings (Wcapp) from honey cells (Hcell) and brood cells (Bcell) in honey chamber (Hch) and brood chamber (Bch) - hives treated with 5 mM and 20mM of Li-cit (Field-2 experiment)

Sample	n	Mean	Std. Dev.	Minimum	Maximum	Coef. of Var.
Wf-Control	10	297.20	10.57	278.40	313.80	3.56
Wcomb-Bch-5	10	300.60	80.02	240.40	449.80	26.62
Wcomb-Bch-20	10	292.60	48.90	232.90	379.50	16.71
Wcomb-Hch-5	10	259.80	30.24	205.70	300.50	11.64
Wcomb-Hch-20	10	282.60	45.99	224.70	387.90	16.27
Wcapp-Bcell_Bch-5	10	234.30	23.12	199.20	280.10	9.87
Wcapp-Bcell_Bch-20	10	267.70	46.55	211.50	363.30	17.39
Wcapp-Hcell_Bch-5	10	255.80	21.74	214.90	294.20	8.50
Wcapp-Hcell_Bch-20	10	262.80	34.26	194.20	319.60	13.04
Wcapp-Hcell_Hch-5	10	253.40	29.59	203.70	300.50	11.68
Wcapp-Hcell_Hch-20	10	266.30	16.02	243.00	304.20	6.01