

BIOLOGICAL ACTIVITY OF TRIS-[N-(PROP-2-EN-1-YL)HYDRAZINE-CARBOTHIOAMIDE]COBALT(III) CHLORIDE

Olga GARBUZ¹, Vasiliu GRAUR², Nadejda RAILEAN¹, Victor TSAPKOV²,
Veronica SARDARI³, Donald POIRIER⁴, Jenny ROY⁴, Ion TODERAS¹, Aurelian GULEA²

¹Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, Moldova State University, 1 Academiei Street, MD-2028 Chisinau, Moldova

²Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova

³Department of Biochemistry and Clinical Biochemistry, State University of Medicine and Pharmacy "Nicolae Testemitanu", MD-2004 Chisinau, Moldova

⁴Laboratory of Medicinal Chemistry, CHU de Québec Research Center, Université Laval, Québec, QC G1V4G2, Canada;

Abstract

This study investigates the biological activity of the coordination compound tris-[N-(prop-2-en-1-yl)hydrazinecarbothioamide]-cobalt(III) chloride, [Co(TSC)₃]Cl₃. The antioxidant activity of [Co(TSC)₃]Cl₃ was evaluated by its ability to inhibit the ABTS radical, with IC₅₀ of 7.3 μmol/L. The toxicity of [Co(TSC)₃]Cl₃ toward *Daphnia magna* was relatively low, with LC₅₀ of 56.3 μmol/L. Experiments with bees of the *Apis mellifera* species showed that the complex increases the total antioxidant status (TAS) in the hemolymph of adult bees and larvae by 5 and 8 times, respectively. The IC₅₀ values for the antioxidant effect were 2.5 mg/mL in adult bee hemolymph and 1.3 mg/mL in larval hemolymph, which are lower than the corresponding control values of 13.6 mg/mL and 10.0 mg/mL. The stimulatory effect of the tested complex on TAS exceeded that of ascorbic acid by up to five times. In addition, [Co(TSC)₃]Cl₃ exhibited high acaricidal activity against *Varroa destructor*, with LC₅₀ of 0.2 μmol/L.

Keywords: tris-[N-(prop-2-en-1-yl)hydrazinecarbothioamide]-cobalt(III) chloride; antioxidant activity; total antioxidant status; biological activity.

ACTIVITATEA BIOLOGICĂ A COMPLEXULUI CLORURĂ DE TRIS[N-(PROP-2-EN-1-IL)HIDRAZINECARBOTHIOAMIDĂ]COBALT(III)

Rezumat

Acest studiu investighează activitatea biologică a complexului clorură de tris-[N-(prop-2-en-1-il)hidrazinecarbothioamidă]cobalt(III), [Co(TSC)₃]Cl₃. Activitatea antioxidantă a complexului [Co(TSC)₃]Cl₃ a fost evaluată prin capacitatea de inhibare a radicalului ABTS, obținându-se o valoare a IC₅₀ de 7,3 μmol/L. Toxicitatea complexului față de *Daphnia magna* a fost relativ scăzută, cu o valoare a LC₅₀ de 56,3 μmol/L. Experimentele efectuate pe albinele din specia *Apis mellifera* au arătat că acest complex crește statusul antioxidant total (TAS) în hemolimfa albinelor adulte și a larvelor de 5, respectiv 8 ori. Valorile IC₅₀ pentru efectul antioxidant au fost de 2,5 mg/mL în hemolimfa albinelor adulte și de 1,3 mg/mL în hemolimfa larvară, fiind mai mici decât valorile corespunzătoare ale matorului, de 13,6 mg/mL și 10,0 mg/mL. Efectul stimulator al complexului testat asupra TAS a depășit efectul acidului ascorbic de până la cinci ori. În plus, [Co(TSC)₃]Cl₃ a prezentat o activitate acarică ridicată împotriva *Varroa destructor*, cu o valoare a LC₅₀ de 0,2 μmol/L.

Cuvinte-cheie: clorură de tris-[N-(prop-2-en-1-il)hidrazinecarbothioamidă]cobalt(III); activitate antioxidantă; status antioxidant total; activitate biologică.

