Research | Egyptian Journal of Agricultural Research | Plant Protection

Efficacy of 2-(*p*-tolylamino)acetohydrazide and its Co(II), Ni(II) complexes on the shell of *Eobania vermiculata* under laboratory conditions

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Received: 31-07-2023 ; **Accepted:** 09-09-2023 ; **Published:** 23-09-2023 **DOI**: 10.21608/ejar.2023.226363.1422

ABSTRACT

Two new divalent metal constructions were synthesized upon chelation reactions between 2-(*p*-tolylamino)acetohydrazide ligand **(HL)** and Co(II), Ni(II) chloride salts. The structure of achieved compounds was identified by conducting a series of spectral and physicochemical techniques. A mixture of distilled water and DMSO was used for preparing four different concentrations of the tested compounds; (50, 100, 200 and 300 ppm), which were then used in treating *Eobania vermiculata* under laboratory conditions. The thin-layer film technique was utilized for performing the *in vitro* treatments. The sub-lethal concentrations; (LC_{25} and LC_{50}) values were estimated. The investigated chemical compounds displayed remarkable toxicological effects against the screened brown garden snails. *Eobania vermiculata* species showed the highest sensitivity level toward Ni(II) complex (LC_{50} = 53.55 ppm). However, the **(HL)** ligand displayed a higher toxicity against *Eobania vermiculata* than Co(II) complex (LC_{50} = 87.92 and 95.59 ppm, respectively). The investigated land snails were treated *in vitro* with the LC_{25} concentration of the obtained compounds for 7 days to evaluate their efficacy on the shell of those species. The level of some elements composing the snail shell; calcium, magnesium, phosphorus and potassium, was assessed. The obtained data emphasized that the tested compounds caused a considerable decrease in the level of estimated shell elements.

Keywords: Metal complexes, *Eobania vermiculata*, shell components, laboratory trials.

INTRODUCTION

Land snails have rapidly increased over the last few years and a lot of species have been recorded in many governorates of Egypt (Ghamry *et al.*, 1993; El-Deeb *et al.*, 1997; El-Masry, 1997; Ismail, 1997; Mahrous *et al.*, 2002; Arafa, 2006 and Abed, 2017). *Eobania vermiculata* species (Mollusca-Helicidae) are considered to be one of the most serious pests of many crops, vegetables, orchards and ornamental plants (Ismail, 1997; 2004; Bayoumi *et al,* 2023), resulting in heavy economic damage because of feeding on plant leaves, roots and fruits (Hussein *et al.*, 2019). Contamination of agricultural products by bodies, feces, or slime of snails leads to financial loss and deterioration of the quality (Ali, 2017). Locomotory activity cares only under particular physical conditions such as; temperature, photoperiod and air humidity, which can be considered as important determinants (Cameron, 1970; El-Kady *et al.,* 2023). Controlling of snail species using chemical methods is remaining the most effective route, especially for wide areas (De Ley *et al.*, 2020). Shell represents the first defense line in land snails that plays important role in protecting them from outside risks and hence impedes controlling them in fields. Moreover, the snails' shell covers their soft body and allows them to live under severe conditions of drought and heat (Crowell, 1977; Ahmed *et al.*, 2023). Hydrazide compounds have gained prominence because of their antifungal, antiplatelet, anti-inflammatory, antimalarial, analgesic, antimicrobial, antibacterial, anticancer, anticandidal, anti-tubercular, anticonvulsant, and antioxidant activities (Shelke *et al.*, 2011; Altıntop *et al.*, 2012; Yaul *et al.*, 2014; Netalkar *et al.*, 2014 and Xu *et al.*, 2017). The role of transition metals as micronutrients and co-factors of various metalloenzymes in living systems is promoting the rationale beyond the synthesis and screening of new transition metal complexes for their promising biological effects (Prajapati *et al.*, 2019). Based on the aforementioned facts, the main target of this study was to synthesize two new metal chelates derived from a hydrazide ligand and estimate their *in vitro* toxicity and efficacy on some shell elements of *Eobania vermiculata.*

