Article

Valorization of Hesperidin from Citrus Residues: Evaluation of Microwave-Assisted Synthesis of Hesperidin-Mg Complex and Their Insecticidal Activity

**Danielle F. da Silva,[a] João P. A. Bomfim,[b] Rafael C. Marchi,[a] Jéssica C. Amaral,[a]
Luciano S. Pinto,[a] Rose M. Carlos,[a] Antonio G. Ferreira,[a] Moacir R. Forim,[a]
João B. Fernandes,[a] Maria F. G. F. da Silva,[*,a] Regiane C. de Oliveira,[b] David Buss,[c]
William D. J. Kirk[c] and Toby J. A. Bruce[c]**

[a]*Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos-SP, Brazil*

[b]*Faculdade de Ciências Agronômicas de Botucatu, Universidade Estadual Paulista Júlio de Mesquita Filho,
Avenida Universitária 3780, Altos do Paraíso, 18610-034 Botucatu-SP, Brazil*

[c]*School of Life Sciences, Keele University, Staffordshire, ST5 5BG, UK*

The aim of the current study was the valorization of hesperidin, the dominant flavonoid in citrus processing waste, by microwave-assisted synthesis of hesperidin-Mg complex, improving its antioxidant activity and insecticidal potential. Here we show, for the first time, that microwave-assisted synthesis of [Mg(hesp)$_2$(phen)]OAc (**1**) (hesp: hesperidin, phen: phenanthroline) improve the reaction rate and yield. The nuclear magnetic resonance (NMR) experiments proved to be powerful tools for the identification of three isomers in metal complexes. Moreover, we explore the insecticidal potential of **1** and [Mg(phen)$_2$(Isov)]OAc (**2**) (Isov : isovanillic acid) complexes against three insects. Complex **1** killed 80% of adults whitefly at 0.14 µmol L^{-1}, and **2** 76% at 0.36 µmol L^{-1}. There was a total mortality of *Spodoptera frugiperda* with **2** at 0.39 µmol L^{-1}, and 83% with **1** at 0.14 µmol L^{-1}. Both have similar activity, and represent a novel group of insecticide for *Bemisia tabaci* and *S. frugiperda*; nevertheless, the benefits of both as *Myzus persicae* repellent require further evaluation.
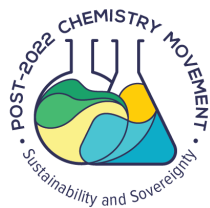
Keywords: *Citrus*, citrus waste, hesperidin, magnesium, microwave

Introduction

About 15 million tons of citrus waste accumulates annually from the food and drink processing industry and other minor contributors, mainly in Brazil. Citrus waste is often simply disposed or is incorporated into animal feed, meaning that this naturally occurring resource is not being fully exploited. It has long been recognized that orange peel represents a promising source of hesperidin, a flavonoid. Extraction methods has been developed to obtain large amounts of hesperidin and other flavonoids, however, the one using microwave was the most efficient for obtaining most of flavonoids studied for us, mainly for hesperidin.[1,2]

The antioxidant activity of flavonoids compounds is mainly due to their redox properties. Some works comment that the complexation of flavonoids with metals improves their antioxidant activities, as in a previous synthesis of

[Mg(hesp)$_2$(phen)]OAc complex, where hesp is hesperidin and phen is 1,10'-phenanthroline.[3,4] However, the mechanism by which these metals increase the antioxidant activities in flavonoids remain unknown. In addition, this complex has not yet been tested against insects, only against *Xylella fastidiosa*, a bacterium that develops in the xylem of plants that has long been known to cause citrus variegated chlorosis (CVC) in sweet orange. *In vitro* assays with *X. fastidiosa* after 15 days of growth showed that bacterial growth was affected by [Mg(hesp)$_2$(phen)]OAc complex.[5]

The beneficial effect of flavonoids and magnesium(II) against oxidative stress motivated us to synthesize [Mg(phen)$_2$(Isov)]OAc complex, also containing 1,10'-phenanthroline (phen) and isovanillic acid (Isov) as ligands.[6] [Mg(phen)$_2$(Isov)]OAc complex showed absence of toxicity to Zebrafish and Wistar male rats, emphasizing its potential as an environmentally friendly insecticide candidate, encouraging the authors to test it against the leaf-cutting ants *Atta sexdens rubropilosa*. The combination of delayed toxicity that suppresses populations of leaf-cutting

ants together with the ability to exterminate the ant colony after 30 days of treatment was clear evidence of the insecticidal potential of the [Mg(phen)$_2$(Isov)]OAc complex.[7]

As part of a continuing study of complexes with flavonoids from citrus waste, recently, we prepared naringin, naringenin, and hesperidin Cu^{II} complexes, and assayed against *Spodoptera frugiperda*. [Cu(naringin)(phenanthroline)], [Cu(naringenin)(2,2'-bipyridine)] and [Cu(hesperidin)(phenanthroline)] were prepared and they showed higher larval mortality than the limonoid gedunin used as a positive control in the bioassay of ingestion at a concentration of 100 mg kg^{-1}.[8]

Information that [Mg(hesp)$_2$(phen)]OAc has not yet been tested as an insecticide, and that a green and sustainable analytical method using microwave to obtain hesperidin from citrus residues in large amounts was effective, stimulated an investigation of a new reaction system involving the microwave-assisted synthesis for hesperidin-Mg complex. To determine the mechanism by which metals increase the antioxidant activities in flavonoids, we have now undertaken an investigation of the antioxidant properties in flavonoids.

Additionally, we tested the insecticidal potential of [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)]OAc complexes against *S. frugiperda*, *Bemisia tabaci* and *Myzus persicae*. The fall armyworm *S. frugiperda* J.E. (Smith) (Lepidoptera: Noctuidae) is an economically important pest in the production of grain and many other crops in North, Central, and South America. This insect is polyphagous, feeding on a broad range of host plants in different families and genera, in consequence it has developed the ability to detoxify plant xenobiotics, which makes it even more difficult to control, especially using natural insecticides.[9] Thus, the discovery of novel natural insecticidal compounds is essential before the development of resistance to those currently in use becomes widespread. The whitefly, *B. tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important global agricultural pest that can damage a wide range of hosts, including several green-house and field crops, ornamental plants, and weeds, worldwide. In addition, whiteflies are also considered to be a super vector, transmitting approximately 300 plant viruses, including viruses of the genera *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, and *Torradovirus*, highlighting the group of *Begomovirus*, which represents approximately 90% of the viruses transmitted by whitefly.[10] Thus, a wider range of natural insecticides for the control of all life stages of *B. tabaci* (eggs, larvae, and adults) is urgently required.

