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SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF NEW SOLID SOLUTION CONTAINING MG(II) AND CU(II) COMPLEXES WITH HEXADENTATE 1,3-PROPANEDIAMINE-*N,N,N',N'*-TETRAACETATE (1,3-PDTA) LIGAND: *IN VITRO* ANTIFUNGAL ACTIVITY OF 1,3-PDTA-CU(II) COMPLEXES[†]

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Abstract. *New solid solution containing Mg(II) and Cu(II) complexes with hexadentate 1,3-propanediamine-*N,N,N',N'*-tetraacetate ligand (1,3-pdta), $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ was synthesized and structurally characterized by elemental microanalyses, molar conductivity, and spectroscopic (IR and UV-Vis) measurements. The spectroscopic data of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ were compared with those for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ complex of the known molecular structure determined by single-crystal X-ray diffraction analysis. In vitro growth inhibition activity of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ against* Colletotrichum acutatum *and their effects on this fungus sporulation level are investigated. The obtained results showed that the highest percentage of inhibition for mycelium growth was achieved at a concentration of 500 µg/mL for the investigated complexes. The biological activities of the investigated complexes were compared with those for the commercial formulation of fungicide captan (Method 480 SC).*

Key words: *3-pdta-copper(II) complexes, IR spectra, electronic absorption spectra, antifungal activity,* Colletotrichum acutatum

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1. INTRODUCTION

The fungus *Colletotrichum acutatum* is one of the most frequently reported species that causes disease on numerous host plants worldwide commonly known as anthracnose. The typical symptoms of this disease are manifested on fruits, stolons, leaf and flower stems, and, in more severe cases, on the leaves, crown, and root. This fungus can attack the plant at all stages of development and can provoke serious problems for fruit (in particular strawberry) and plant production (Freeman et al., 1998; Maas et al., 1997; Wedge et al., 1999). The control of anthracnose is usually achieved by the treatment of plants with fungicides, to which *C. acutatum* becomes more and more resistant. The resistance development and lack of the appropriate agents for the disease treatment are two main factors driving the need for novel plant protectants.

Despite the production of various organic fungicides, copper-based fungicides still predominate in the field of fungicidal plant disease control (Gharieb et al., 2004). Among them, copper(II) oxychloride is the most widely used, being efficient against different plant pathogenic fungi such as *Rhizoctonia solani*, *R. bataticola*, *Botrytis cinerea*, *Fusarium semitectum*, *F. culmorum*, *F. moniliforme*, *F. solani*, *F. oxysporum*, *Stemphylium radicinum*, *Hirschmanniella oryzae*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides* (Gharieb et al., 2004). One of the approaches to tune the activity of Cu(II) is to administer this ion in the form of a complex (Szymański et al., 2012). The advantage of this approach is the selective delivery of Cu(II) ions to the diseased tissues and the modification of pharmacokinetics and pharmacodynamics due to the ligand used for the synthesis of a complex (Szymański et al., 2012).

In the last several decades, great attention has been dedicated to diaminopolycarboxylate ligands structurally related to edta (ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetate anion) and their metal complexes because of their importance in chemistry, medicine, and agriculture (Anderegg, 1987; Bulman, 1987; Douglas and Radanović, 1993). In different forms, edta has been used as a powerful antimicrobial agent, which manifests significant activity against a range of clinically important microorganisms, including different Gram-negative and Gram-positive bacteria, yeasts, amoeba, and fungi (Brown and Richards, 1965; Kite and Hatton, 2014). Besides that, edta is effective in the elimination of the pre-existing biofilms as well as in the prevention of biofilm formation (Finnegan and Percival, 2015; Sherertz et al., 2006).

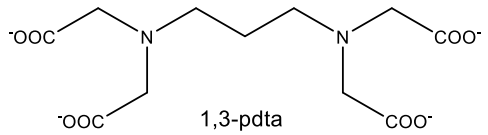

Fig. 1 Structure of 1,3-propanediamine-*N*,*N*,*N'*,*N'*-tetraacetate anion (1,3-pdta).