Introduction

The European honey bee (*Apis mellifera*) plays a key role in the pollination of natural ecosystems and agricultural crops, and is also the foundation of beekeeping. Maintaining their health and productivity is directly linked to the effectiveness of their antioxidant system, which

neutralizes reactive free radicals generated by high metabolic activity. Insects, including bees, produce significant amounts of free radicals, making them particularly vulnerable to oxidative stress. Its development disrupts cellular function, promotes disease, and reduces colony viability [1–5].

A significant factor in increasing oxidative stress is the infestation of the parasitic mite *Varroa destructor*, which feeds on hemolymph and damages key bee tissues. This leads to increased free radical generation, overload of antioxidant systems, accelerated aging, and decreased immunity, which can ultimately lead to colony collapse.

Assessing antioxidant activity (AOA) is an important indicator of the functional state of the antioxidant defenses of bees and their larvae [6–10]. An important characteristic is the total antioxidant status (TAS) of *Apis mellifera*, reflecting the combined activity of both enzymatic and non-enzymatic antioxidants [11–12]. Dietary supplements based on ascorbic acid are widely used to increase TAS; however, their relatively weak antioxidant activity limits their practical effectiveness.

In recent years, the attention of researchers has shifted to transition metal compounds and their coordination complexes, which can significantly enhance antioxidant protection [13–20]. However, the use of such complexes in beekeeping remains poorly studied, and the literature data are contradictory: some studies indicate a pronounced protective effect, while others note potential toxicity or low efficacy [21–25]. This emphasizes the need to develop new substances that combine high antioxidant activity and low toxicity.

This study examined the antioxidant activity of the hemolymph of adult bees and *Apis mellifera* larvae, and assessed the effect of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on antioxidant defense. The complex was shown to exhibit antioxidant properties, suppressing free radical activity and increasing the effectiveness of the antioxidant system after addition to bee feed. These properties indicate that $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ is a promising agent for stimulating TAS and enhancing resistance.

Furthermore, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ exhibits pronounced acaricidal activity against *Varroa destructor*, opening the door to the development of multifunctional apiculture products that combine antioxidant support and protection against varroaosis.

Materials and methods

Chemicals and reagents

All chemicals were of analytical grade and used as received. Cobalt(II) chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, Merck) was employed without additional purification. Allyl isothiocyanate, 50–60% (w/w) aqueous solution of hydrazine, and hydrochloric acid were obtained from Sigma-Aldrich. The ligand, 4-allylthiosemicarbazide (TSC), was synthesized by the reaction of a four-fold excess of 50–60% hydrazine solution with allyl isothiocyanate according to the published procedure. The composition of the complex was verified by elemental analysis on a Perkin Elmer 2400 elemental analyzer, and its molar conductivity in 1 mM methanol solution was measured with an R-38 conductometric bridge at 20 °C.

Synthesis of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$

$[\text{Co}(\text{TSC})_3]\text{Cl}_3$ was synthesized by dissolving 4-allylthiosemicarbazide (TSC, 3.94 g, 30 mmol) in 25 mL of hot ethanol (55 °C). Cobalt(II) chloride hexahydrate (2.38 g, 10 mmol) was added to the solution, followed by 0.83 mL of 37% (w/w) hydrochloric acid (10 mmol). The reaction mixture was stirred for 1 h at 55 °C under continuous air bubbling. After cooling to room temperature, the resulting brown precipitate was filtered, washed with cold ethanol, and dried in vacuo. The yield of the complex was 87%. Elemental analysis and conductivity data were consistent with the formula $\text{C}_{12}\text{H}_{27}\text{Cl}_3\text{CoN}_3\text{S}_3$.

Enriched feeding technique on *Apis mellifera* colonies

Biological trials were conducted on colonies of *Apis mellifera*. The coordination compound $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ was added to a sugar syrup (50% sugar, 50% water) at concentrations of 0.2 mg/L and 0.6 mg/L. The feeding was carried out at the beginning of spring, when nectar and pollen sources were limited.