Aphids are members of the family Aphidoidea, about 5,000 species and they are small sap-sucking insects.

Approximately 400 species are found on food and fiber crops, and many are serious pests of agriculture and forestry. The plant family Brassicaceae consists of many important agricultural crops, and they are one of the secondary hosts of the aphid *Myzus persicae* (Sulzer). This aphid is a particularly important pest, not only because of the direct damage it causes but also because it is the vector for more than 100 plant viruses.[11]

Experimental

Material

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The organic solvents were all high-performance liquid chromatography grade. Aqueous solutions were prepared with Milli-Q water. All nuclear magnetic resonance (NMR) analyses were performed in 9.4 and 14.1 T Bruker equipment's (600 MHz), AVANCE III model (Karlshure, Germany), using a conventional 5 mm BB probe head and a cryogenic TCI probe head; the temperature was maintained at 298 K during all experiments. The 2D NMR determinations were ran using standard Bruker pulse sequences with the same parameters for acquisition time (0.29 s), relaxation delay (1 s), time domain in F2 (4K points), and number of experiments in F1 (256). However, for homonuclear correlation spectroscopy (COSY), heteronuclear single-quantum correlation spectroscopy (HSQC), and heteronuclear multiple bonding correlation (HMBC) (long-range coupling constants equal to 8 Hz), the number of scans was 16, 16, and 128, respectively, and the sweep widths in F2 were 7122, 7122, and 6798 Hz, respectively. ESI-QTOF-MS (electrospray ionization quadrupole time-of-flight mass spectrometry) measurements were performed in the positive mode on a Xevo G2 instrument (Waters Co, Milford, MA, USA). The full-scan MS data were produced across the mass range of 50-1200 Da. For the MS/MS experiments by collision-induced dissociation (CID), argon was used as collision gas and collision energy was increased until sufficient fragmentation of the precursor was observed. Samples were dissolved in methanol and introduced into the ESI source via direct infusion through a 250 μL glass syringe and were purged with > 1 mL of pure methanol between each sample. The ESI source conditions were as follows: capillary voltage 1.5 kV, sample cone voltage 30 V, cone gas flow (N$_2$) 50 L h^{-1}, desolvatation gas flow 750 L h^{-1}, source and desolvation temperatures were set to 100 and 300 °C, respectively. Data were analyzed with use of MassLynx 4.1 software. Microwave synthesis of complex [Mg(hesp)$_2$(phen)]OAc (**1**) was conducted in microwave

synthesizer system (Model Discover SP-909150, CEM Corp., North Carolina, USA). The bioassays with *S. frugiperda* and *B. tabaci* were carried out in the laboratory of the Research Group on Integrated Pest Management, in Agriculture of the Faculty of Agronomic Sciences of the São Paulo State University, Júlio de Mesquita Filho, Botucatu, SP, Brazil. The bioassays with *M. persicae* were carried out in the School of Life Sciences, Keele University (UK).

Methods

Synthesis of [Mg(hesp)₂(phen)]OAc

Solid Mg(CH₃COO)₂·4H₂O (0.055 g; 0.25 mmol) and subsequently 1,10'-phenanthroline (0.045 g; 0.25 mmol) were added to a solution of hesperidin (0.305 g; 0.5 mmol) and triethylamine (140 μL; 1 mmol) in methanol (40 mL). The solution was stirred under a N_2 atmosphere for 2 h under reflux. The mixture was filtered at room temperature and the remaining solution was evaporated to 5 mL under reduced pressure. Then, 10 mL of cold water was added, and the solution was stored at 4 °C for 3 days. The resulting yellow precipitate was filtered, washed with cold water, and dried under vacuum (65% yield).

Microwave-assisted synthesis of [Mg(hesp)₂(phen)]OAc

Solid Mg(CH₃COO)₂·4H₂O (0.055 g; 0.25 mmol) and subsequently 1,10'-phenanthroline (0.045 g; 0.25 mmol) were added to a solution of hesperidin (0.305 g; 0.5 mmol) and triethylamine (140 μL; 1 mmol) in methanol (50 mL), and were placed in a glass tube, purged with oxygen-free nitrogen during 10 min sealed and irradiated during 15 min in a microwave oven at 80 °C and 150 W. The yellow precipitate of **1** was filtered, washed with methanol, and dried under a vacuum (75% yield).

Conformation and energy minimization of hesperidin

Firstly, the 2D representation of the flavonoid hesperidin was drawn by using ChemDraw Ultra 10.0 software.[12] After that, the structure was converted into 3D representation by using Chem3D Pro 10.0 software.[12] The structure of hesperidin was optimized by minimizing energy using Molecular Mechanics (MM2) method. The MM2 method was applied because it considers the interaction among the atoms including electrons and orbital.[12]

Insecticidal activity assay

The experiments consisted of five treatments, [Mg(hesp)₂(phen)]OAc complex 20 mg mL⁻¹, 22 mg mL⁻¹,

and [Mg(phen)₂(Isov)]OAc complex 20 mg mL⁻¹, 22 mg mL⁻¹, dissolved in H_2O and control. Bean leaves were dipped into each dilution for 10 s then allowed to air dry, then being fixed in floral foam inside glass tubes (2 × 8 cm). Ten adults of *B. tabaci* were then placed on the leaves and covered with black tissue. Control tests were also carried out using only H_2O. The evaluations were made at 24, 48 and 72 h after application.

For *S. frugiperda* assay, pieces of corn leaf (5 × 5 cm) were dipped into each dilution for 10 s then allowed to air dry, then placed in 100 mL plastic cups containing moist filter paper, to maintain the turgidity of the leaves. For each treatment and control one second instar larvae of *S. frugiperda* was placed, and then closed with plastic lid. The evaluations were made at 24, 48 and 72 h after application.