Considering the use of copper(II) compounds as fungicides and biological activity of ligands from the edta family, two copper(II)-containing complexes with 1,3-pdta ligand (1,3-propanediamine-*N*,*N*,*N'*,*N'*-tetraacetate anion; Fig. 1), $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ (Rychlewska et al., 2000), were synthesized and characterized. In the present study, 1,3-pdta ligand was chosen as metal chelator because it shows a higher ability to act as a hexadentate, compared to edta, which

can be a consequence of the increased length and flexibility of the 1,3-propanediamine ring (Rychlewska et al., 2000; Weyh and Hamm, 1968). The growth inhibition activity of both copper(II) complexes against *C. acutatum* was evaluated, as well as their effect on this fungus sporulation levels.

2. MATERIALS AND METHODS

2.1. Materials

1,3-Propanediamine and chloroacetic acid were obtained from Acros Organics. All other common chemicals were of reagent grade and used without further purification.

2.2. Preparation of Ba_2(1,3-pdta)·$2H_2O$

Ba_2(1,3-pdta)·$2H_2O$ was obtained by the following procedure which was used previously in our lab for the preparation of the corresponding barium salts of different diaminopolycarboxylate-type of ligands: 1,3-propanediamine-*N,N,N',N'*-tetraacetic (1,3-H_4pdta) (Rychlewska et al., 2000; Weyh and Hamm, 1968), 1,3-propanediamine-*N,N'*-diacetic-*N,N'*-di-3-propionic (1,3-H_4pddadp) (Radanović et al., 1992), 1,4-butanediamine-*N,N,N',N'*-tetraacetic (1,4-bdta) (Radanović et al., 2007) and 1,3-pentanediamine-*N,N,N',N'*-tetraacetic (1,3-H_4pndta) (Drašković et al., 2012) acids. The mixture of water solution containing 1,3-propanediamine (0.05 mol; 4.2 mL; ρ = 0.88 g/mL) and neutralized chloroacetic acid (0.2 mol; 18.9 g) was stirred with heating at 80 ºC with the slow addition of solution of NaOH (0.2 mol; 8.0 g) for 4 h. Deposited inorganic salt was removed by filtration and filtrate was added to the hot solution of $BaCl_2$·$2H_2O$ (0.1 mol; 24.4 g) with stirring and heating at 60 ºC for 2 h. The white precipitate of Ba_2(1,3-pdta)·$2H_2O$ was filtered and air-dried. Yield: 18.7 g (61%). Anal. Calcd. for Ba_2(1,3-pdta)·$2H_2O$ = $Ba_2C_{11}H_{18}N_2O_{10}$ (FW = 612.93): C, 21.56; H, 2.96; N, 4.57. Found: C, 21.42; H, 2.78; N, 4.53%. IR (KBr, ν, cm^{-1}): 3446br, 2914w, 2821w, 1570vs, 1436m, 1408s, 1329m, 1263w, 1172w, 1158w, 1002w, 979w, 927w, 727m, 632w, 550w.

2.3. Preparation of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}$(1,3-pdta)]·$2H_2O$

$CuSO_4$·$5H_2O$ (1.25 g; 0.005 mol) was dissolved in 25 mL of distilled water at 60 ºC. To this solution, solid Ba_2(1,3-pdta)·$2H_2O$ (6.13 g; 0.01 mol) was added and the reaction mixture was heated at 60 ºC with stirring for 20 min. Then, $MgSO_4$·$7H_2O$ (3.70 g; 0.015 mol) was added and stirring with heating at 60 ºC was continued for the next 20 min. Deposited $BaSO_4$ was filtered out. To the obtained filtrate, 5–6 mL of ethanol was added and the solution was left to stand in a refrigerator overnight. The blue crystals of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}$(1,3-pdta)]·$2H_2O$ complex were collected, washed with ethanol, and air-dried. Yield: 3.7 g (72%). Anal. Calcd. for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}$(1,3-pdta)]·$2H_2O$ = $C_{11}H_{30}Mg_{1.50}Cu_{0.50}N_2O_{16}$ (FW = 514.59): C, 25.67; H, 5.88; N, 5.44. Found: C, 25.87; H, 6.09; N, 5.38%. IR (KBr, ν, cm^{-1}): 3376br, 2976w, 2877w, 1691s, 1599vs, 14047vs, 1334s, 1273m, 1233m, 1161w, 1072w, 1041w, 988w, 922m, 771s, 681s, 535w. UV-Vis (H_2O, λ_{max}, nm): 706.0 (ε = 45.8 $M^{-1}cm^{-1}$). Λ_M (H_2O): 198.9 $\Omega^{-1}cm^2mol^{-1}$.

