Cobalt(II) Coordination Compounds of Ethyl 4-Methyl-5-Imidazolecarboxylate: Chemical and Biochemical Characterization on Photosynthesis and Seed Germination.

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ABSTRACT

In this work we present the synthesis, structural and spectroscopic characterization of Co²⁺ coordination compounds with ethyl 4-methyl-5-imidazolecarboxylate (emizco). The effects of emizco, the metal salts CoCl₂·6H₂O, CoBr₂, Co(NO₃)₂·6H₂O and their metal coordination compounds [Co(emizco)₂Cl₂], [Co(emizco)₂Br₂]·H₂O, [Co(emizco)₂(H₂O)₂](NO₃)₂·2H₂O were evaluated on photosynthesis in spinach chloroplasts. Seed germination and seedling growth of the monocotyledonous species Lolium multiflorum and Triticum aestivum and the dicotyledonous species Trifolium alexandrinum and Physalis ixocarpa were also assayed under the effect of the compounds and salts. The results showed that cobalt(II) salts and their emizco coordination compounds inhibit photosynthetic electron flow and ATP-synthesis, behaving as Hill reaction inhibitors. Coordination compounds are more potent inhibitors than the salts. It was found that the salts target is at the b₆f level while the complexes targets are at Qₐb(D1)-protein and b₆f level. The Qₐb inhibition site was confirmed by variable chlorophyll a fluorescence yield. On the other hand, emizco inhibits seed germination, root and shoot development, in both weed and crop species. Cobalt(II) coordination compounds are the most effective photosynthesis inhibitors, but they are less potent than emizco in germination and seedling growth, while the metal salts are the least active of all.

Key words: Ethyl 4-methyl-5-imidazolecarboxylate, Co²⁺ emizco coordination compounds, cobalt(II) salts, photosynthesis, seed germination.

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INTRODUCTION

It is known that imidazole derivatives are extensively used in pharmaceutical /1/ and agrochemical industries /2,3/. Ethyl 4-methyl-5-imidazolecarboxylate (emizco) derivatives present antiviral /4/ and herbicidal /5/ activities. Most commercial herbicides are organic synthetic compounds with a wide variety of structures and with different targets, selectivity, mode of action, and weed spectrum. In order to exert toxicity, all pre-emergence herbicides (or post-emergence) must be absorbed into the root (or foliage) and move to the site of action, where they must be present at an active concentration and a proper toxic form for a long-enough period /6/. Any factor that interferes with this sequence may account for differential selectivity or sensitivity of herbicides between species among herbicides. Properties, or spatial distribution of herbicides functional groups are also important factors that may affect their selectivity.

It is known that the phytotoxicity of the organic compound is either enhanced or decreased upon coordination with metal ions /7/. The primary interest in our screening program is directed to compounds that affect seed germination, seedling root and shoot development or energy metabolism. We have previously studied the effects of transition metal coordination compounds on different photosynthetic activities. Ni$^{2+}$ coordination compounds of emizco and nickel salts inhibited thylakoid electron transport chain at two different targets, being nickel salts less potent as Hill reaction inhibitors. Emizco was inactive on photosynthetic activities /8/. Continuing with this work, we extended our research to the effect of cobalt salts and their emizco coordination compounds on photosynthesis and germination.

Some transition metal ions participate in a great variety of biological roles, among them, as essential components of several enzymes /9/. These elements are plant micronutrients affecting their growth and metabolism /9,10/. For example, cobalt sulphate decreased seed germination of Pinus sylvestris /11/ and Nicotiana tabacum /12/. This metal ion stimulates yeast mitochondria respiration when α-keto glutarate is employed, but it was irreversibly inhibited in the presence of succinate. Pre-incubation of mitochondria with cobalt sulphate inhibited respiration and oxidative phosphorylation /13/. The effect of Co$^{2+}$ on photosynthesis is controversial. Tripathy et al /14/ reported that cobalt inhibits the oxidizing side of the PSII reaction centre. Furthermore, it interacts with Q$_B$ site /10,15/. Here we report the synthesis and characterization of emizco cobalt(II) coordination compounds. We also investigated the effect on photosynthesis, seed germination, seed respiration and root and shoot development of emizco, its Co$^{2+}$ complexes, and cobalt(II) salts.