For bioassays using *M. persicae*, individual *Brassica rapa* (cultivar "F1 Hanakan" (Moles Seeds Ltd., UK)) plants were grown to the three- to four leaf stage. Six to eight adult apterous *M. persicae* were confined in clip cages on the underside of the flattest of the older leaves, one cage *per* plant, and the plants then kept under controlled conditions (16:8 h light:dark, 20 (± 2) ºC). After 48-72 h, the adult aphids were removed, but the clip cages were left in place for a further 24 h to allow the nymphs to resettle. This provided small colonies of approximately 30-40 adults, which were used for testing.[11] Aphids were obtained from this laboratory colony. They were collected with a brush, and with the help of a stereoscopic, and in a number of 10 aphids were laid on a healthy leaf of cut cabbage (*B. oleracea*) in the shape and size of a 1-pound coin, dipped in solutions of [Mg(hesp)₂(phen)]OAc and [Mg(phen)₂(Isov)]OAc complexes at 10 mg mL⁻¹, dissolved in H_2O. These were maintained in sealed Petri dishes containing moist filter paper, to maintain the turgidity of the leaves. The results were compared with insects fed leaves that had been immersed in pure water (negative control). The evaluations were made at 24, 48 and 72 h after application.

Data were subjected to analysis of variance and means compared by the Tukey's test at 5% significance.

Results and Discussion

Synthesis and structural characterization of the complex **1**

Here we show, for the first time, that microwave-assisted synthesis of **1** improve the reaction rate and yield, despite the use of temperatures a little higher than the conventional reaction and pressure-assisted. This preparation was also advantageous, since it eliminated the need of the final

reaction mixture to be stored at 4 °C for 3 days to get the solid complex (Scheme 1).

The microwave synthesis protocol was based on references cited in the review by Abe *et al.*[13] This methodology made the synthesis of Mg-flavonoid complex profitable, and there is also strong evidence to suggest that this technology is suitable for large-scale use. The complex was characterized by nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS).

The HRMS spectrum was acquired in the positive mode, and the charged complex ion was observed at m/z 1422.4181 [M]$^+$, suggesting the chemical formula $C_{68}H_{74}N_2O_{30}Mg^+$ (1422.4177), and indicating hexacoordinated [Mg(hesp)$_2$(phen)]OAc (**1**). The literature registers X-ray crystallographic studies, which have shown that the metal center always presents an optimum coordination number of six in magnesium complexes, being linked to the oxygen and nitrogen groups in an octahedral arrangement (hybridization sp^{3}d^2), as in compound **1**.[4] The covalent interaction via the oxygen at C-5 of the hesperidin anion and one empty

sp^{3}d^2 orbital of Mg^{II} prevents the ligand-exchange reaction from occurring.

The conformational analysis and energy minimization are important tool to aid the understanding of the steric demand, spatial arrangement in metal complexes, and their NMR data. Thus, the most stable conformation of hesperidin, and with energy minimization was obtained through the software ChemDraw Ultra 10.0 (Figure 1).[12]

The NMR spectra showed characteristic signals for only one phenanthroline in the complex (Figures S1-S9, Supplementary Information (SI) section). The ^{1}H and ^{13}C NMR signals were closely comparable to those for pure phenanthroline (Figures S3 and S4, and Table 1). The magnesium isotope ^{25}Mg is of moderate natural abundance (10.0%), and quadrupolar (spin, I = 5/2). In magnesium-containing organometallics, chemical shifts are particularly broadened due to the presence of the isotope ^{25}Mg, deshielding the hydrogens close to the ion. In fact, all phenanthroline signals were broadened due to the presence of Mg isotopes, but this may also be attributed to the ligand-exchange reaction. Of note, 1,10 phenanthroline contains
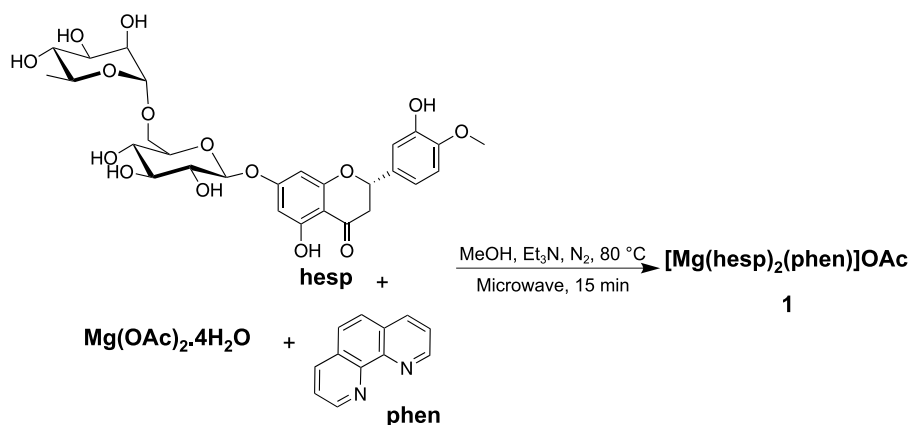
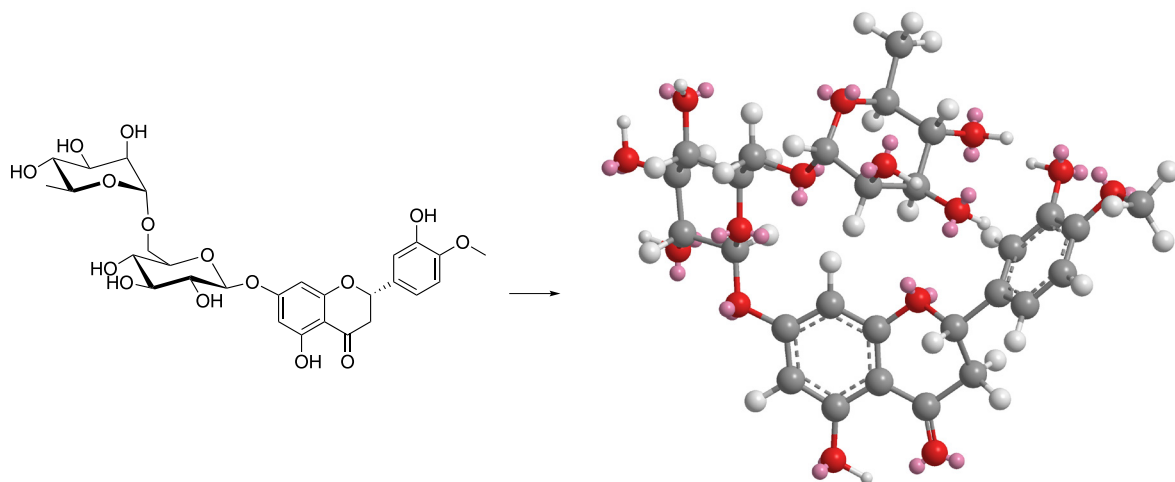**Scheme 1.** Synthetic route for the hexacoordinated [Mg(hesp)$_2$(phen)]OAc (**1**).

