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# STUDY ON FUNGICIDAL ACTIVITY OF NEWLY SYNTHESIZED COMPLEX COMPOUNDS OF Cu (II), Zn (II) AND Ni (II) WITH PYRAZOLE-DERIVED LIGANDS AGAINST THE PHYTOPATHOGENIC FUNGUS *Phomopsis viticola Sacc*. IN LABORATORY CONDITIONS

## SUMMARY

In the continuation of our detailed studies on pyrazole and its derivatives, Cu (II), Zn (II) and Ni (II) complexes were synthesized with ligand 4-Bromo-2-(1H-pyrazol-3-yl) phenol (HL) and their potential fungicidal activity against the phytopathogenic fungus *Phomopsis viticola* Sacc. (causal agent of Phomopsis cane and leaf spot disease of grapevine) was tested. Based on elemental (C, H, N) analysis and conductometric measurements, the formulas of complex compounds were determined. Biological research based on determining the inhibitory effects of commercial fungicide with active substances pyraclostrobin and metiram, ligand, and all newly synthetized complexes on *Ph. viticola* has been carried out.

**Keywords:** *4-Bromo-2-(1H-pyrazol-3-yl) phenol; complexes of Zn, Ni and Cu; Phomopsis viticola; active fungicidal substances, Phomopsis cane and leaf spot disease of grapevine*

## INTRODUCTION

Grapevine production is very important for Montenegrin agriculture. Vineyards cover an area of 2783.2 ha (MONSTAT, 2017). Cultivation of grapevine is endangered by numerous diseases, and one of the most significant is Phomopsis cane and leaf spot caused by the phytopathogenic fungus *Phomopsis viticola* Sacc. (Latinovic and Latinovic, 2011). The disease occurs every year and makes more or less damages depending on the intensity of the disease, while in exceptional cases it can lead to vine declining. In conditions of Montenegro, the symptoms usually occur on canes and leaves (Latinovic, 2007), while in the

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world they can also appear on berries (Pscheidt and Pearson, 1989). According to Latinovic *et al*. (2003), particular problem is the fact that the most widespread varieties in Montenegrin vineyards Vranac and Kratosija are very susceptible to this disease. To control the disease, preventive measures are used that enable better aeration of the plant canopy and removal of infected plant parts, however treatments with fungicides are still essential. For this purpose, fungicides from the chemical groups' dithiocarbamates and strobilurins are the most commonly used (Latinovic, 2007; Gubler *et al*., 2015).

Pyrazole-based compounds and their transition metal complexes have attracted considerable research interest because of their potentially beneficial biological properties. The wide biological activity of this class of compounds (anticancer, antimicrobial, antiviral, anti-inflammatory, antifungal and others) is described in several reviews (Alex and Kumar, 2014; Kumar *et al*., 2013; Chimenti *et al*., 2006; Trofimenko, 1986). These results represent a part of our continued work with pyrazole-based complex (Jaćimović *et al*., 2013; Jaćimović *et al*., 2017a; Jaćimović *et al*., 2017b). In agriculture, they are in the use as pesticides (Lemaire *et al*., 2006; Vicentini *et al*., 2004; Singh *et al*., 2000).

In this paper, the syntheses of tree new Cu(II) complexes, Zn(II) and Ni(II) compounds of formulas $Cu(L\text{-}H)_2$, $[Cu(L\text{-}H)_2]Cl_2$, $[Cu(L\text{-}H)_2](NO_3)_2$, $Zn(L\text{-}H)_2$ and $Ni(L\text{-}H)_2{\cdot}4H_2O$ obtained in reaction of $Cu(OAc)_2$, $CuCl_2$, $Cu(NO_3)_2$, $Zn(OAc)_2$ and $Ni(OAc)_2$ with 4-Bromo-2-(1H-pyrazol-3-yl)phenol (HL) is described. Their activity was examined to the mycelial growth of *Ph. viticola in vitro*. Obtained results were compared with the commercial fungicide whose one active substance is pyraclostobin that belongs to pyrazole derivates.

## MATERIAL AND METHODS

### Preparation of complexes

Microcrystals of the complex of formula $Cu(L\text{-}H)_2$ were obtained by mild heating in the reaction on warm methanolic solutions of $Cu(OAc)_2{\cdot}H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with methanol.

Microcrystals of the complex of formula $[Cu(L\text{-}H)_2](NO_3)_2$ were obtained by mild heating in the reaction on warm methanolic solutions of $Cu(NO_3)_2{\cdot}3H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. After two days, the formed microcrystals were filtered and washed with methanol.

Microcrystals of the complex of formula $[Cu(L\text{-}H)_2]Cl_2$ were obtained by mild heating in the reaction on warm ethanolic solutions of $CuCl_2{\cdot}2H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with ethanol.

Microcrystals of the complex of formula $Zn(L\text{-}H)_2$ were obtained by mild heating in the reaction on warm ethanolic solutions of $Zn(OAc)_2{\cdot}2H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with ethanol.