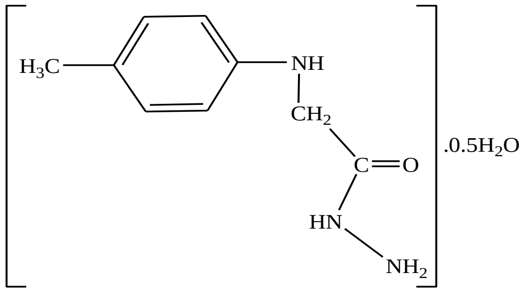
MATERIALS AND METHODS

Chemicals and Instruments:

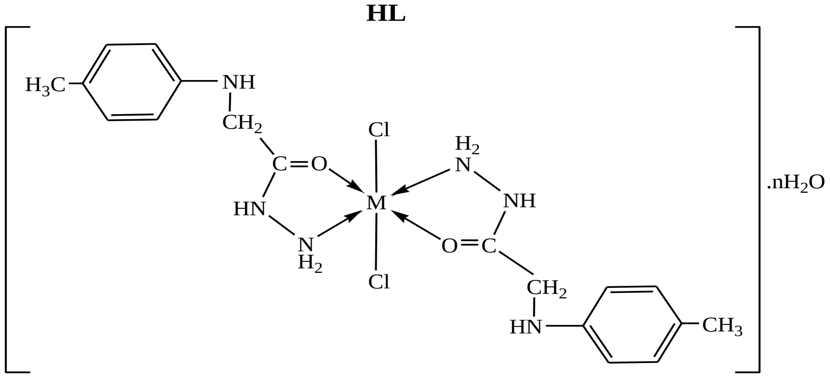
Hydrazine hydrate, ethyl 2-(*p*-toluidino)acetate, Co(II), Ni(II) chlorides as well as solvents and reagents were utilized without any further purifications, and were of analytical grade (Merk, Sigma Aldrich and BDH). Elemental analyses; (C, H, and N) were carried out at micro analytical center of Cairo University by using a Perkin Elmer-2400 elemental analyzer. FT-IR spectra were obtained by the Nicolet FT-IR spectrophotometer within range (4000–400 cm^{-1}).

Preparation of the (HL) ligand and its Co(II), Ni(II) complexes:

The hydrazide ligand; 2-(*p*-tolylamino)acetohydrazide **(HL)** was prepared as reported (Aminabhavi *et al*., 1987; Rollas *et al*., 2007). The newly synthesized metal complexes were synthesized by the same route. Hot ethanolic solutions (0.01 mol) of Co(II) and Ni(II) chloride salts were mixed with (0.01 mol) of the hydrazide ligand **(HL)** dissolved in hot ethanol with continuous stirring and reflux at 70ºC for 4 hrs. The gained solutions were evaporated to about 20% of their original volumes, filtered off, washed several times with ethanol and dried under vacuum over anhydrous $CaCl_2$. The suggested structure of prepared compounds is shown in Fig.1.



HL



M	n
Co	2
Ni	3

Fig. 1. Proposed structure for prepared compounds

Tested Land Snails:

Laboratory treatments were performed at Sers Ellyan Agricultural Research Station, Menoufia governorate. Adults of healthy *Eobania vermiculata* species having nearly the same shell diameters were collected manually from an untreated infested ornamental nursery at Sadat City, Menoufia governorate, Egypt in February, 2022. The collected brown garden snails were directly transferred to the laboratory inside plastic containers covered with muslin and kept at 27ºC and 65% R.H in small plastic boxes filled with a suitable layer of moist optimal soil. The snails were daily fed on fresh green lettuce leaves until needed, allowing them to be fully adapted to laboratory conditions for two weeks. Snails with approximately the same shell diameter were starved for 24 hrs before experiments.

Laboratory Tests:

Treatments were conducted by employing the thin layer film method (Mourad, 2014) and (Abbott, 1925) formula was utilized to assess the corrected mortality. Four different concentrations of the tested compounds; (50, 100, 200 and 300 ppm) were prepared using a mixture of distilled water and DMSO. Two ml of each tested concentration was spread on the surface of a Petri-dish and moved to ensure an equal spreading. Under room temperature, evaporation of the solvents then occurred, leaving a residual layer of the investigated compound. All experiments were performed through three replicates, everyone with ten snails, along 72 hrs. Dead snails were daily counted and removed, and a parallel control test was carried out utilizing distilled water and DMSO only.