The experiments were performed on three groups of 10 hives each. The control group (I) received plain sugar syrup without the complex. The experimental groups (II and III) were fed syrup fortified with $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ at concentrations of 0.2 mg/L (2 mg per hive) and 0.6 mg/L (6 mg per hive), respectively. The syrup (100 mL per frame) was poured into a container placed in the upper part of the hive and was replaced every two days over a period of two weeks.

Determination of antioxidant activity (ABTS assay)

Antioxidant activity was assessed by the ABTS radical cation scavenging method, modified from the procedure described by Re et al. The radical cation was generated by reacting ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) with potassium persulfate in the dark at 25 °C for 12–20 h. The stock solution was diluted with ethanol (pH adjusted to 6.5) to obtain an absorbance of 0.70 ± 0.01 at 734 nm. Aliquots of test solutions (20 μL) were mixed with 180 μL of the working ABTS solution in a 96-well microplate, and the decrease in absorbance at 734 nm was recorded after 30 min of incubation at 25 °C using a Synergy H1 microplate reader (BioTek). The percent inhibition (I, %) of ABTS was calculated from the difference in absorbance between control and sample wells, and IC_{50} values were derived from dose-response curves.

Determination of antioxidant activity (DPPH assay)

The DPPH radical scavenging assay was performed according to Brand-Williams et al. with minor modifications. A 0.002% (w/v) methanolic DPPH solution was adjusted to an absorbance of 0.70 ± 0.01 at 517 nm. Test solutions (20 μL) were mixed with 180 μL of DPPH working solution in a 96-well microplate, briefly shaken, and incubated for 30 min at 25 °C. Absorbance at 515 nm was measured on the same microplate reader. The percent inhibition (I, %) of DPPH was calculated analogously to the ABTS assay, and IC_{50} was obtained by nonlinear fitting of the dose-response data.

Assessment of *Varroa destructor* infestation

The level of *Varroa destructor* infestation was evaluated using the powdered-sugar method. Bees (300 individuals, approximately 30 g) harvested from a well-developed brood frame were placed in a jar with a perforated lid, and 10 g of powdered sugar was added. After gently shaking the jar for 2 min, mites fell through the perforations onto a light-coloured surface, while the bees were returned to the colony. The mites were counted, and the number per 100 bees (10 g) was calculated by dividing the total count by 3 and expressing the result as a percentage. The level of brood infestation was estimated by inspecting 100 larvae per colony.

Statistical analysis

Antioxidant-assay results are presented as mean $\pm$ standard deviation (SD) of triplicate determinations. IC_{50} values were calculated from dose-response curves using the least-squares fit method. The acaricidal effect of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ was characterized by the percentage reduction in *Varroa destructor* infestation relative to the control group and by the median lethal concentration (LC_{50}) determined using GraphPad software. All data were evaluated statistically, and differences were considered significant at $p < 0.05$.

Results and discussion

The total antioxidant status (TAS)

Properties of the complex $[\text{Co}(\text{TSC})_3]\text{Cl}_3$

A new cobalt(III) coordination compound, tris-[*N*-(prop-2-en-1-yl)hydrazine-carbothioamide]cobalt(III) chloride ($[\text{Co}(\text{TSC})_3]\text{Cl}_3$), has been developed to regulate the total

antioxidant status (TAS) of *Apis mellifera* hemolymph. The compound was synthesized in ethanol from 4-allylthiosemicarbazide (TSC), cobalt(II) chloride hexahydrate, and hydrochloric acid in a 3:1:1 molar ratio under air bubbling. The composition and structure of the complex were confirmed by elemental analysis and molar conductivity, with a value of $312 \Omega^{-1} \cdot \text{cm}^2 \cdot \text{mol}^{-1}$ in a 0.001-mol/L methanol solution at 20 °C, corresponding to a 1:3-type electrolyte, i.e., $[\text{Co}(\text{TSC})_3]^{3+}$ and three Cl^- ions in the outer sphere.