Microcrystals of the complex of formula $Ni(L\text{-}H)_2 \cdot 4H_2O$ were obtained by mild heating in the reaction on warm methanolic solutions of $Ni(OAc)_2$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with methanol.

Elemental analysis (C, H and N) of air-dried compounds was carried out by standard micromethods.

The molar conductivity of freshly prepared $1 \cdot 10^{-3}$mol $dm^{-3}$ solutions of the complexes in DMF was determined at room temperature using a digital conductivity meter Jenway 4510.

These complexes were used to study the inhibition of *Ph. viticola* mycelial growth in laboratory conditions. A commercial fungicide which contains pyrazole derivate pyraclostrobin and methiram as active components is used as a standard. Potato dextrose agar (PDA) was prepared as a nutrient medium to test the growth of the fungus. After sterilization of the medium, it was cooled in a water bath at 60 °C when aqueous solutions of the complexes or a commercial fungicide in certain concentrations were added. For each complex and for a fungicide, five different concentrations were made: 0.12; 0.06; 0.03; 0.015; 0.0075 (%). 10 ml of a solution of each chemical complex and a fungicide in certain concentration was added in 100 ml of PDA. After homogenization of the solution of chemicals or fungicide with PDA, the medium was poured into 9cm Petri dishes. As a control, the medium with no amendments was used. After agar solidification, mycelial fragments 0.6 cm in diameter (taken from the edge of 10-day old fungal culture) were placed in the centre of Petri dishes. Inoculated Petri dishes were maintained in incubator at 25 °C. Ten days after the inoculation, mycelial growth of *Ph. viticola* was measured (in the control the fungus had covered 2/3 of the Petri dish).

Diameters of fungal mycelium as parameters of the growth inhibition effect were statistically analysed by analysis of variance, and mean values were compared using LSD test. If their difference was greater than the LSD test, they were considered statistically significant (Stankovic *et al*., 1990). The percentage of fungal inhibition in treatments compared to the control was also calculated for each chemical and for each concentration (Kaiser *et al*., 2005).

## RESULTS AND DISCUSSION

Results of elemental analysis (C, H and N) and molar conductivity is given in Table 1.

Based on the obtained results, it can be concluded that the synthesised complexes and a commercial fungicide have shown statistically significant inhibition of *Ph. viticola* in comparison to control. The growth of fungal mycelium (cm) depending on the applied chemical and concentration is given in Table 2.

Average percentage of inhibition achieved by different chemical compounds and certain concentration in comparison to control is presented in Table 3.

Table 1. Elemental analysis and molar conductivity results for obtained complexes compounds

| Formulas of the complex's compounds | C (%) Found ( ) Calculated | N (%) Found ( ) Calculated | H (%) Found ( ) Calculated | $\lambda_M$(DMF) $Scm^2\ mol^{-1}$ |
|---|---|---|---|---|
| $Cu(L\text{-}H)_2$ | (39.82) 40.06 | (10.36) 10.38 | (2.33) 2.24 | 5.95 |
| $[Cu(L\text{-}H)_2]\ Cl_2$ | (32.70) 33.20 | (9.11) 8.61 | (1.95) 1.86 | 33.10 |
| $[Cu(L\text{-}H)_2](NO_3)_2$ | (32.10) 32.54 | (12.15) 12.66 | (1.59) 1.82 | 41.00 |
| $Zn(L\text{-}H)_2$ | (40.02) 39,89 | (10.42) 10.34 | (2.40) 2.23 | 3.60 |
| $Ni(L\text{-}H)_2{\cdot}4H_2O$ | (35.97) 35.63 | (8.90) 9.23 | (2,84) 3.32 | 4.20 |

Table 2. The growth of fungal mycelium (cm) depending on the applied chemical and concentration

| **Concentrations %** | **Studied compound** | | | | | |
|---|---|---|---|---|---|---|
| | Commercial fungicide | $[Cu(L\text{-}H)_2]\ (NO_3)_2$ | $Cu(L\text{-}H)_2$ | $Zn(L\text{-}H)_2$ | $Ni(L\text{-}H)_2{\cdot}4H_2O$ | $[Cu(L\text{-}H)_2]\ Cl_2$ |
| **0.12** | 0.0 | 5.2 | 5.1 | 3.3 | 3.2 | 1.6 |
| **0.06** | 0.0 | 6.1 | 5.5 | 4.1 | 4.6 | 3.6 |
| **0.03** | 0.0 | 6.6 | 5.8 | 5.4 | 5.2 | 5.2 |
| **0.015** | 1.0 | 6.7 | 6.4 | 5.6 | 5.5 | 5.5 |
| **0.0075** | 1.1 | 6.8 | 6.7 | 6.5 | 5.6 | 6.0 |
| **Control** | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| **$LSD_{0,01}$** | 0.208 | 0.995 | 0.526 | 0.544 | 0.316 | 0.383 |

Compared to the complex compounds studied, a commercial fungicide expressed the best inhibitory effect. All applied concentrations of a commercial fungicide have shown statistically significant inhibition of *Ph. viticola* colony growth in relation to control.