Determination of Shell Elements:

Biochemical assessments were carried out at Reclamation and Development Laboratory for Desert Soil, Cairo University. After 7 days of treating adults of *Eobania vermiculata* with LC_{25} concentration of the tested compounds, the shell of the treated species was removed and well grinded to estimate the percentage of some elements composing it; (calcium, magnesium, phosphorous and potassium). Also, the level of some shell elements of untreated snails (control) was assayed. An acid digestion method for samples was run according to (Parkinson *et al.*, 1975). The percentage of Ca, Mg and K elements was determied by conducting (Christian *et al.*, 1970) method, utilizing Thermo Scientific iCE 3300, atomic absorption spectrometer, German. However, the level of phosphorous was determined by using a spectrophotometer at 660 nm; a phosphomolybdate complex was formed between phosphorous (which found in shell solution as phosphate) and molybdic acid. This complex undergoes reduction by the action of stannous chloride ($SnCl_2$) to a blue color which in turn can be measured according to (El-Merzabani *et al*., 1977) method. The environmental conditions; (temperature and R.H.) during biochemical analyses were 21.8ºC and 44.6%, respectively.

Statistical Analysis:

The SPSS statistical software (Ver. 21, SPSS Inc., Chicago, IL) was utilized to analyze the obtained data. Before analyses, the normality assumption of the data and homogeneity of variance among the different groups were investigated and data were found to hold the above two assumptions. Different parameters were monitored in treated and untreated species. The variance one-way ANOVA analysis, followed by Bonferroni post hoc test was performed to estimate the significant difference in means.

RESULTS

Construction of prepared compounds:

The most characteristic infrared spectral bands of the ligand and its metal complexes along with their assignments as well as elemental analyses data are displayed in Table 1 and shown in Fig. 2. The arbitrage between the IR spectrum of the hydrazide ligand **(HL)** and those of the target complexes concluded the neutral complexation mode of the ligand toward metal ions, through carbonyl oxygen and amino nitrogen atoms. The ligand stretching bands of NH_2 and NH groups appeared at (3353, 3303) and (3199, 3111 cm^{-1}), respectively (Adaji *et al*., 2022; Bala *et al*., 2022). Upon complexation with Co(II) and Ni(II) ions, those bands were subjected to changes in their position as well as their shape (Table 1). Moreover, the hydrazide ligand spectrum displayed a strong band at 1658 cm^{-1}, compatible with υ(C=O) (Ndiaye-Gueye *et al*., 2022; Ashma *et al*., 2022). After chelation with metal ions, the ligand υ(C=O) band undergone shift to (higher/lower) wavenumbers by (51/42 cm^{-1}).