In the Toxicity Screen assay with *Daphnia magna*, a relatively low LC_{50} of $56.3 \pm 3.1 \mu\text{mol/L}$ was observed, indicating that the complex can be considered potentially safe for use in biological systems of bees.

The activity of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ toward free radicals was evaluated in model systems using the ABTS assay. The ABTS test showed that $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ exhibits high antioxidant activity, exceeding that of the standard compounds Trolox and ascorbic acid.

Table 1 presents the IC_{50} values for $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ and the reference antioxidants.

Table 1. Comparative antioxidant activity of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ and reference compounds in the ABTS radical-scavenging assay, expressed as IC_{50}

Compound	IC_{50} , $\mu\text{mol/L}$
Trolox	33.3 ± 0.3
Ascorbic acid (vitamin C)	14.0 ± 0.3
$[\text{Co}(\text{TSC})_3]\text{Cl}_3$	7.3 ± 0.3

As can be seen, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ requires approximately five times lower concentration than Trolox and twice lower concentration than ascorbic acid to achieve 50% inhibition of ABTS, which indicates a high antioxidant efficiency of the complex.

Effect of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on the TAS of bee and larval hemolymph

To evaluate the influence of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on total antioxidant status (TAS), biological samples of *Apis mellifera* (hemolymph of adult bees and larvae) were used. The samples were collected in sterile containers and stored under cooling conditions prior to analysis. Homogenization was performed in 96% ethanol, followed by incubation at 25 °C for 2 days; the samples were then analyzed across a concentration range of 0.01–100.0 mg/mL.

In the control group (I), where bees received plain sugar syrup without the complex, the TAS values of adult-bee and larval hemolymph reflect the level of natural antioxidant protection (Table 2).

Table 2. Total antioxidant status (TAS) of hemolymph in the control group, expressed as IC_{50} in the ABTS and DPPH radical-scavenging assays

Hemolymph	ABTS ; IC_{50} (mg/mL)	DPPH; IC_{50} (mg/mL)
Adult bees	13.56 ± 0.37	13.85 ± 0.19
Larvae	9.95 ± 0.70	13.44 ± 2.95

These results indicate a significant baseline antioxidant activity in bees, especially in the ABTS assay, but this level may be insufficient to prevent cellular damage under stress and parasitic pressure.

In experimental group II, bees were fed syrup enriched with $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ at a concentration of 0.6 mg/L. In this group, the total antioxidant status (TAS) of hemolymph increased (Table 3).

Table 3. TAS in the group II (IC₅₀, mg/mL)

Hemolymph	ABTS IC ₅₀ (mg/mL)	DPPH IC ₅₀ (mg/mL)
Adult bees	2.5 ± 0.22	7.08 ± 0.34
Larvae	1.3 ± 0.12	6.70 ± 0.12

Compared with the control group, the level of antioxidant activity in adult bees increased by approximately 5.4 times, and in larvae by up to 7.8 times. This indicates a pronounced stimulating effect of [Co(TSC)₃]Cl₃ on the antioxidant systems of *A. mellifera* both at the adult stage and in the early stages of development.

Comparison of [Co(TSC)₃]Cl₃ with vitamin C and its biological significance

The stimulating effect of [Co(TSC)₃]Cl₃, compared to vitamin C, is manifested in higher efficiency: according to ABTS assay data, the stimulation of TAS in adult bees reaches approximately a 5.2 times increase, and in larvae about a 4.8 times increase. This effect may be due to the ability of the cobalt complex to act as a catalyst in redox reactions, as well as to the participation of sulfur and nitrogen containing fragments of the ligand in binding and neutralizing reactive oxygen species.

Elevating TAS in hemolymph leads to reduced oxidative stress, decreased lipid peroxidation, and preservation of the structure of proteins and nucleic acids. Thus, [Co(TSC)₃]Cl₃ exhibits a dual physiological role: as a strong antioxidant that increases the TAS of bee and larval hemolymph; as a non toxic compound with pronounced acaricidal activity.

These properties make the complex a promising candidate for the further development of agents aimed at enhancing the viability and resilience of bee colonies against environmental and parasitic stressors.