Figure 1. Hesperidin structure and the most favorable spatial orientation of rhamnoglucoside moiety.

Table 1. ^{1}H and ^{13}C NMR data for phenanthroline moiety in [Mg(hesp)$_2$(phen)]OAc **1**, and 1,10'-phenanthroline

H	Phenanthroline	H	Phenanthroline moiety in **1**	C	Phenanthroline	C	Phenanthroline moiety in **1**
2/9	9.10 dd (1.8; 4.3)	2/9	9.10 brs	2/9	149.9	2/9	150.0
4/7	8.49 dd (1.8; 8.1)	4/7	8.49 brs	4/7	136.2	4/7	136.2
5/6	7.98 s	5/6	7.99 brs	5/6	126.7	5/6	126.7
3/8	7.77 dd (4.3; 8.1)	3/8	7.78 brs	3/8	123.3	3/8	123.4
				11, 12	145.5	11, 12	145.5
				13,14	128.5	13,14	128.5

^{1}H and ^{13}C NMR spectra were acquired in DMSO-d_6 at 600 and 150 MHz, respectively. The chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses. Assignments are based on COSY, HSQC, and HMBC.

four pairs of equivalent hydrogens (2/9, 4/7, 5/6, 3/8). The complex [Mg(narin)(phen)$_2$]OAc, containing naringenin (narin) was previously synthesized and was used as a model to assign the chemical shifts for the phenanthroline hydrogens in complex **1**.[5]

In addition, the NMR spectra showed characteristic signals for two flavonoids in the complex (Figure S5-S9, SI section). Hesperidin, in its stable conformation, is an unsymmetrical ligand and may give rise to four diastereoisomers for the Mg[II] complexes as represented in Figure 2. Due to its plane of symmetry, in the structure corresponding to form b, the hesperidin ligands are the farthest from each other, whose spectroscopic properties accord with the NMR signals. In the ^{1}H NMR spectrum of **1** (Table 2) four methoxy hydrogens appeared at δ 3.74 (3H), 3.75 (6H) and 3.77 (3H), clearly indicating the presence of a mixture of two diastereoisomers.

Since **1b** isomer has an axis of symmetry, the methoxy hydrogens at δ 3.75 (6H) were consistent with their symmetry elements, a pair of equivalent methoxy groups, and supported the proposed isomer **1b** for the hexacoordinated [Mg(hesp)$_2$(phen)]OAc.

The ^{1}H NMR, in addition, revealed the presence of two methoxy hydrogens at δ 3.74 (3H), and 3.77 (3H), suggesting **1a/1d** isomers and led their assignments as 4'-OMe held near to the ligand phenanthroline, and 4'-OMe in a steric compression, near to ring-A of the second hesperidin, respectively. This steric compression produced an anisotropic magnetic deshielding effect on the 4'-OMe near to ring-A of the second hesperidin. In flavonoid, the fused benzene ring is designated as ring A and the 3,4-dihydro-2*H*-pyran or the pyran as ring C, along with a phenyl group (ring B) on ring C. The **1a/1d** isomers have one flavonoid with ring-B near to the ligand phenanthroline, defined as hpa, and the second one with ring-B near to ring A of the second hesperidin, defined as hpb, just to facilitate the assignment of chemical shifts in all isomers in Table 2. The structure represented by **1c** would be consistent with

the methoxy hydrogens equivalents, however both should be equally deshielded, in comparison with pure hesperidin and **1a/1d**, thus, the form **1c** was ruled out.

HSQC and HMBC experiments with complex **1** permitted the assignments of all signals for isomer **1a/1b/1d** (Table 2). From HMBC, the observed correlation between the ^{1}H signal at δ 4.97 (HSQC δ 99.4) and the ^{13}C signal at δ 165.1 led to their assignments as H-1'' and C-7. The oxymethine hydrogen at δ 4.79 showed one-bond correlation with the ^{13}C signal at δ 99.2, thus indicating the presence of one more glycosyl group, and permitting the assignment of the signal at δ 4.79 to H-1'' of the second flavonoid. Signals at δ 4.59 and 4.52 showed cross peaks with the ^{13}C signals at δ 100.6 and 100.8, respectively, in HSQC, and in HMBC both showed correlations with oxymethine carbon signals at δ 66.1-73.9, suggesting that these signals (δ 4.59, 4.52; δ 100.6, 100.8) can be attributed to H-1''' and C-1''' of the two glycosyl groups.

Moreover, one broadened ^{1}H signal appeared at the deshielded position δ 5.71 like phenanthroline hydrogens, which showed cross peaks with the ^{13}C signals at δ 100.4. These observations can be explained by the presence of isomer **1a** (**1a'**) with the glycosyl group oriented on the β-face of the complex, and H-1''' located near Mg, where the chemical shifts are probably broadened due to the moderate presence of the isotope [25]Mg (natural abundance 10.0%), and the ^{1}H signal is deshielded.[5] The glycosyl substituents in both isomers **1a** and **1d** are arranged on β-face and α-face of the complex, respectively, indicating that they are less stable then **1b** isomer due to a steric effect in *syn* isomers. Thus, one glycosyl group moves as showed in isomer **1a'** in Figure 2, in which the steric interactions are as weak as possible, whose spectroscopic properties accord with the above data.

In the same way, the signal for H-2 was visible as a multiplet at δ 5.47-5.51 and showed one-bond correlation with the ^{13}C signal at δ 78.8. However, beside this multiplet there is one broadened signal at δ 5.52, which showed