2.4. Preparation of $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$

This complex was prepared by following the method which was described in the literature (Rychlewska et al., 2000). Equimolar amounts of $Ba_2(1,3\text{-pdta})\cdot 2H_2O$ and $CuSO_4\cdot 5H_2O$ were mixed in water. The obtained mixture was stirred with heating at 70 ºC for 2 h. Deposited $BaSO_4$ was removed by filtration and obtained filtrate was passed through the column supplied with Dowex 1-X8 anion exchange resin in the Cl^- form. The column was washed with water and eluted with 0.05 M $MgCl_2$. Only one blue band with −2 charge which corresponds to the $[Cu(1,3\text{-pdta})]^{2-}$ complex anion appeared on the column. The eluate of the band was evaporated to a volume of 15-20 mL and then desalted by passage through a G-10 Sephadex column, with distilled water as the eluent. The desalted eluate containing the hexadentate 1,3-pdta-Cu(II) complex was evaporated to 20 mL and allowed to stand at room temperature for several days and the crystals of $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ were collected and air-dried. Yield: 64%. Anal. Calcd. for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O = MgCuC_{11}H_{30}N_2O_{16}$ (FW = 534.21): C, 24.73; H, 5.66; N, 5.24. Found: C, 24.79; H, 5.47; N, 5.14%. IR (KBr, ν, cm^{-1}): 3285br, 2980w, 2885w, 1692s, 1593vs,1404vs, 1333vs, 1275m, 1232m, 1154m, 1112m, 1070m, 1040m, 989m, 911m, 786s, 680vs, 530w. UV-Vis (H_2O, λ_{max}, nm): 706.0 (ε = 89.6 $M^{-1}cm^{-1}$). Λ_M (H_2O): 193.5 $\Omega^{-1}cm^2mol^{-1}$.

2.5. Measurements

Elemental microanalyses for carbon, hydrogen, and nitrogen were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade. The IR spectra were recorded as KBr pellets on a Perkin Elmer Spectrum One spectrometer over the wavenumber range 4000–450 cm^{-1}. The electronic absorption spectra were recorded over the wavelength range of 900–200 nm on a Shimadzu double-beam spectrophotometer after dissolving the corresponding copper(II) complex in water. For these measurements, $1\cdot 10^{-2}$ M solutions of the copper(II) complexes, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$, were used. Molar conductivities were measured at ambient temperature on a digital conductivity-meter Crison Multimeter MM 41. The concentration of the solutions of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ in water used for the conductivity measurements was $1\cdot 10^{-3}$ M.

2.6. Biological tests

The antifungal activity of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ complexes was tested on potato-dextrose agar (PDA) in Petri dishes of 90 mm diameter. Substrates were inoculated by mycelial fragments of *C. acutatum* isolates taken from the edge of culture at seven days of age. The isolate of *C. acutatum* was obtained from strawberries grown in Serbia and determined based on morphological, pathogenic, and molecular characteristics, and maintained on PDA at 25 °C. The tested complexes were dissolved in water at three different concentrations: 100, 500, and 1000 µg/mL. These solutions were incorporated into the autoclaved PDA, which was cooled to 55 °C. Cultures prepared in this way were grown for seven days in a thermostat at 25 °C. The commercial formulation of fungicide captan (Method 480 SC, Galenika phytopharmacy) was used as a positive control and was applied at a concentration

recommended for practical application. The negative control variant, fungi isolates grown on PDA were used under identical conditions without the described treatments (Zhang et al., 2012). After seven days, the radial increase in the studied cultures was measured. The inhibition of the fungal growth expressed in percentage terms was determined from the growth in the test plate relative to the respective control plate as given below: Inhibition (%) = (C−T) 100 / C where, C = diameter of fungal growth in the control plate and T = diameter of fungal growth in the test plate.