EXPERIMENTAL

Materials

All chemicals were reagent grade: solvents (Merck); ethyl 4-methyl-5-imidazolecarboxylate (emizco) (Aldrich); CoCl$_2$·6H$_2$O, CoBr$_2$, Co(NO$_3$)$_2$·6H$_2$O (J. T. Baker) were used without further purification. Sorbitol, sucrose, tricine, KCN, KCl, MgCl$_2$, KOH, NH$_4$Cl, [Fe(CN)$_6$], Methyl viologen (MV), N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (DCMU), 2,6-dichlorophenol indophenol (DCPIP), 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone
(DBMIB), sodium silicomolybdate (SiMo) and reduced tetramethyl-p-benzoquinone (TMQH₂) were purchased from Sigma-Aldrich.

**Synthesis of emizco coordination compounds**

[Co(emizco)₂Cl₂], [Co(emizco)₂Br₂]·H₂O and [Co(emizco)₂(H₂O)₂](NO₃)₂·2H₂O, were prepared by adding the appropriate salt (0.5 mmol) dissolved in 15 cm³ of methanol to emizco (1 mmol), (1:2 molar ratio) dissolved in 15 cm³ of hot methanol, and refluxed for five hours. The solution was allowed to stand at room temperature for two to six weeks. The coordination compounds were characterized by different spectroscopic and chemical techniques (IR, electronic absorption spectroscopy, elemental analyses, thermogravimetric studies and magnetic susceptibility).

**[Co(emizco)₂Cl₂]**

A solution of emizco (0.1542 g, 1.0 mmol) in hot methanol (15 cm³) was added to a solution of CoCl₂·6H₂O (0.24 g, 0.5 mmol) in hot methanol (15 cm³). The mixture was heated under reflux for 5 h. A navy blue microcrystalline precipitate was obtained by slow evaporation of the solvent and it was vacuum filtered. Anal. Calc. for C₁₄H₂₀Cl₂N₄Co: C, 38.38%; H, 4.60%; N, 12.79%. Found: C, 37.66%; H, 4.57%; N, 12.86%. IR (KBr, cm⁻¹): 1730, 1690 ν(C=O), 1600 ν(C=N).

**[Co(emizco)₂Br₂]·H₂O**

A solution of emizco (0.1542 g, 1.0 mmol) in hot methanol (15 cm³) was added to a solution of CoBr₂ (0.238 g, 0.5 mmol) in hot methanol (15 cm³). The mixture was heated under reflux for 5 h. A navy blue microcrystalline precipitate was obtained by slow evaporation of the solvent and it was vacuum filtered. Anal. Calc. for C₁₄H₂₂O₂Br₂N₄Co: C, 30.80%; H, 4.07%; N, 10.28%. Found: C, 29.20%; H, 3.17%; N, 10.80%. IR (KBr, cm⁻¹): 1736, 1690 ν(C=O), 1599 ν(C=N).

**[Co(emizco)₂(H₂O)₂](NO₃)₂·2H₂O**

A solution of emizco (0.1542 g, 1.0 mmol) in hot methanol (15 cm³) was added to a solution of Co(NO₃)₂·6H₂O (0.291 g, 0.5 mmol) in hot methanol (15 cm³). The mixture was heated under reflux for 5 h. A pink microcrystalline precipitate was obtained by slow evaporation of the solvent and it was vacuum filtered. Anal. Calc. for C₁₄H₂₄O₄N₄Co: C, 29.85%; H, 5.00%; N, 14.92%. Found: C, 28.75%; H, 4.22%; N, 15.81%. IR (KBr, cm⁻¹): 1676 ν(C=O), 1600(sh) ν(C=N), 1384 ν(NO₃).
buffers (such as HEPES and Tricine) varying the concentration of the complexes were performed in order to know if the coordination compounds under study interacted with the buffers. The buffer concentration was varied from 20 to 40 mM, for two different pH values: 7.0 and 8.0.