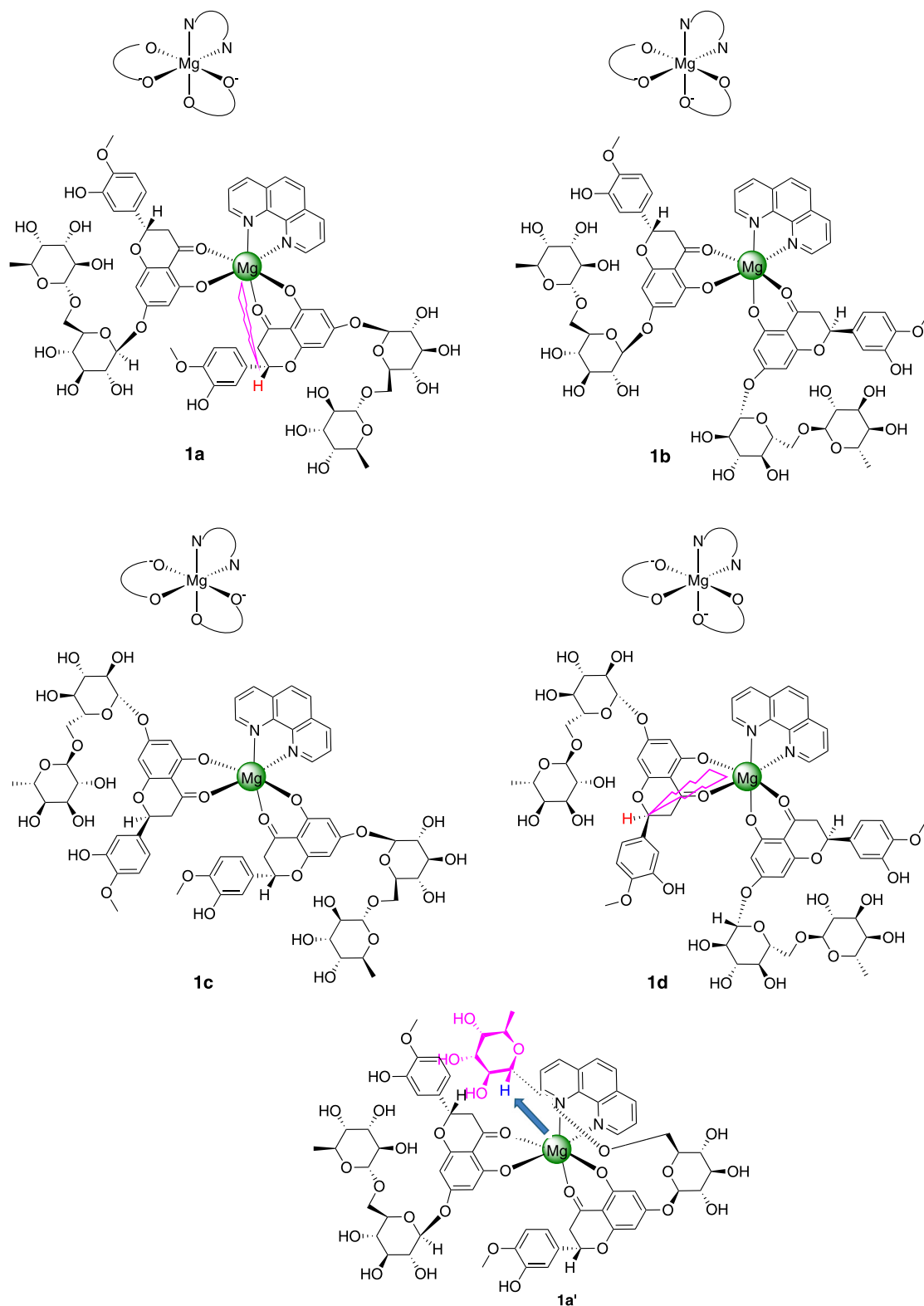**Figure 2**. Molecular structures of the four diastereoisomers of [Mg(hesp)₂(phen)]OAc (**1**).

one-bond correlation with the ^{13}C signals at δ 89.5, and long-range correlations with the ^{13}C signals at δ 103.3 (C-10) and 162.5 (C-9), permitting the assignment of the signal at δ 5.52 to H-2. The deshielded resonance

observed here for C-2 at δ 89.5 could not be explained by the presence of isomer **1b**, indicating that C-2 held directly above the magnesium isotope ^{25}Mg in isomer **1a** and below in isomer **1d**, causing the unexpectedly large deshielded

Table 2. ^{1}H and ^{13}C NMR data for hesperidin moiety in [Mg(hesp)$_2$(phen)]OAc **1** and hesperidin

H	Hesperidin	Hesperidin moiety in **1**	C	Hesperidin	Hesperidin moiety in **1**
			1'	130.9	130.9
2'/5'	6.92-6.95 m	6.90-6.94 m	2'	114.1	114.0
			3'	146.4	146.4
			4'	147.9	147.9
			5'	112.1	112.0
6'	6.90 brd (8.5)	**1a/1d**: 6.86 m (hpb); 6.90 (hpa); **1b**: 6.90 m	6'	117.9	**1a/1d**: 117.4 (hpb); 117.7 (hpa); **1b**: 117.7
2	5.49 dd (12.0; 3.0)	**1a/1d**: 5.52 brs (hpb), 5.20 brd (hpa); **1b**: 5.47-5.51 m	2	78.3; 78.4	**1a/1d**: (5.52) 89.5; (5.20) 76.4, **1b**: 78.8
3α	3.25 m	**1a/1d**: 2.91 m (hpb); 2.76 (hpa); **1b**: 2.76 m	3	42.1; 42.3	42.3; 42.1
3β	2.77 dd (12.0; 3.0)	**1a/1d**: 2.58 m (hpb); 2.50 (hpa); **1b**: 2.50 m			
			4	197.0	191.7
			5	163.0	163.1
6	6.14 d (2.2)	6.14 m	6	96.4	96.4
			7	165.1	165.1
8	6.12 d (2.4)	6.11 m	8	95.6	95.6
OMe	3.77 s	**1a/1d**: 3.74 s (3H) (hpa), 3.77 s (3 H) (hpb); **1b**: 3.75 s (6H)	OMe	55.7	55.7
5-OH	12.01 s		9	162.5	162.5
3-OH	9.12 s		10	103.3	103.3
1''	4.97 d (7.8)	4.79 m; 4.97 m	1''	99.4	99.2; 99.4
2''	3.21 m	3.22 m	2''	73.0	73.1
3''	3.26 m	3.26 m	3''	76.3	76.4
4''	3.62 m	3.64 m	4''	70.1	70.2
5''	3.53 m	3.62 m	5''	75.5	75.5
6a''	3.84 d (13)	3.78-3.80 m	6''	66.1	70.1
6b''	3.40 m	3.68 m			
1'''	4.52 brs	**1a/1d**: 5.71 m (hpb); 4.59 m (hpa); **1b**: 4.52 m	1'''	100.6	**1a/1d**: 100.4 (5.71); 100.6 (4.59); **1b**: 100.8
2'''	3.43 m	3.44 m	2'''	70.7	70.7
3'''	3.15 m	3.12 m	3'''	69.6	68.3
4'''	3.15 m	3.16 m	4'''	72.1	72.1
5'''	3.38 m	3.78-3.80 m	5'''	68.3	66.1
6'''	1.09 d (3.5)	1.12 brs	6'''	17.9	17.9