Acaricidal activity

One of the key challenges of modern beekeeping is the protection of *Apis mellifera* colonies against the parasitic mite *Varroa destructor*. Infestation with *V. destructor* affects not only the mortality of brood and adult bees, but also the functioning of their antioxidant system: under heavy infestation, the activity of key antioxidant enzymes decreases, aging processes accelerate, and the average lifespan of individuals is shortened [27–28]. Under these conditions, the search for new agents that combine high efficacy with low toxicity for bees and the environment becomes particularly relevant.

Varroa destructor mite morphology and epizootiology

The parasitic mite *Varroa destructor* is widely distributed in apiaries of the Republic of Moldova. Adult females measure approximately 1.1–1.8 mm in length and 1.5–2.0 mm in width. The body is strongly sclerotized, dorso-ventrally flattened, and transversely oval in shape, with a slightly convex dorsal region (idiosoma). The dorsal scutellum extends along the entire body and is covered with dense pubescence composed of thin, spirally curved setae, which facilitate the mite's movement within sealed brood cells between the larva and the cocoon. Adult individuals possess four pairs of robust, six-segmented legs, terminating in strong sucking cups.

The major epizootiological role is played by adult females (Figure 1 d): they can remain outside cells for extended periods, attaching to the bodies of bees and overwintering on adult hosts. Immature stages (protonymph, deutonymph, tritonymph) and males (Figure 1 a–f) develop primarily within sealed brood cells, where reproduction and ontogenesis of the mite take place.

In spring, the population of *V. destructor* grows exponentially, and the greatest damage is inflicted on brood during the period of active preparation of bees for honey collection. In 2025, in *Apis mellifera* colonies of the Republic of Moldova, the level of brood infestation reached

26.4% in control groups (Table 4). In individual cells up to three mites of different developmental stages were found, while their numbers on adult bees were noticeably lower.

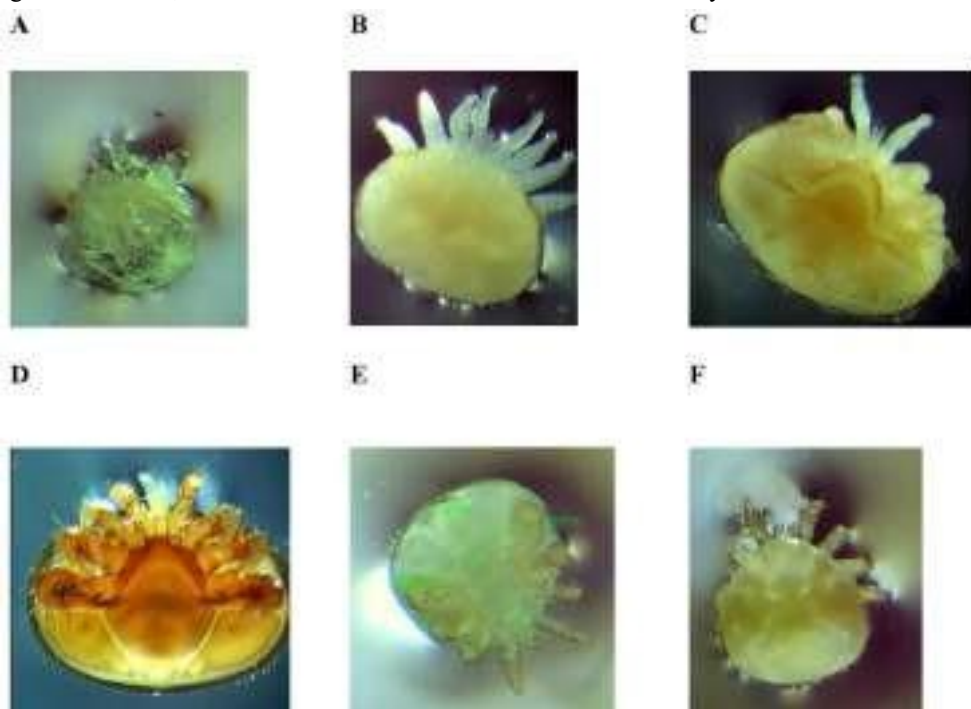


Figure 1. Microscopic images of *Varroa destructor*: protonymph (a), deutonymph (b), tritonymph (c), adult female (d), male ventral view (e) and dorsal view (f).