Chloroplasts isolation and chlorophyll determination

Intact chloroplasts were isolated from market spinach leaves (*Spinacea oleracea* L.) as previously described /16,17/, and suspended in 400 mM sucrose, 10 mM KCl, 5 mM MgCl₂ and 30 mM tricine buffer (pH 8.0 with the addition of KOH). They were stored as a concentrated suspension in the dark for 1 hour at 0°C. Intact chloroplasts were lysed to yield free thylakoids previous to each experiment by incubating them in 100 mM sorbitol, 0.5 mM KCN, 10 mM KCl, 5 mM MgCl₂ and 20 mM HEPES-KOH buffer (pH 8.0). Chlorophyll was determined according to the reported method /18/.

Physical Measurements

A FTIR spectrometer (Perkin Elmer 599 B) was used for obtaining spectra of solid samples in KBr pellets (4000 – 400 cm⁻¹). The UV-Vis spectra (diffuse reflectance and solution, 40000 – 40000 cm⁻¹) were recorded on a Cary-SE (Varian) spectrometer. Elemental analyses were carried out with a Fisons EA 1108 analyser. Magnetic susceptibility measurements at room temperature, of powdered samples, were recorded on a Johnson-Matthey DG8 5HJ balance using the Gouy’s method.

Measurement of electron transport and ATP-synthesis

ATP-synthesis was determined titrimetrically using an Orion Mod. 8103 Ross microelectrode connected to a Model 12 Corning potentiometer, with expanded scale as reported by Dilley /19/. The ATP-synthesis medium contained 100 mM sorbitol, 0.5 mM KCN, 10 mM KCl, 5 mM MgCl₂, 50 μM MV, and 1 mM HEPES-KOH buffer (pH 8.0).

Photosynthetic non-cyclic electron transport was monitored with an YSI (Yellow Springs Instrument) Model 5300 oxygen monitor using a Clark electrode in a temperature-regulated flask at 20 °C. The reaction medium was similar to that for ATP-synthesis but HEPES concentration was changed to 15 mM at the same pH. 20 μg/mL chloroplasts were added (whole electron chain transport). The sample was illuminated for 1 minute in the presence or absence of 6 mM NH₄Cl /16,20/.

PSII was measured by photo-reduction of DCPIP monitored polarographically by O₂ evolution. The reaction medium for assaying PSII activity contained the same whole electron chain transport medium (H₂O→MV) above mentioned, without methylviologen but in the presence of 1 μM DBMIB, 100 μM DCPIP, 300 μM [Fe(CN)₆]₃⁻ and 6 mM NH₄Cl.

PSI electron transport was determined in a similar form to non-cyclic electron transport. The following reagents were added: 100 μM DCPIP, 300 μM ascorbate, 10 μM DCMU and 6 mM NH₄Cl /21/.

Electron transport chain of the partial reactions of the PSII and PSI were measured using specific inhibitors: 10 μM DCMU, 1 μM DBMIB, 30 mM KCN, and the following electron donors and acceptors:
Chlorophyll $a$ fluorescence assays

Chl $a$ fluorescence induction curves of freshly lysed chloroplasts were measured at room temperature using a PEA (Plant Efficiency Analyser) fluorometer (Hansatech UK), as described /8, 22/. Aliquots of dark-adapted thylakoids containing 15 µg/cm$^3$ of Chl $a$ were suspended in the electron transport medium and transferred with a dot-blot apparatus (Bio Rad USA) to filter paper. The thylakoids were immediately transferred to vials containing 3 cm$^3$ of solutions of the tested compounds, which contained different concentrations and were incubated for 5 min in the dark.