^{1}H and ^{13}C NMR spectra were acquired in DMSO-d_6 at 600 and 150 MHz, respectively. The chemical shifts are shown in the δ scale with J values (Hz) in parentheses. Assignments are based on COSY, HSQC, and HMBC; H-2 and anomeric signals indicated the presence of 2R and 2S isomers. Hesperidin with ring-B near to phenanthroline: hpa; with ring-B near to ring A of the second hesperidin hpb. The hydrogen shift for methyl group of the CH$_3$COO ligand is δ 1.82 s, and the chemical shifts for methyl and carboxyl groups of the CH$_3$COO ligand are δ 23.6 and 180.1, as *per* HSQC and HMBC, respectively.

effect. Moreover, one broadened doublet appears at δ 5.20 (δ 76.4), allowing to assign it to H-2 and C-2 of the second flavonoid of **1a/1d**, both close to phenanthroline, which presumably shields the hydrogen and carbon resonances. The HSQC also showed signals at δ 2.91 (m), and 2.58 (m) attributed to H-3α and H-3β in isomer **1a/1d**, and other shielded signals at δ 2.76 (m) and 2.50 (m), also assigned to H-3α and H-3β in isomer **1b** and indicating that these hydrogens are held closer to the phenanthroline, which imposes an anisotropic magnetic shielding effect on the

H-3α and H-3β. Altogether, the NMR data supported the proposed structures of [Mg(hesp)$_2$(phen)]OAc depicted as **1a**, **1b** and **1d** (Figure 2).

Advanced analytical technology has provided evidence of the existence of stereoisomers of many flavanones, which might have two different configurations at C-2 existing in many plants, as in citrus fruits.[14] The ^{1}H NMR of pure hesperidin revealed the presence of both isomers, H-2 and anomeric signals indicated the presence of 2R and 2S isomers (Figure S1, SI section). The presence of

NMR signals for two hesperidin flavanones in complex **1**, and with both isomers made the spectra very complex (Figure S5-S9, SI section).

Oliveira *et al.*[4] previously synthesized [Mg(hesp)$_2$(phen)] OAc (**1**) under an N$_2$ atmosphere using glassware, and it was identified on the basis of elemental analysis (C, H, N), atomic absorption and spectroscopic (Fourier transform infrared (FTIR), UV-Vis, ^{1}H NMR) techniques. We noted that as flavanones are asymmetrical ligands, the reaction may give rise to diastereoisomers. Thereby, we developed several NMR experiments with high resolution and sensitivity to identify the diastereoisomers. Importantly, the complex **1** obtained in the present work represent a novel group of diastereoisomers. Our results showed that the NMR experiments performed should be more used in coordination chemistry.

The main physicochemical properties of complex **1** were successfully studied by Oliveira *et al.*[4] In contrast, we still have not enough evidence for developing an understanding of the mechanism by which metals increase the antioxidant activities in flavonoids. In an effort to find more evidence, we have now undertaken an investigation of the antioxidant properties in flavonoids.

Antioxidant properties in flavonoids

Overall, the reviews of literature on flavonoids showed that the mechanisms of antioxidant action can include suppressing reactive oxygen species (ROS) formation either by inhibition of enzymes or chelating trace elements involved in free radical production. For example, free iron is potential enhancers of reactive oxygen species formation, as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical, through Fenton reaction (H$_2$O$_2$ + Fe^{+II} → $^\bullet$OH + $^-$OH + Fe^{+III}).[15] A number of flavonoids efficiently chelate trace metals, however, the proposed binding sites for trace metals are the 3',4'-dihydroxyl moiety in ring B, and the 4-oxo, 5-hydroxyl groups in ring C and A, respectively, in which the last two are chelated by Mg^{II} in complex **1**.

The mechanisms of antioxidant can also include scavenging reactive oxygen species (ROS), and upregulating or protecting antioxidant defenses. Hydroxylated flavonoids are thermodynamically able to reduce highly oxidizing free radicals, as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation. The aryloxy radical (ArO$^\bullet$) may react with a second radical, acquiring a stable quinone structure, as exemplified in the Figure 3.[15-17] However, in complex **1** and hesperidin there are not a 3',4'-dihydroxyl moiety in ring B to form stable quinone (Figure 3). Overall, the presence of only one hydroxyl

in ring B diminishes the activity, and methoxylation of the hydroxy groups on flavonoids resulted in a decrease in activity, as in hesperidin. Flavanonols and flavanones (as hesperidin), due to the lack of conjugation provided by the 2,3-double bond with the 4-oxo group, are weak antioxidants.[18,19]

A wide range of methods is available in the scientific literature to measure the antioxidant capacity of various types of substances.[20] These assays are based on the ability of an antioxidant to scavenge a preformed radical or radical cation, and the sequence of chemical structures and reactions proposed to be involved in the oxidative conversion of hesperidin are associated with oxidation of the 3'-OH group of B ring (Figure 3).

The complexation of flavonoids with metals improves their antioxidant activities, as confirmed by the scavenging effect of complex **1** evaluated by MET/VitB2/NBT assay, where the reaction of methionine (MET) with vitamin B2 (VitB2) produces the superoxide radicals and the nitro blue tetrazolium (NBT) is the O$_2$$^{-\bullet}$ indicator. In a previous study[4] on classic synthesis of complex **1**, it was found that **1** is a better radical scavenger for superoxide radical (concentration of complex **1** which gives half-maximal response, IC$_{50}$ = 68.3 μmol L^{-1} at pH 7.8) than free hesperidin (IC$_{50}$ = 116.7 μmol L^{-1}). Due to the lack of conjugation provided by the 2,3-double bond with the 4-oxo group in hesperidin, this observation could not be explained by the resonance effects (electron delocalization). Thus, the effect of increasing the antioxidant activity in complexation of flavonoids with metals can only be attribute to the field effect.[21] Inductive effect, the permanent dipole induced in one bond by another, may also be transmitted in ways other than directly along a chain, for example, through space and through solvent molecules, which is known as field effect.[21] The field effect of cation Mg in the position of the 3'-OH group of B ring lowers the activation energy required to reach the transition state for ionization, or radical formation. This hypothesis is also supported by the effect of cation Mg observed in C-4, electron delocalization from the oxygen group at C-7 (by the 7,6,5,10-diene) to the oxygen atom of the ketone at C-4 strengthened the interaction between oxygen and cation Mg, shielding C-4 absorption in ^{13}C NMR of complex **1** (δ 197.0 in hesperidin and 191.7 in **1**).