Morphometric measurements of mites collected from bee larvae showed that the average size of pale males was length $L = 0.91 \pm 0.15$ mm and width $D = 1.11 \pm 0.38$ mm, whereas adult females had larger dimensions of $L = 1.15 \pm 0.05$ mm and $D = 1.74 \pm 0.05$ mm. Incompletely sclerotized tritonymphs measured $L = 1.11 \pm 0.06$ mm and $D = 1.55 \pm 0.12$ mm, which corresponds to the diagnostic characters described in the keys of De-Guzman, Fernandez, and Coineau (2007).

Table 4. *Varroa destructor* infestation in the control and experimental groups.

Nº	Mites per 10 g bees (control)	0.2 mg/L [Co(TSC) ₃]Cl ₃	0.6 mg/L [Co(TSC) ₃]Cl ₃
1	32	4	0
2	25	3	0
3	37	4	1
4	26	2	0
5	28	1	1
6	24	5	1
7	30	1	0
8	20	2	0
9	24	2	1
10	18	3	0
M	26.4	2.7	0.4

Effect of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on *Varroa destructor* infestation

In biological studies, bees were fed sugar syrup enriched with $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ at concentrations of 0.2 and 0.6 mg/L. The control group received plain sugar syrup with no complex added. The level of infestation was assessed by the number of mites per 10 g of bees (equivalent to 100 individuals) and by the percentage of infested brood cells.

In the control group, the mean infestation level was 26.4 mites per 10 g of bees. After supplementation with $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ at 0.2 mg/L, this value decreased to 2.7, and at 0.6 mg/L it further dropped to 0.4.

To evaluate the acaricidal efficacy, the percentage reduction in infestation relative to the control group was calculated, and the median lethal concentration (LC_{50}) was determined.

At 0.2 mg/L, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ reduced the level of infestation by 89.8%, and at 0.6 mg/L by 98.5% (Table 5). The obtained LC_{50} of 0.2 $\mu\text{mol/L}$ confirms the high acaricidal activity of the complex, even at very low doses.

Table 5. Reduction of *Varroa destructor* infestation

$[\text{Co}(\text{TSC})_3]\text{Cl}_3$ concentration	Reduction in infestation (%)
0.2 mg/L	89.8
0.6 mg/L	98.5

Mechanism of the acaricidal action of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on *Varroa destructor*

The possible mechanism of the acaricidal effect of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ on *Varroa destructor* may be associated with the induction of oxidative stress and disruption of mitochondrial redox homeostasis in the mite, rather than direct interaction with classical neurotoxic targets. In other studies, redox-active cobalt(III) complexes have been shown to increase reactive oxygen species (ROS) generation, disturb mitochondrial membrane potential, and alter the activity of key antioxidant enzymes, which ultimately leads to cytotoxicity in small ectoparasitic organisms that have limited antioxidant reserves. Given the very low LC_{50} of 0.2 $\mu\text{mol/L}$ observed for $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ against *V. destructor*, it is plausible that the complex, after entering the mite's hemolymph or through its cuticle, disturbs mitochondrial electron transport and increases oxidative load, thereby reducing the parasite's viability and reproductive capacity [30].

At the same time, the sulfur- and nitrogen-containing fragments of the TSC ligand may contribute to the binding and neutralization of ROS, as well as interfere with the activity of detoxification enzymes such as cytochrome P450-like systems and esterases, which are known to be involved in acaricide resistance in *V. destructor*. By combining a pronounced pro-oxidative effect on the mite with relatively low toxicity to *Daphnia magna* (LC_{50} 56.3 $\mu\text{mol/L}$) and to bees, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ appears to act as a selective stressor that preferentially targets the parasite. However, these mechanistic interpretations remain hypothetical, and confirmation of the involvement of mitochondrial pathways and specific enzyme systems in the mite will require targeted biochemical and molecular studies in *V. destructor* tissues [31].