Seed germination bioassay

Uniform size seeds were selected. For the germination bioassays, 40 wheat seeds (Triticum aestivum) and 100 seeds of Physalis ixocarpa, Lolium multiflorum and Trifolium alexandrinum, were set into 10 cm Petri dishes containing a 10 cm filter paper and 10 cm$^3$ of a solution containing the test compound, or 10 cm$^3$ deionised water as control /23/. The seeds were imbibed in water, or an aqueous solution of the ligand, cobalt salts, or coordination compounds, at different concentrations. They were let to stand for 5 days (3 days for germination and 2 more days for growth) in the dark at 28°C. Emergence of radicle from the seed was taken as indication of germination.

Seed respiration bioassays

Experiments on respiration followed the same procedure as that for germination bioassay. However, the seed respiration was measured as O$_2$ uptake using a Clark type electrode attached to a Yellow Spring Instrument (YSI) model 5300 oxymeter. The current generated during O$_2$ reduction to water was converted to voltage, and the signal recorded on a Gilson chart recorder. The current was stoichiometrically related to the oxygen consumed at the cathode.

The seeds were imbibed in water on Petri dishes, and an aqueous solution of the ligand, cobalt salts, or coordination compounds, was added at concentrations ranging from 100 to 400 µM. The Petri dishes were placed in the respiration chamber of an oxymeter. O$_2$ uptake was monitored for 3 min and then the seeds were discarded. The oxygen consumption rate was calculated and reported in nano atom O$_2$ x h$^{-1}$ x seed$^{-1}$. Temperature was kept at 20°C.

RESULTS AND DISCUSSION

The IR spectrum of emizco shows absorption bands corresponding to the stretching modes $\nu$(C=O), $\nu_{as}$(C-O-C), $\nu$(C-O-C) of the ester functional group at 1698, 1320, and 1178 cm$^{-1}$ respectively, and for the $\nu$(C=N) at 1510 cm$^{-1}$. In all cobalt coordination compounds $\nu$(C=N) shifts to higher frequencies (1514-1600
cm\(^{-1}\)), due to the coordination of the imidazole nitrogen atom N3 to the metal ion. In the spectra of [Co(emizco)_2Cl_2] and [Co(emizco)_2Br_2_H_2O the ν(C=O) band is splitted and shifted to higher energy (1730 and 1690 cm\(^{-1}\)) indicating that the ligands are not coordinated to Co\(^{2+}\) by the ester oxygen atoms, only by the heterocyclic nitrogen, as has been previously observed for analogous cobal(II) imidazolic compounds, [24,25]. On the other hand, for [Co(emizco)_2(H_2O)_2(NO_3)_2_H_2O], the ester bands were shifted to ca.1672, 1328 and 1214 cm\(^{-1}\) respectively, indicating that the two ligands are coordinated to the metal ion in a bidentate mode, through the oxygen of the ester group and the imidazolic nitrogen. There were observed vibration bands at 1384 and 1094 cm\(^{-1}\) indicating the presence of ionic NO_3\(^{-}\).

The blue Co\(^{2+}\) complexes [Co(emizco)_2Cl_2] and [Co(emizco)_2Br_2_H_2O] show the expected magnetic moments (4.52 and 4.51 BM). Their electronic spectra (diffuse reflectance) are typical of tetrahedral complexes, with transitions \(\nu_2 \leftrightarrow 4A_1(F) \leftrightarrow 4A_2(F)\) at 1302 and 600 nm for the chloro compound, and for the bromo complex at 1295 and 610 nm. This difference between the spectra of both compounds indicates that the halides are coordinated (Scheme 1). The electronic spectrum of [Co(emizco)_2(H_2O)_2(NO_3)_2_H_2O] shows bands as expected for an octahedral complex, \(\nu_1 1075 \text{ nm}, \nu_2 600 (\text{sh})\) and \(\nu_3 500 \text{ nm}\), corresponding to \(4T_2g(F) \leftrightarrow 4T_1g(F), 4A_2g(F) \leftrightarrow 4T_1g(F), 4T_1g(P) \leftrightarrow 4T_1g(F)\) and its magnetic moment is as expected.