The studied complex compounds had a lower inhibitory effect than a commercial fungicide but with obvious inhibition activity. $[Cu(L\text{-}H)_2]\ Cl_2$ complex expressed the best inhibitory effect at concentration of 0.12%.

Concerning $[Cu(L\text{-}H)_2]\ (NO_3)_2$ complex, concentrations of 0.12 and 0.06% exhibited inhibitory activity, while in the other three concentrations used, inhibition was not expressed at the level of a significant statistical difference in regard to control. $Cu(L\text{-}H)_2$ complex showed better inhibitory activity than the previous one since all five applied concentrations expressed inhibition effects that were statistically significant compared to control. The best inhibition achieved was at concentration of 0.12%. In relation to similar Cu (II) complexes examined in previous studies (Jaćimović *et al*., 2013; Jaćimović *et al*., 2017a; Jaćimović *et al*., 2017b), the tested Cu complexes in this research showed significantly higher inhibition of the growth of *Ph. viticola*. In previous studies, inhibition in relation to control at the highest concentration of the complexes (0.12%) was only 9.3%, 12.1% and 14.7%.

Table 3. Average % of inhibition achieved by different chemical compounds and certain concentration in comparison to control

| **Concentrations %** | **Studied compound** | | | | | |
|---|---|---|---|---|---|---|
| | Commercial fungicide | $[Cu(L-H)_2](NO_3)_2$ | $Cu(L-H)_2$ | $Zn(L-H)_2$ | $Ni(L-H)_2·4H_2O$ | $[Cu(L-H)_2]Cl_2$ |
| **0.12** | 100.0 | 30.7 | 32.0 | 56.0 | 57.3 | 78.7 |
| **0.06** | 100.0 | 18.7 | 26.7 | 45.3 | 38.7 | 52.0 |
| **0.03** | 100.0 | 12.0 | 22.7 | 28.0 | 30.7 | 30.7 |
| **0.015** | 86.7 | 10.7 | 14.7 | 25.3 | 26.7 | 26.7 |
| **0.0075** | 85.3 | 9.3 | 10.0 | 13.3 | 25.3 | 20.0 |

In comparison to $[Cu(L-H)_2]$ $(NO_3)_2$ and $Cu(L-H)_2$ complexes, $Zn(L-H)_2$ and $Ni(L-H)_2·4H_2O$ complexes expressed better inhibitory activity. Complex of formula $Zn(L-H)_2$ caused fungal inhibition growth at all concentrations, which differed statistically from the control, and between each individual concentration, except between the third and the fourth. Maximum inhibition was achieved by the first concentration and it was 56.0%. A similar percentage of inhibition was obtained also in the previous study, where Zn complex expressed an inhibition of 53.5% at the highest concentration (Jaćimović *et al*., 2013). In the testing of Ni(L-H)2·4H2O complex, the compound applied in all investigated concentrations expressed significant inhibition compared to control. The best results were obtained at 0.12 and 0.06% concentrations, while at the other three concentrations applied inhibitory effects were lower and relatively uniform. Compared with the inhibition achieved by the similar Ni complex previously examined (14.7%) by Jaćimović *et al*. (2017b), the percentage of inhibition in this study is almost 4 times higher and amounts to 57.3%.

The complex of formula $[Cu(L-H)_2]Cl_2$ achieved the best results from other studied complexes (excluding a commercial fungicide), and especially at concentration of 0.12% when it inhibited the growth of fungal mycelium at a level of 78.7%, which is the best result in this experiment compared to other synthesised complexes and concentrations and also in comparison to previously reported experiments.

## CONCLUSIONS

In the reaction on warm alcoholic solution (methanol or ethanol) of metal salts $Cu(OAc)_2$, $CuCl_2$, $Cu(NO_3)_2$, $Zn(OAc)_2$ and $Ni(OAc)_2$ and 4-Bromo-2-(1H-pyrazol-3-yl)phenol (HL) as ligand in molar ratio 1:2, tree new Cu(II) complexes, Zn(II) and Ni(II) complexes with formulas: $Cu(L\text{-}H)_2$, $[Cu(L\text{-}H)_2]Cl_2$, $[Cu(L\text{-}H)_2](NO_3)_2$, $Zn(L\text{-}H)_2$ and $Ni(L\text{-}H)_2 \cdot 4H_2O$ were obtained. Based on elemental analysis (C, H and N) and conductivity measurements, formulas of complex compounds were determined.

All complex compounds used in the experiment showed inhibitory activity against the growth of the phytopathogenic fungus *Phomopsis viticola*, causal agent of Phomopsis cane and leaf spot disease of grapevine. The best inhibition was achieved by the complex $[Cu(L\text{-}H)_2]$ $Cl_2$ at concentration of 0.12%. At this concentration the inhibition reached 78.7%. These results are of importance for the preparation of the pyrazole compound which will have satisfactory fungicidal activity against *Ph. viticola*, since it has shown the best results so far both in this experiment and in previous studies carried out by the same researchers.

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