Ten days after treatment, the effect of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ on *C. acutatum* sporulation levels was determined. Determination of sporulation levels was performed using a Thom haemocytometer. For this purpose, a spore suspension was prepared by adding 5 mL of water to the Petri dish with a culture of isolate *C. acutatum*. The sporulation level is expressed according to the scale by Quesada and Lopez (1980), where: + = poor sporulation (< 5.000 spores/mL), ++ = medium sporulation (5.000−10.000 spores/mL) and +++ = abundant sporulation (> 10.000 spores/mL). The experiment was set in five repetitions.

The results obtained during the research were processed by ANOVA analysis with the statistical program StatSoft STATISTICA 8.0. Duncan's test was conducted to analyze the difference between various pre-treatments. A value of P = 0.05 was considered statistically significant.

3. Results and Discussion

This paper reports synthesis, spectroscopic characterization, and antimicrobial evaluation of new solid solution containing Mg(II) and Cu(II) complexes with hexadentate 1,3-propanediamine-*N,N,N',N'*-tetraacetate (1,3-pdta), $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and Cu(II) complex with 1,3-pdta ligand, $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$. The purity of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ was checked by elemental microanalyses, while hexadentate coordination of 1,3-pdta ligand and *cis*(N)-octahedral geometry of these complexes were confirmed by IR and electronic absorption spectroscopy. The crystal structure of complex $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ was determined previously by single-crystal X-ray diffraction analysis (Rychlewska et al., 2000) and herein its IR and electronic absorption spectra are compared to those for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$.

3.1. Spectroscopic characterization of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$

3.1.1. Electronic absorption spectra

The electronic absorption spectra of the investigated complexes and its numerical data (the wavelengths of the maximum absorption (λ_{max}, nm) and molar extinction coefficients (ε, $M^{-1}cm^{-1}$) are compared. As it can be seen from Fig. 2, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ exhibit a single quasi-symmetric band, which can be assigned to the d_{z^2}, d_{xy}, d_{xz}, $d_{yz} \rightarrow d_{x^2-y^2}$ transitions with a $d_{x^2-y^2}$ ground state (Drašković et al., 2012; Hathaway, 1987; Rychlewska et al., 2000). The electronic absorption spectra of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-}$

pdta)]·2H_2O are nearly identical in the shape and have the same wavelengths of the maximum absorption (see Fig. 2), indicating that the same N_2O_4 coordination mode of 1,3-pdta ligand occurs in $[Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]^{2-}$ and $[Cu(1,3\text{-pdta})]^{2-}$ ions. Moreover, it can be noticed that the absorption intensity for $[Cu(1,3\text{-pdta})]^{2-}$ is 2-fold higher than for $[Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]^{2-}$. The lower absorption intensity for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ can be attributed to the presence of Mg(II) and Cu(II) ions coordinated to 1,3-pdta ligand in respect to $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ having only Cu(II) inside the coordination sphere. No significant changes in the intensity and position of the absorption maxima and the shape of the time-dependent UV-Vis spectra of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ were noticed during 48 h, indicating the appreciable stability of the complexes.

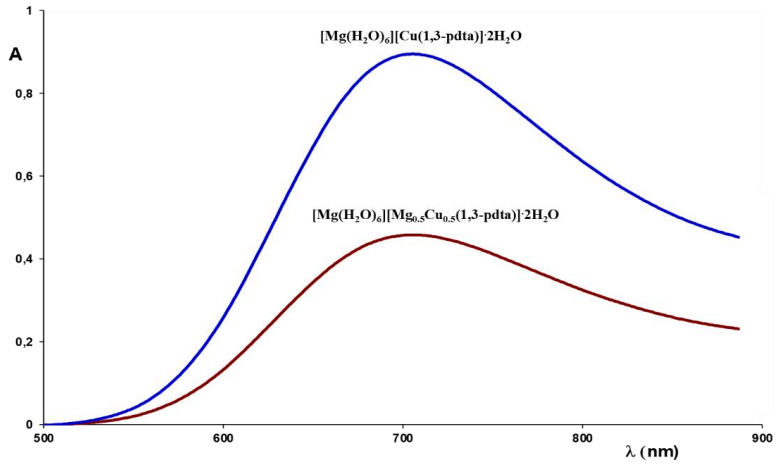

Fig. 2 Electronic absorption spectra of the investigated complexes, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$, measured in water at room temperature ($c = 1\cdot 10^{-2}$ M).