From its chemical and spectroscopic characterization, an octahedral geometry is proposed for [Co(emizco)_2(H_2O)_2(NO_3)_2-H_2O], similar to that of the X-ray structure of its Ni\(^{2+}\) analogue /8/. Where emizco behaves as a chelate ligand, bonded to the metal ion through the oxygen atom from the ester group and the imidazolic nitrogen, and two water molecules coordinated to the metal atom (Scheme 1).

![Scheme 1. [Co(emizco)_2X_2], where X = Cl', Br', and [Co(emizco)_2(H_2O)_2]^{2+}](image)

**Solution characterization of the coordination compounds**

The tetrahedral [Co(emizco)_2Cl_2] and [Co(emizco)_2Br_2-H_2O] compounds become hexa-coordinated in aqueous solution, with an octahedral arrangement, adding two coordinated water molecules. The hexa-coordinated complex [Co(emizco)_2(H_2O)_2](NO_3)_2-2H_2O conserves its octahedral geometry in aqueous solution, as indicated by its UV-visible spectrum in solution [26]. The emizco ligand remains coordinated in a bidentate mode through the imidazolic nitrogen and the oxygen atom. For the chloro and bromo compounds
it can be inferred, from the UV-visible absorption spectra, that water molecules substituted the halides, Fig. 1.

It was shown that the buffer did not coordinate to the metal ion in any of the compounds (Fig. 1). The UV-visible spectra of the compounds remained unchanged for solutions of increasing buffer concentration (Fig. 1). Emizco remains bonded to the metal ion in aqueous solution for at least three days, as indicated from kinetic studies. Therefore, the active species in all the experiments contains the emizco ligand coordinated to Co²⁺.

\[ \text{[Co(emizco)₂Cl₂]} \]

![UV-Visible absorption spectra of [Co(emizco)₂Cl₂]. Left: diffuse reflectance spectrum, right: 6 x 10⁻³ M buffered aqueous solution in 6 x 10⁻² M HEPES, pH 8.0.]

**ATP-synthesis and electron transport chain**

The effect of emizco, the cobalt(II) salts and their coordination compounds on ATP-synthesis and on the electron transport chain was determined on spinach thylakoids. The results indicated that emizco has no activity. Cobalt(II) salts and their coordination compounds behave as Hill reaction inhibitors, as they inhibited basal, phosphorylating and uncoupled electron flow; and ATP-synthesis in a concentration-dependent manner (Fig. 2). Coordination compounds are more potent inhibitors than the salts. Fig. 2 shows the effect of CoCl₂ (2A) and its coordination compound [Co(emizco)₂Cl₂] (2B) on ATP-synthesis and basal, phosphorylating and uncoupled electron transports from H₂O to MV. The I₅₀ values for the uncoupled electron transport were 50 and 400 µM respectively.

In order to localise the inhibition site of the salts and the coordination compounds on the electron transport chain, the effect on photosystems II and I was measured on partial photosynthesis reactions. Artificial electron donors and acceptors are used to study partial reactions of the electron transport chain, as shown in Fig. 3.