Dual acaricidal and antioxidant role of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ in the control of *Varroa destructor* mite

The results indicate that $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ is a promising acaricidal agent for the control of *Varroa destructor* under apiary conditions. Its administration as an additive to sugar syrup leads to a marked reduction in mite populations without exerting a significant negative impact on bee viability at the studied dosages. In combination with the previously described antioxidant activity, the complex can simultaneously enhance the total antioxidant status (TAS) of the

hemolymph of adult bees and larvae, thereby increasing their resistance to infections and stressors.

Thus, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ demonstrates a dual benefit: a substantial reduction in the abundance of *Varroa destructor*; strengthening of the overall antioxidant defense in bees.

This makes the complex a candidate for the development of multifunctional agents aimed at sustainably improving the health and productivity of bee colonies, reducing economic losses in apiculture, and addressing the problem of chemical contamination of bee products.

The possible mechanism of action of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$, in view of its high antioxidant and acaricidal activity, may involve several factors. First, the sulfur and nitrogen atoms in the thiosemicarbazide ligands are capable of taking part in electron transfer and in direct scavenging of reactive oxygen species, including the ABTS and DPPH radicals. In an octahedral coordination, the Co(III) ion provides high kinetic stability to the complex and an optimal geometry for interaction with biomolecules, which explains the low IC_{50} values in the model antioxidant assays. At the same time, such a structure may disrupt metabolic processes in the *Varroa destructor* mite, leading to an LC_{50} of only $0.2 \mu\text{mol/L}$, even though toxicity is low for *Daphnia magna* and for bees, indicating a selective action of the compound.

The literature describes various agents against *Varroa destructor*, including synthetic acaricides (e.g., amitraz, flumethrin, acarodectin) and organic compounds, mostly based on terpenes and fatty acids, many of which are active in the range of $1\text{--}10 \mu\text{mol/L}$ or higher [29–32]. In contrast, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ exhibits pronounced acaricidal activity at $0.2 \mu\text{mol/L}$ while maintaining low toxicity and simultaneously increasing the total antioxidant status in bee hemolymph. This combination of properties opens the perspective of developing new multifunctional agents that suppress the mite population and strengthen the host's protective systems.

The combined effect of elevated TAS and reduced *Varroa destructor* infestation allows $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ to be viewed as a dual-action regulator: on one hand, it reduces the main stress factor by suppressing the parasite, while on the other hand, it diminishes oxidative stress, protecting bee cells and tissues from damage. Such an approach meets the modern requirements of sustainable beekeeping, as it enables a reduction in the use of conventional chemical acaricides and enhances the natural resilience of bee colonies to infections and ecological stressors.

In the present study, long-term consequences of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ application were not evaluated, including possible effects on queen fecundity, brood development, and honey quality, as well as residual concentrations of the complex in bee products (honey, wax, and pollen). An important direction for future work will be the investigation of the pharmacokinetics of the compound in bee hemolymph, its distribution in different organs, and the assessment of its potential impact on resistant strains of *Varroa destructor*. In the future, it will also be advisable to study the combined use of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ with biopesticides and existing acaricides in order to increase the efficiency of varroa control and reduce the risk of resistance development.

Conclusions

In this work, a new cobalt(III) coordination compound, tris-[N-(prop-2-en-1-yl)hydrazinecarbothioamide]-cobalt(III) chloride ($[\text{Co}(\text{TSC})_3]\text{Cl}_3$), was synthesized and studied as a potential regulator of the total antioxidant status (TAS) of *Apis mellifera* hemolymph. Physicochemical investigations showed that the compound forms a stable octahedral 1:3-type electrolyte complex, highly soluble in polar solvents, and exhibiting high electrical conductivity. The antioxidant activity of the complex in model systems (ABTS and DPPH) exceeds that of standard reference compounds such as Trolox and ascorbic acid; the IC_{50} in the ABTS assay is $7.3 \mu\text{mol/L}$, which indicates high efficiency of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ as an antioxidant.