3.1.2. IR spectra

The IR spectra of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ recorded in the range of 4000–450 cm^{-1} show the expected bands attributed to the hexadentately coordinated 1,3-pdta and crystalline water molecule (see data in the Materials and Methods section). The asymmetric carboxylate stretching frequencies of 1,3-pdta ligand can be used for the determination of the environment of the carboxylate group in this type of complexes (Busch and Bailar, 1953; Busch and Bailar, 1956; Morris and Busch, 1956; Nakamoto, 1963).

The parts of IR spectra related to the region of carboxylate stretching frequencies of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ are shown in Fig. 3. As can be seen, the studied complexes show strong band in the asymmetric carboxylate spectral region, 1593 cm^{-1} for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ and 1599 cm^{-1} for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$, indicating that all carboxylate groups in these compounds are coordinated. However, these bands show tendency for splitting at

the higher energy side at 1691 and 1690 cm^{-1} for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$, respectively. Besides that, one strong band due to the symmetric carboxylate stretching frequencies, $\nu_s(COO^-)$, at 1404 cm^{-1} for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ and 1407 cm^{-1} for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ was also observed.

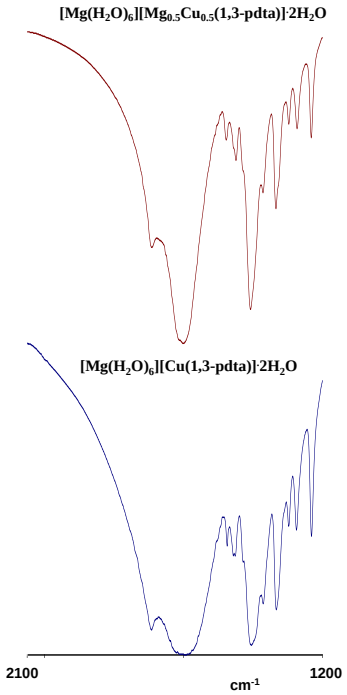

Fig. 3 The parts of IR spectra related to the region of carboxylate stretching frequencies of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$.

3.2. Antifungal activity of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$

The investigated complexes, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ inhibited the growth of *C. acutatum* mycelia. The percentage of inhibition was dependent on the applied concentration of the complex. The inhibition of mycelial growth was observed at concentrations of 100 μg/mL and higher for the complexes. Specifically, the highest percentage of inhibition of the mycelium of the *C. acutatum* fungus, compared to the commercial formulation of captan (Method 480 SC Galenika phytopharmacy), was achieved at a concentration of 500 μg/mL for $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ complexes (Table 1 and Fig. 4). No statistically significant difference was found between the studied complexes in the percentage inhibition of mycelial growth of the tested *C. acutatum* fungus.

Table 1 Influence of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ on the inhibition of *C. acutatum*

Complex	100 µg/mL	500 µg/mL	1000 µg/mL	Method 480 SC
$[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$	14.82[d]	25.56[b]	18.75[c]	100[a]
$[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$	18.77[d]	39.30[b]	19.40[c]	100[a]

* The data in rows marked by the same letter are not statistically significantly different based on Duncan test (p=0.05)

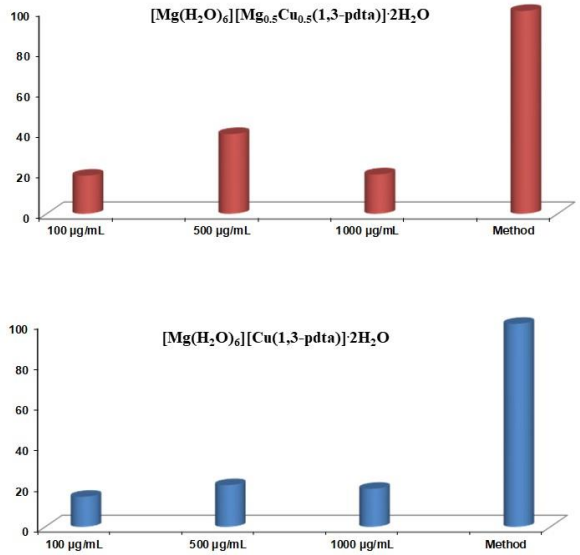

Fig. 4 The effect of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ on the growth of *C. acutatum* isolate *in vitro* after seven days.