The cobalt(II) salts inhibited the electron flow measured from TMQH₂ to MV (Table 1) and they did not affect the electron flow of PSI, measured from H₂O to DCPIP, neither PSI, measured from DCP1PH₂. These results indicate that the target is at the b₆f level.
A. Effect of CoCl₂ on ATP-synthesis (○) and photosynthetic electron transport from water to MV on freshly lysed spinach chloroplasts: basal (■), phosphorylating (●) and uncoupled (▲) electron transport rate. Emizco ligand did not present any effect (▲). Figure 2B. Effect of [Co(emizco)Cl₂] on ATP-synthesis (○) and photosynthetic electron transport from water to MV on freshly lysed spinach chloroplasts: basal (■), phosphorylating (●) and uncoupled (▲) electron transport rate. Emizco ligand did not present any effect (▲). Control average rates are 311, 640, 1346 μequiv e'/mg chl per h., for basal, phosphorylating and uncoupled electron flows, respectively, and 1117 μM Pi/mg chl per h for ATP-synthesis. Each curve is the average of three replicates.

On the other hand, the coordination compounds inhibited PSII and electron flow from TMQH₂ to MV, but did not inhibit PSI or electron transport from water to SiMo (Table 1); indicating that their targets are at Qb-protein and at b₆f level. The Qb inhibition site was confirmed by variable chlorophyll a fluorescence...
yield. An increase in relative variable fluorescence yield as a function of the cobalt complexes concentration was indicative of a loss in $Q_A^-$ re-oxidation capacity (Fig. 4). The salts and the complexes have one common inhibition site located at $b_{6f}$, while the coordination compounds have a second target at $Q_B$.

![Graph](image)

**Fig. 4:** Relative variable fluorescence, $F(V)$, corresponding to the electron transfer from $Q_A$ to $Q_B$ as a function of concentration: (A) $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (■) and $[\text{Co(emizco)}_2\text{Cl}_2]$ (●); (B) $\text{CoBr}_2$ (■) and $[\text{Co(emizco)}_2\text{Br}_2] \cdot \text{H}_2\text{O}$ (●); (C) $\text{Co(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (■) and $[\text{Co(emizco)}_2(\text{H}_2\text{O})_2(\text{NO}_3)_2] \cdot 2\text{H}_2\text{O}$ (●).

**Chlorophyll a fluorescence measurements**

The reduction of the relative $Q_A^-$ re-oxidation capacity of thylakoids on addition of coordination compounds was significantly concentration dependent (Fig. 4); while cobalt(II) salts showed no effect. Inhibition of PSII electron transport activity, from $\text{H}_2\text{O}$ to DCPIP, was determined by polarography. A correlation was found between the accumulation of $Q_A^-$ and the inhibition on PSII (Table 1 and Fig. 4). These observations strongly suggest that the target site of the coordination compounds is located at the acceptor side of the PSII at the $Q_B$-protein.
Table 1

Effect of cobalt(II) salts and emizco coordination compounds at 500 μM on uncoupled electron transport rate, PSII, PSI and partial reactions.

| Compound                  | Uncoupled | PIS | PSI | TMQH₂ to MV | H₂O to SiMo |
|---------------------------|-----------|-----|-----|-------------|-------------|
|                           | a         | b   | a   | b           | a           | b           |
| Control                   | 1346      | 100 | 662 | 100         | 1750        | 100         |
| CoCl₂·6H₂O                | 538       | 40  | 649 | 98          | 1750        | 100         |
| [Co(emizco)₂Cl₂]          | 202       | 15  | 238 | 36          | 1750        | 100         |
| CoBr₂                     | 538       | 40  | 569 | 86          | 1750        | 100         |
| [Co(emizco)₂Br₂]·H₂O      | 175       | 13  | 238 | 36          | 1750        | 100         |
| Co(NO₃)₂·6H₂O             | 942       | 57  | 602 | 91          | 1750        | 100         |
| [Co(emizco)₂(H₂O)₂]       | 269       | 20  | 311 | 47          | 1750        | 100         |
| (NO₃)₂·2H₂O               |           |     |     |             |             |             |

a = μequiv c/h x mg Chl
b = Activities percentage. Control = 100 %

Seed germination, respiration and growth

The effects of emizco, its Co²⁺ coordination compounds and the cobalt(II) salts were investigated on seed germination, seed respiration and seedling growth. The following monocotyledonous (Lolium multiflorum and Triticum aestivum) and dicotyledonous (Trifolium alexandrinum and Physalis ixocarpa) species were employed.