In biological trials with bees, supplementation of sugar syrup with $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ at concentrations of 0.2 and 0.6 mg/L significantly increased the TAS of hemolymph in adult bees and larvae. The corresponding IC_{50} values in these groups decreased to 2.5 mg/mL and 1.3 mg/mL, respectively, which represents more than a 5-fold improvement over the protective effect of ascorbic acid. At the same time, the complex exhibited pronounced acaricidal activity against *Varroa destructor*, reducing bee infestation by 89.8–98.5 % at 0.2–0.6 mg/L and showing an LC_{50} of only 0.2 $\mu\text{mol/L}$, while toxicity remained relatively low in the *Daphnia magna* bioassay.

Thus, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ demonstrates a dual biological effect: on one hand, it enhances the antioxidant protection of bees, reducing oxidative stress and improving their resistance to diseases; on the other hand, it effectively suppresses the population of *Varroa destructor*, one of the key factors weakening and causing the collapse of bee colonies. The obtained results indicate that this cobalt complex is a promising nontoxic and highly effective agent for the comprehensive support of bee health, an increase in colony productivity, and a reduction of economic losses in apiculture. In the future, it will be advisable to continue studies on the pharmacokinetics of the compound, residual concentrations in bee products, and the possible use of $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ in combined protective schemes against *Varroa destructor*.

Thus, $[\text{Co}(\text{TSC})_3]\text{Cl}_3$ demonstrates a dual biological effect: on one hand, it enhances the antioxidant protection of bees, reducing oxidative stress and improving their resistance to diseases; on the other hand, it effectively suppresses the population of *Varroa destructor*, one of the key factors weakening and causing the collapse of bee colonies. The obtained results indicate that this cobalt complex is a promising nontoxic and highly effective agent for the comprehensive support of bee health, an increase in colony productivity, and a reduction of economic losses in apiculture.

Acknowledgment

This research was funded by the subprograms 010602 and 010701 (Moldova State University).

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Authors:



Olga Garbuz, Dr., Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, Moldova State University, 1 Academiei Street, MD-2028 Chisinau, Moldova
e-mail: olga.garbuz@sti.usm.md
ORCID: 0000-0001-8783-892X



Vasilii GRAUR, PhD, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: vasilii.graur@usm.md
ORCID: 0000-0001-8153-2153



Nadejda RAILEAN, Dr., Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, Moldova State University, 1 Academiei Street, MD-2028 Chisinau, Moldova
e-mail: nadejdarailean@yahoo.com
ORCID: 0000-0001-7818-9200



Victor TSAPKOV, Dr., Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: vtsapkov@gmail.com
ORCID: 0000-0003-1732-3116



Veronica SARDARI, Dr., Department of Biochemistry and Clinical Biochemistry, State University of Medicine and Pharmacy “Nicolae Testemitanu”, MD-2004 Chisinau, Moldova
e-mail: veronica.sardari@usmf.md
ORCID: 0000-0002-1047-9145



Donald POIRIER, Professor, Laboratory of Medicinal Chemistry, CHU de Québec Research Center, Université Laval, Québec, QC G1V4G2, Canada
e-mail: donald.poirier@crchudequebec.ulaval.ca
ORCID: 0000-0002-7751-3184



Jenny ROY, Dr., Laboratory of Medicinal Chemistry, CHU de Québec Research Center, Université Laval, Québec, QC G1V4G2, Canada

e-mail: jenny.roy@crchudequebec.ulaval.ca

ORCID: 0000-0002-9928-3009



Ion TODERAS, Academician, Doctor Habilitate, University Professor, Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, Moldova State University, 1 Academiei Street, MD-2028 Chisinau, Moldova

e-mail: iontoderas@yahoo.com

ORCID: 0000-0003-1599-838X



Aurelian GULEA, Academician, Doctor Habilitate, University Professor, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova

e-mail: guleaaurelian@gmail.com

ORCID: 0000-0003-2010-7959