Effects of [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)]OAc on *Bemisia tabaci*, *Spodoptera frugiperda* and *Myzus persicae*

Bemisia tabaci is considered a complex of cryptic species with at least 44 species. Among them, the species Middle East-Asia Minor 1 (MEAM1, formerly B biotype)
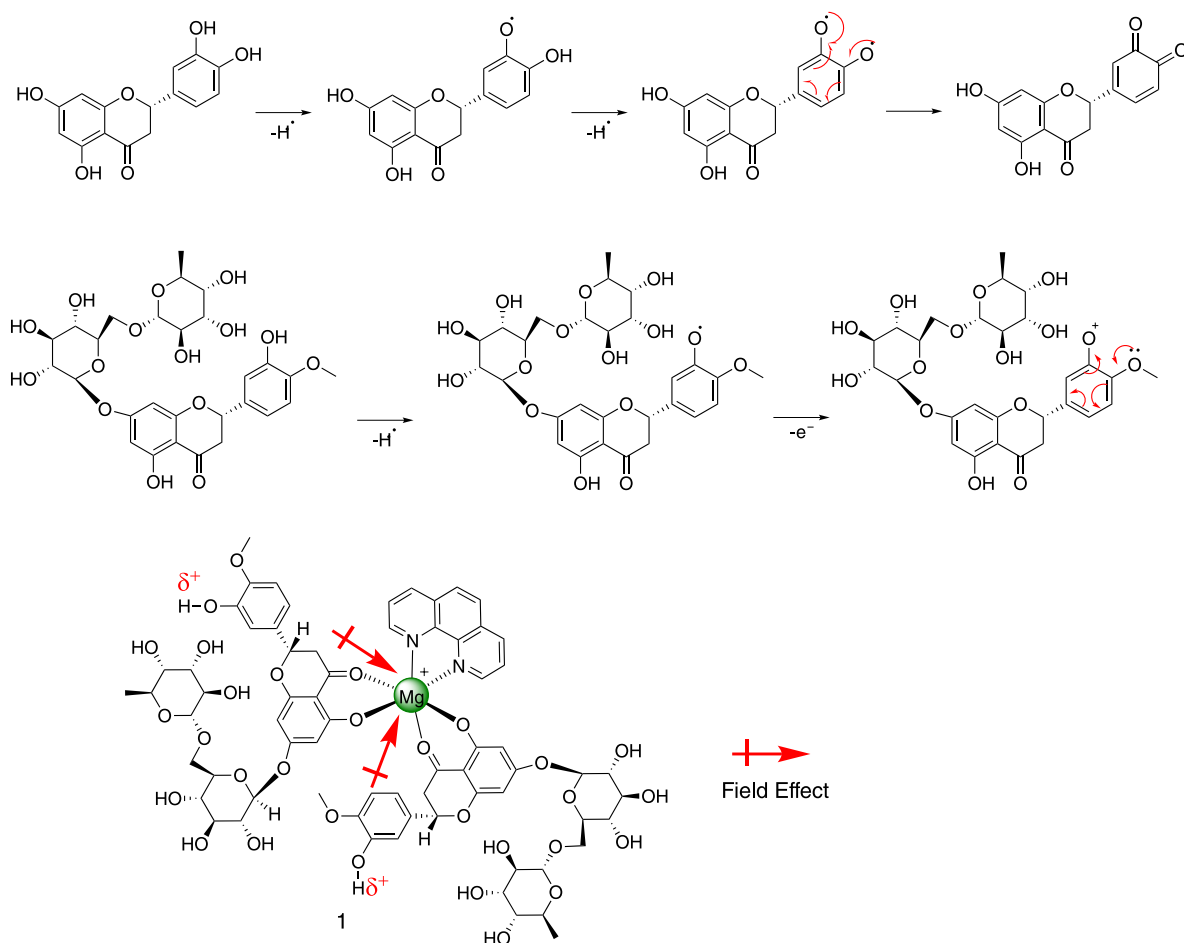
Figure 3. Scavenging of R• by model flavonoid, hesperidin and complex **1**.

and Mediterranean (MED, formerly Q biotype) are the most important, and they have attained global status. In Brazil, MEAM1 was first reported in the 1990s and is currently the predominant species in the country, meanwhile, MED was recently reported in the South and Southeast regions and was found to be mainly associated with ornamental plants.[22] *B. tabaci* occurs in tropical and subtropical habitats and is multivoltine, producing 11 to 15 generations *per* year under favorable conditions, and females deposit 100 to 300 eggs in a lifespan of three to six weeks. Eggs are laid and immature stages develop on the undersides of leaves of host plants. Adults usually congregate on younger leaves, where oviposition is heaviest. The life cycle from egg to adult requires 2.5 to 3 weeks in tropical weather. [Mg(hesp)$_2$(phen)]OAc (**1**) and [Mg(phen)$_2$(Isov)]OAc (**2**) (Figure 4) complexes were evaluated against *B. tabaci* MEAM1, and leaf dip treatments were used as they ensure good coverage of the leaf undersides.

There was a significant mortality of adult after bean leaf (*Phaseolus vulgaris* L.) dipping with [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)]OAc complexes at 20 and 22 mg mL^{-1}, that were higher than the water control
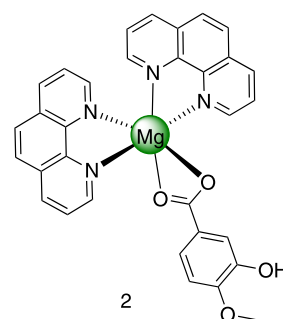

Figure 4. Molecular structures of the [Mg(phen)$_2$(Isov)]OAc (**2**) complex.