Emizco inhibited germination and root and shoot growth (Fig. 5). The extent of inhibition increased with concentration up to 200 μM. The pattern of inhibition of germination and shoot development was similar for both monocotyledonous and dicotyledonous species. While P. ixocarpa was the most sensitive (Fig. 5) and Trifolium alexandrinum was the least (20% germination is still observed at 400 μM). On the other hand, root development for monocotyledonous species was more sensitive to emizco than dicotyledonous plants as shown in Fig. 5. The I₅₀ for germination was on the range 27 to 133 μM, and for growth 33 to 102 μM (Table 2). These I₅₀ values are in the low range value of many phytochemical compounds tested as reported by Einhellig /27/, therefore emizco is a potent inhibitor for germination and seedling growth. On the activities assayed, emizco showed a similar phytotoxic potency to sorgoleone (10 to 125 μM) /28/.

In order to inhibit seed respiration, higher concentrations of emizco were needed. It was observed that emizco at 400 μM inhibited seed respiration, ranging from 0.0 to 77% (Table 3). The largest effect was observed for T. aestivum seeds, where maximum inhibition of the activity was 33% on the first day of imbibitions, and then started to diminish. The other studied species were less affected by emizco (Table 3). Since seed respiration was only partially inhibited, our results suggest that mitochondria might not be a major target of these compounds.
Emizco

Fig. 5: Root length □, shoot elongation □, and seed germination □, under emizco effect at 50 μM, 200 μM and 400 μM. Control without compound = 100%.

Table 2

10 value in μM for the coordination compounds, emizco and cobalt salts on different seeds. Root growth (r), shoot elongation (s) and seed germination (g). i means increased growth, elongation, or germination.

ne means no effect.

| Compounds             | T. vulgare | L. multiflorum | P. ixocarpa | T. alexandrinum |
|-----------------------|------------|----------------|-------------|-----------------|
|                       | r  s  g    | r  s  g        | r  s  g     | r  s  g         |
| [Co(emizco)₂Cl₂]     | 178 269 200 | 130 >400 394  | 208 270 287  | 314 >400 384    |
| [Co(emizco)₂Br₂].H₂O | >400 ne ne  | 288 ne ne      | 178 334 87  | 246 37 278      |
| [Co(emizco)₂(H₂O)₂(NO₃)₂].2H₂O | 355 315 >400   | 135 ne ne    | 68 254 369   | 232 331 ne      |
| emizco                | 33 36 85    | 102 122 133   | 41 41 27    | 78 55 46        |
| CoCl₂.6H₂O           | 339 396 400 | 465 ne ne     | >400 ne ne  | >400 >400 ne    |
| CoBr₂                 | 432 >400 >400 | >400 i i     | 322 384 >400 | >400 i >400     |
| Co(NO₃)₂.6H₂O        | 517 593 ne  | ne i ne       | 400 ne ne   | 355 295 >400    |
Phytotoxicity of the coordination compounds: [Co(emizco)₂Cl₂], [Co(emizco)₂Br₂]-H₂O and [Co(emizco)₂(H₂O)₂(NO₃)₂]-2H₂O, was studied. Seed germination, growth (root growth and shoot development) (Table 2) and respiration (Table 3) were either slightly inhibited or non-affected by these compounds, even at high concentrations (400 μM). As an example, Fig. 6 shows the effect of [Co(emizco)₂Cl₂] on seed germination and seedling growth. The latter was less potent than emizco (Fig. 5). The results for the remaining coordination compounds, at 400 μM, are listed in Table 2. [Co(emizco)₂Cl₂] had the highest phytotoxic activity on germination. However, coordination compounds were less potent than the ligand (Table 2). Interestingly, the presence of the metal ion diminished the inhibitory potency of emizco (compare Fig. 5, 6 and Table 3). Therefore, its coordination to Co²⁺ may have diminished emizco phytotoxicity.