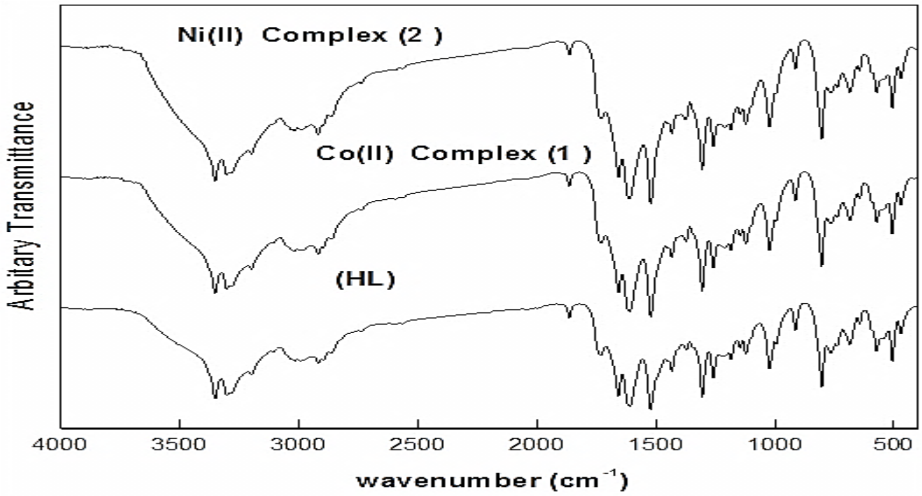


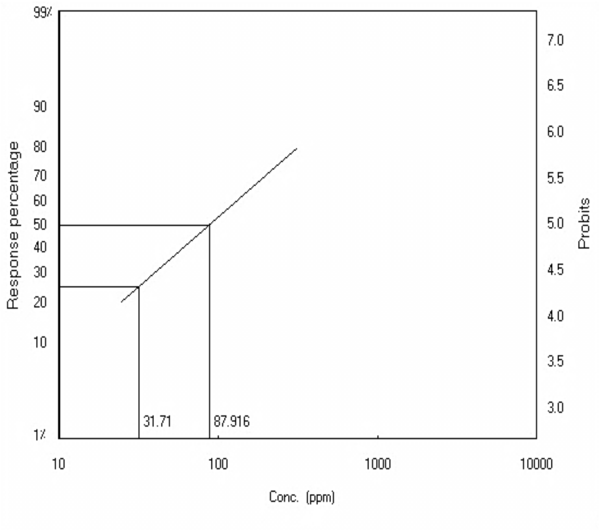
Fig. 2. IR spectra of prepared compounds

Table 1. Elemental analyses and IR spectra of the **(HL)** ligand and its metal complexes:

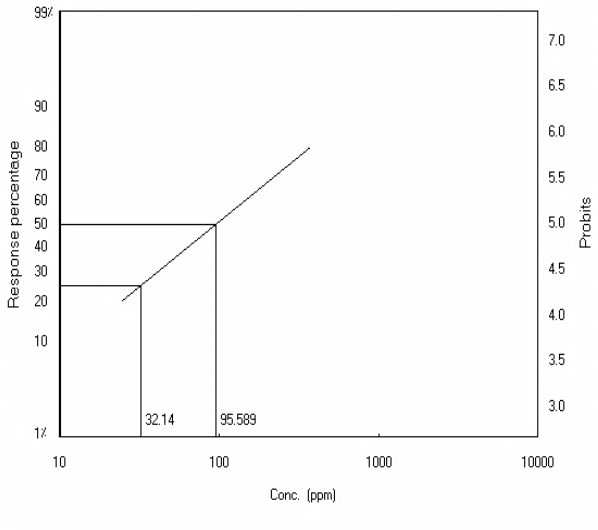
No.	Compounds	Elemental analyses Found/(Calcd.)%			IR Spectra		
		C	H	N	υ(NH_2)	υ(NH)	υ(C=O)
	$HL.0.5H_2O$	56.95 (57.42)	7.36 (7.50)	22.58 (22.32)	3353 3303	3199 3111	1658
1	$[Co(HL)_2Cl_2].2H_2O$	40.88 (41.23)	5.83 (5.77)	16.43 (16.03)	3398 3337	3279 3191	1709
2	$[Ni(HL)_2Cl_2].3H_2O$	40.17 (39.88)	5.60 (5.95)	15.11 (15.50)	3398 3267	3068 -	1616

In vitro Toxicity:

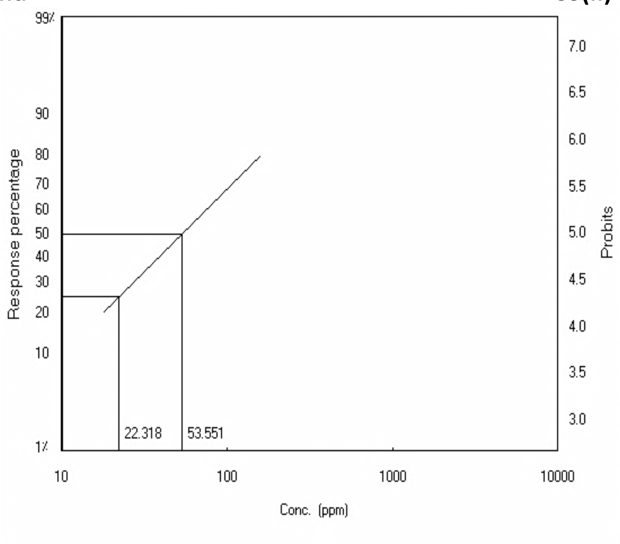
The laboratory trials of the 2-(*p*-tolylamino)acetohydrazide ligand **(HL)** and Co(II), Ni(II) chelates emphasized their significant toxicological efficacy (Table 2 and Fig. 3). The data authenticated that the mortality percentage elaveted as the the concentration of investigated chemical compounds increased. The obtained results demonstrated that the hydrazide ligand **(HL)** had (LC_{25} = 31.71 and LC_{50} = 87.92 ppm). Furthermore, the results shown in (Fig. 3) proved the promising high cytotoxicity of Co(II) and Ni(II) complexes **(1, 2)** (LC_{25} = 32.14, 22.32 and LC_{50} = 95.59, 53.55 ppm, respectively).



(HL) ligand



Co(II) Complex



Ni(II) Complex

Fig. 3. Toxicity of prepared compounds against *Eobania vermiculata*

Table 2. Results of laboratory trials of investigated chemicals on *E. vermiculata***:**

No.	Compounds	Concentrations (ppm)	Corrected Mortality (%)	LC_{25} (ppm)	LC_{50} (ppm)	Slope ± S.E.
	$HL.0.5H_2O$	50	33.33	31.71	87.92	1.523 ± 0.225
		100	57.45			
		200	69.21			
		300	78.82			
1	$[Co(HL)_2Cl_2].2H_2O$	50	32.09	32.14	95.59	1.425 ± 0.223
		100	55.28			
		200	66.87			
		300	75.11			
2	$[Ni(HL)_2Cl_2].3H_2O$	50	46.08	22.32	53.55	1.775 ± 0.245
		100	72.29			
		200	82.22			
		300	91.23			

Efficacy of Tested Compounds on some Shell Elements:

The results of the tested compounds on the shell of *Eobania vermiculata* are tabulated in (Table 3). The outcome data authenticated the comparatively significant effects of the investigated compounds on the shell of treated snails compared to control ones. The naked eye examination of treated snails clarified the weakness of their shell compared to control species. Also, a noticeable weight loss of treated snails was detected, emphasizing the decrease of some shell component levels, especially calcium element. The data showed that the percentage of calcium level was highly affected upon treatments with prepared compounds. Calcium content of treated snails was reduced to 84.75, 86.77 and 83.13% after treatment with the ligand **(HL)**, Co(II) and Ni(II) complexes, respectively, compared to 89.58% for control species. While, the percentage of magnesium, phosphorous and potassium elements of treated snails was slightly affected after treatments (Table 3). The phosphorous and potassium level of control snails (0.29, 0.18%) was decreased to (0.26, 0.15), (0.28, 0.13) and (0.27, 0.15%) after treatments with LC_{25} concentrations of the ligand **(HL)**, Co(II) and Ni(II) complexes, respectively.

Table 3. Percentage of some shell elements of *E. vermiculata*:

Compounds	Ca (%)	Mg (%)	P (%)	K (%)
Control	89.58	0.09	0.29	0.18
$HL.0.5H_2O$	84.75	0.07	0.26	0.15
$[Co(HL)_2Cl_2].2H_2O$	86.77	0.08	0.28	0.13
$[Ni(HL)_2Cl_2].3H_2O$	83.13	0.09	0.27	0.15

DISCUSSION

The relatively high toxicity of the **(HL)** ligand is assigned to the existence of nitrogen atoms in its chemical skeleton (El-Samanody *et al*., 2017a). Also, the presence of free amino group in the ligand structure is responsible for the initiation of carcinogenic process (Garrigós *et al.*, 2002).