(Figure 5). Complex **1** was the most active, remarkably, at 0.14 μmol L^{-1} (20 mg mL^{-1}) it killed 80% of whitefly adults. Significant activity was also found in complex **2**, which at 0.36 μmol L^{-1} (20 mg mL^{-1}) killed 76% of whitefly adults. However, the activity appeared to be affected by the concentration, since both complexes were less active at 22 mg mL^{-1}. Complex **1** killed 74% of whitefly adults at 0.15 μmol L^{-1}, while complex **2** killed only 54% at 0.39 μmol L^{-1}. The complexes in higher concentration probably did not show translaminar activity, therefore with less efficacy in sucking pests, such as *B. tabaci*. Overall,
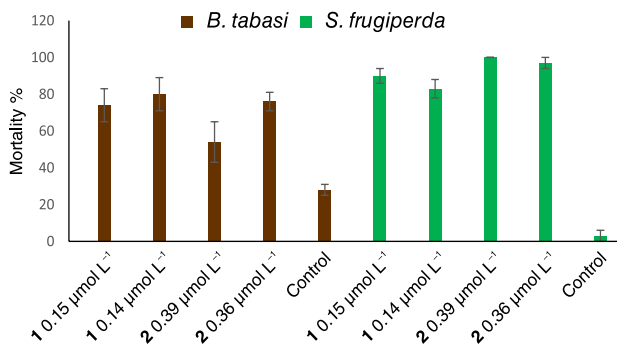
Figure 5. The efficacy of [Mg(hesp)$_2$(phen)]OAc (**1**) (0.15 and 0.14 µmol L^{-1}) and [Mg(phen)$_2$(Isov)]OAc (**2**) (0.39 and 0.36 µmol L^{-1}) treatments against adults *B. tabaci* (brown) and second-instar *S. frugiperda* (green); mortality assessed after 72 h for all treatments; means followed by the same letter do not differ from each other using the Tukey's test ($p < 0.05$) {brown, **1** 0.15 µmol L^{-1}, a; **1** 0.14 µmol L^{-1}, a; **2** 0.39 µmol L^{-1}, ab; **2** 0.36 µmol L^{-1}, a; control b; green, **1** 0.15 µmol L^{-1}, ab; **1** 0.14 µmol L^{-1}, b; **2** 0.39 µmol L^{-1}, a; **2** 0.36 µmol L^{-1}, ab}.

the results showed that the two complexes at optimal concentration are effective against *B. tabaci*.

Spodoptera frugiperda, or fall armyworm, is a nocturnal insect and its mating occurs at night. The female lays most of her eggs during the first four to five days of life, but some oviposition may occur for up to three weeks. Eggs are laid mainly on the underside of leaves, covered with dense scales. It has four stages to complete its life cycle, which takes two to four days, at temperatures in the range of 21-27 degrees Celsius. The larva has six developmental instars, and the older stages cause higher damage. A single larva can masticate about 140 cm^2 of maize leaf area to complete the larval development period.[9] [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)] OAc complexes were evaluated against *S. frugiperda*, and leaf dip treatments were also used as they ensure good coverage of the leaf undersides. There was a total mortality of second-instar *S. frugiperda* after maize leaf (*Zea mays* L.) dipping with [Mg(phen)$_2$(Isov)]OAc complex at 22 mg mL^{-1} (0.39 µmol L^{-1}) and 96.6% at 20 mg mL^{-1} (0.36 µmol L^{-1}). The activity of [Mg(hesp)$_2$(phen)]OAc (**1**) was comparable to that of **2**, at 0.15 µmol L^{-1} (22 mg mL^{-1}) it killed 90% of second-instar fall armyworm and 83% at 0.14 µmol L^{-1} (20 mg mL^{-1}). The complex **2** was active at a concentration around two times greater than that of the complex **1**, suggesting that both have the same activity. We did not repeat the trials with the two complexes at the same concentration, as the data indicated both with potential to be used as alternative control agents of *S. frugiperda*, at the point where it causes the greatest damage to the corn crop.

The [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)]OAc complexes were also tested against *M. persicae* in a similar assay. Unfortunately, these complexes did not also show

significant activity against *M. persicae*, they caused 8% and 15% adults' mortality, respectively. However, the insect's outward displacement of the cabbage leaf containing the test solutions was perceived as a repellent signal. Compared to the water control both complexes **1** and **2** caused 70 and 85% adults' displacement, respectively.

Conclusions

The microwave-assisted synthesis of [Mg(hesp)$_2$(phen)]OAc (**1**) improve the reaction rate and yield. Mg^{II} complex with naringenin and phenanthroline was previously synthesized and NMR analysis revealed the generation of two [Mg(narin)(phen)$_2$] OAc diastereoisomers.[5] Here, we verified that using the flavanone hesperidin, the probability of diastereoisomer formation increased, since the NMR spectra showed characteristic signals for two flavonoids in the complex. Hesperidin is as unsymmetrical ligand and may give rise to four diastereoisomers for the Mg^{II} complexes. We also developed several NMR experiments and identified a least three diastereoisomers.

The aim of the current study was also to investigate the efficacy of [Mg(hesp)$_2$(phen)]OAc and [Mg(phen)$_2$(Isov)]OAc complexes to control adult *B. tabaci*, and second-instar *S. frugiperda*, stages that they cause higher damage. The results indicated that both complexes tested were active at concentrations in the micromolar range, comparable to the limonoid azadirachtin used as a positive control in our screening program to search for new insecticides. Due to their high insecticide activity, both can be used in integrated management of *B. tabaci*, and *S. frugiperda*. Complexes **1** and **2** represent a novel class of compounds tested for the first time as insecticide against these insects. However, information on the mode of action of these complexes will be useful for designing predictable and durable strategies to control pests in the field.

Several studies suggest that flavonoids respond to biotic and abiotic factors by providing antioxidant properties. Ingestion of flavonoids by insects can be tolerated or detoxified depending on the levels of antioxidant, cytochrome P450 monoxygenase and esterase enzymes present in the insects as well as the pH of the gut.[18,19] Few studies into these enzymes have been resulted in a better understanding on their role in the ability of insects to detoxify and metabolize ingested flavonoids. However, the antioxidant efficacy of flavonoids against insects has been less thoroughly documented, possibly due to the limited knowledge on their mechanism of action as an antioxidant in inhibiting insect development.

Finally, our results suggest that complexes **1** and **2** can be used for *B. tabaci* and *S. frugiperda* management strategy; nevertheless, the benefits of both as *M. persicae* repellent require further evaluation at the field level.

Supplementary Information

Supplementary information (NMR spectra) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

Author Contributions

DFS was responsible for investigation, data curation, and performed acquisition of data; JPAB was responsible for biological experiments; RCM, JCA, LSP performed acquisition of data; RMC, AGF, MRF, JBF were responsible for conceptualization, formal analysis, funding acquisition, writing original draft and supervision; MFGFS was responsible for planning, project administration, funding acquisition, writing original draft and writing-review and editing, and coordination; RCO, DB, WDJK were responsible for biological experiments; TJAB was responsible for biological experiments, project administration, funding acquisition in UK, and coordination.

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