Phytotoxicity was concentration-dependent for the coordination compounds, the higher effects occurred at 400 μM (Table 2). Of the evaluated processes, respiration was the least affected by the coordination compounds (Table 3).

Root and shoot development, and seed germination were only inhibited by the coordination compounds at high concentration. Fig. 7 shows that emizco prevents germination and seedling growth, contrary to the effect observed with [Co(emizco)₂Cl₂] that allows germination even at 400 μM, Fig. 8.

Phytotoxicity of the cobalt(II) salts was tested as control in parallel experiments. In general, none of the salts had any effect on germination or seedling growth (Table 2); in some cases it slightly inhibited or stimulated these processes. In Table 3, it can be appreciated that respiration is inhibited by the salts only after five days of treatment; the effect being larger in Lolium multiflorum and Physalis ixocarpa. This may be due to the presence of the anions.

### Table 3

Effect of metal salts, coordination compounds and emizco at 400μM on seed respiration at 1, 3 and 5 days.

Range of rate respiration. Control values in nanoatom·h⁻¹·seed⁻¹ are 1000 to 1600.

| Compound                  | T. vulgare | L. multiflorum | P. ixocarpa | T. alexandrinum |
|---------------------------|------------|---------------|-------------|-----------------|
|                           | 1  3  5*   | 1  3  5*      | 1  3  5*    | 1  3  5*        |
| Percentage of Control     |            |               |             |                 |
| Control                   | 100 100 100| 100 100 100   | 100 100 100 | 100 100 100     |
| CoCl₂·6H₂O                | 70 65 39   | 100 100 30    | 17 10 25    | 150 83 75       |
| [Co(emizco)₂Cl₂]          | 64 75 50   | 100 100 100   | 107 50 64   | 80 100 50       |
| CoBr₂                     | 80 81 126  | 120 100 30    | 50 30 13    | 100 100 42      |
| [Co(emizco)₂Br₂]-H₂O      | 80 90 55   | 75 140 133    | 100 100 55  | 80 100 58       |
| Co(NO₃)₂·6H₂O             | 80 80 75   | 200 75 20     | 33 20 25    | 80 100 50       |
| [Co(emizco)₂(H₂O)₂(NO₃)₂]-2H₂O | 79 75 41 | 88 80 117     | 89 79 75    | 80 80 83        |
| emizco                    | 33 40 80   | 100 100 60    | 83 54 88    | 89 40 54        |

*1,3,5 days
Our results show that emizco is a potent phytotoxic compound that inhibits germination, and root and shoot development on monocotyledonous and dicotyledonous species. According to its $I_{50}$ value, emizco is approximately two to three times less active than a standard commercial herbicide, such as methazole (2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione) and butamifos ($o$-ethyl-$o$-(6-nitro-$m$-tolyl)-sec-butyl phosphoramoñothioate).
Fig. 7: *Physalis ixocarpa* seeds in Petri dishes in the presence of emizco at 50, 200 and 400 μM.

Fig. 8: *Physalis ixocarpa* seeds in Petri dishes in the presence of [Co(emizco)Cl₂] at 50, 200 and 400 μM.
CONCLUSIONS

As previously observed \(^8\) neither the anions (Cl\(^-\), Br\(^-\) and NO\(_3\)\(^-\)) nor emizco by themselves contribute to the inhibition of electron transport (Table 1). However, when emizco is bound to cobalt(II), the coordination compounds inhibit electron transport, behaving as more potent Hill reaction inhibitors than the salts, and reducing emizco toxicity. For the coordination compounds there are two inhibition sites on PSII; one of them is also a target for the metal salts. On the other hand, emizco is a potent germination and seedling growth inhibitor, while the metal salts and coordination compounds have no effect.

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