The high toxicity of the tested metal constructions may be attributed to the binding effects between metal cations and enzymes (El-Samanody *et al.*, 2017b). The elevated toxic effects of cobalt chelate are ascribed to the activity of Co(II) cations towards ALP and ACP enzymes that keeps them in an unfavourable conformation after immobilizing them (Alnuaimi *et al*., 2012). Moreover, the enhanced toxicological efficacy of nickel construction is due to the interference with the metabolism of divalent iron, manganese, calcium, zinc, copper or magnesium metals, resulted in modification or suppression of the nickel cation toxicity. It is possible that the toxic impacts of Ni(II) cations resulted primarily from its ability to substitute other cations in enzymes and proteins or bind with the atoms of oxygen, nitrogen and sulfur of cellular components (enzymes and nucleic acids), which are then inhibited. Ni(II) is immunotoxic and can affect the activity of every individual cell type participating in the immunological response (Cempel *et al*., 2006).

The reduction of calcium level may be responsible for the weakness of the treated snail shell (Wilbur *et al*., 1955). Furthermore, the synthesized tested compounds may block the deposition of calcium by inhibiting the activity of some responsible enzymes. As concluded in a previous study by (Mobarak *et al.*, 2014), tannic acid decreased the ACP and ALP activities which were responsible for Ca participation in the shell of *Eobania vermiculata*. The disturbance of phosphorous and calcium content of the body may lead to pathological changes (Taylor *et al.*, 2009). Furthermore, terminals of the prejunctional nerve at several cholinergic synapses are influenced by the opposite and specific effect of Ca and Mg cations (Jenkinson, 1957). The decrease of potassium level in the treated *E. vermiculata* results in disturbance in some shell component (Ahmed *et al.*, 2023).

CONCLUSION

2-(*p*-tolylamino)acetohydrazide ligand **(HL)** and its cobalt, nickel complexes were prepared and their chemical structures were identified by some chemical and spectral techniques. The synthesized compounds were tested *in vitro* against *Eobania vermiculata* by using the thin layer film method. The obtained compounds showed a significant toxicological activity. The level of some shell elements was estimated after 7 days of treatments with the LC_{25} concentrations of tested compounds. The results clarified that the tested compounds displayed significant effects on the shell of treated species. So, these compounds may be used in controlling *E. vermiculata* after further studies to investigate their effects on the health of human and animals.

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تقييم فاعلية 2-(بارا توليل أمينو) أسيتوهيدرازايد و متراكباته مع الكوبلت والنيكل علي صدفة قوقع الحدائق البني تحت الظروف المعملية

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تم تحضير متراكبين معدنيين جديدين من تفاعلات التراكب بين عامل التراكب 2-(بارا توليل أمينو) أسيتوهيدرازايد و كلوريدات الكوبلت والنيكل ثنائية التكافؤ، وتم إثبات التركيب الكيميائي للمركبات المُحضرة بإستخدام بعض التحاليل الطيفية والطرق الفيزيوكيميائية. تم استخدام مخلوط من الماء المقطر و DMSO لتحضير 4 تركيزات مختلفة من المركبات المُحضرة والتي طُبقت معمليًا على قوقع الحدائق البني بطريقة التلامس وتم حساب قيم التركيزات المميته (LC_{50} و LC_{25}). أظهرت المعاملات وجود سُمية فعالة للمركبات المُختبرة ضد قوقع الحدائق البني. أكدت النتائج أن القواقع المُختبرة أكثر حساسية تجاه متراكب النيكل عن متراكب الكوبلت وعامل الترابط. تم معاملة قوقع الحدائق البني بتركيز (LC_{25}) من المركبات المُحضرة لمدة 7 أيام لدراسة تأثيرها علي الصدفة الخارجية للقواقع المُختبرة. تم تقييم مستوي بعض العناصر الكيميائية المكونة لصدفة القواقع المختبرة مثل نسبة الكالسيوم، الماغنيسيوم، الفوسفور و البوتاسيوم. أكدت النتائج التي تم الحصول عليها أن المركبات المُختبرة تسببت في انخفاض ملحوظ في مستوى العناصر المُقدرة.

الكلمات المفتاحية: متراكبات معدنية، قوقع الحدائق البني، مكونات الصدفة، التجارب المعملية.