3.2.1. Sporulation level

The different concentrations of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ greatly influenced the ability of sporulation of the tested fungus *C. acutatum*, the formation of conidia in larger or smaller numbers (Table 2). Complex $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ compared to the negative control (fungi isolates grown on PDA), showed a medium sporulation level at concentrations of 100 and 500 µg/mL, while at 1000 µg/mL, it showed a lower sporulation level. The $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ showed a medium sporulation level at all three applied concentrations compared to the negative control, in which abundant sporulation was observed. The sporulation level is very important for the infection by *C. acutatum*, so the reduction of the number of spores can significantly affect the spread of the infection.

Table 2 Influence of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ on the sporulation level of *C. actatum*

Complex	100 µg/mL	500 µg/mL	1000 µg/mL	Control
$[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$	++	++	+	+++
$[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$	++	++	++	+++

+ = poor sporulation, ++ = medium sporulation, +++ = abundant sporulation

4. CONCLUSION

Herein, we report the synthesis and spectroscopic characterization of new solid solution containing Mg(II) and Cu(II) complexes with hexadentate 1,3-propanediamine-*N,N,N',N'*-tetraacetate ligand (1,3-pdta) ligand, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$. The $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ was characterized by comparison of its spectroscopic data with those for $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ complex of the known crystal structure (Rychlewska et al., 2000). *In vitro* antimicrobial activity and sporulation levels of $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ and $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ were investigated for the first time and obtained results were compared with those for the commercial formulation of fungicide captan (Method 480 SC). The results showed that these complexes significantly inhibited the mycelial growth of the isolate *C. acutatum* and potently induce defense reactions in the plant. Contact copper-based fungicides are economically acceptable and do not lead to resistance, unlike the commercial systemic fungicides whose irrational and unplanned utilization led to increasing fungi resistance. Our latest results should be considered during the preparation of novel copper-based complexes for different applications as antimicrobial agents in agriculture, thus offering a lower risk for resistance development of fungi.

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SINTEZA I SPEKTROSKOPSKA KARAKTERIZACIJA NOVOG RASTVORA U ČVRSTOM STANJU KOJI SADRŽI Mg(II) I Cu(II) KOMPLEKSE SA HEKSADENTATNIM 1,3-PROPANDIAMIN-*N,N,N',N'*-TETRAACETATO (1,3-PDTA) LIGANDOM: *IN VITRO* ANTIFUNGALNA AKTIVNOST 1,3-PDTA-Cu(II) KOMPLEKSA

Opisana je sinteza i spektroskopska karakterizacija (IR i elektronski apsorpcioni spektri) novog rastvora u čvrstom stanju koji sadrži Mg(II) i Cu(II) komplekse sa 1,3-propandiamin-N,N,N',N'-tetraacetato (1,3-pdta) ligandom, $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$. Spektroskopski podaci za $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ kompleks su upoređeni sa odgovarajućim podacima za $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ kompleks poznate kristalne strukture. Ispitivana je in vitro *antimikrobna aktivnost $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ i $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ kompleksa prema fitopatogenoj gljivi* Colletotrichum acutatum, *koja uzrokuje antraknozu. Analiziran je uticaj različitih koncentracija $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ i $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ kompleksa na rast micelija i na intenzitet sporulacije gljive. Dobijeni rezultati su pokazali da ovi kompleksi imaju najveću aktivnost pri koncentraciji od 500 µg/mL. Biološka aktivnost $[Mg(H_2O)_6][Mg_{0.5}Cu_{0.5}(1,3\text{-pdta})]\cdot 2H_2O$ i $[Mg(H_2O)_6][Cu(1,3\text{-pdta})]\cdot 2H_2O$ je upoređena sa odgovarajućom aktivnošću komercijalnog fungicida 480 SC (Kaptan).*

Ključne reči: *3-pdta-bakar(II) kompleksi, IR spektri, elektronski apsorpcioni spektri, antifungalna aktivnost,* Colletotrichum acutatum