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Laboratory and Field Toxicity Evaluation of some Methomyl Derivatives against *Eobania vermiculata* Infesting Citrus Trees

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Methomyl, Metal Chelates, Land Snails, *In vitro* and *In vivo* Tests.

ABSTRACT

Divalent Co, Ni, Cu, Zn and Cd chelates originating from methomyl (Lannate) were prepared, and their anti-molluscicidal activity was screened *in vitro* and *in vivo* against *Eobania vermiculata* using contact and spraying techniques, respectively. The results proved that each tested complex had varying degrees of sensitivity to the tested land snail species, with significant anti-molluscicidal activity. The laboratory test data demonstrated that $CdCl_2$ complex adopted the highest toxicity toward *E. vermiculata* species (LC_{50} = 82.72 ppm). *Eobania vermiculata* was more susceptible to $CoCl_2$ complex (LC_{50} = 100.09 ppm) than $CuBr_2$, $CuCl_2$, $NiCl_2$ and $Cu(ClO_4)_2$ complexes (LC_{50} = 142.06, 147.61, 148.62 and 149.16 ppm, respectively). Whereas, $ZnCl_2$ complex exhibited the lowest toxicity among the screened coordinated constructions (LC_{50} = 181.69 ppm). Field trials of tested compounds were applied by spraying technique and the population reduction percentage of *Eobania vermiculata* was evaluated. The field experiments showed that complexes (1, 3 and 4) achieved high population reduction percentages of *E. vermiculata* (94.22, 94.19 and 92.86%, respectively), while, complex **(7)** had the lowest toxic efficacy; it displayed only 3.27% reduction of *Eobania vermiculata* population.

INTRODUCTION

Terrestrial snails resulted in many dangerous effects on a lot of field crops, orchards trees and ornamentals (El-Okda 1979; De Ley *et al*., 2020), they are considered one of the most severe agriculture pests that eradicate vegetables, seeds, fruit trees, roots, tubers and ornamental plants (Shahawy 2019; El-Deeb *et al*., 1999; El-Okda 1984; Ismail 1997 and, Godan 1983). The mucous secretion produced by land snails' movement caused unfavorable taste and aroma which in turn makes obviation for animals and humans to feed on infected plants (El-Deeb *et al.*, 1999). Moreover, snails may also cause bacterial infection by viruses and fungi owing to their feeding habits through scratching plants (Lindqvist *et al*., 2006). *E. vermiculata* species are usually found in agricultural fields, gardens, hedgerows, vineyards and dry vegetation (Puizina *et al*., 2013). The terrestrial snail; *E. vermiculata* lives in citrus orchards and it may protect itself under stones, plants leaves or clay (Mohamed and Ali, 2013), who recorded the highest *Eobania vermiculata* population in the spring and summer seasons, these results showed a dissimilarity with Yaakoub *et al*., (2016) and Ismail *et al*.,

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(2017), they demonstrated the highest population was in summer than spring, this is due to environmental differences among countries. Ismail *et al.,* (2003) determined the damage caused by *E. vermiculata* snails on citrus leaves (navel and baladi orange). The snails prefer navel orange leaves in their feeding; where damage percentage was 35.80% in the bottom level, while it reached 15.88% only in Baladi leaves for the same level. In the case of heavy infestation, snails were noticed in all parts of the tree.

Control of land snails by chemical pesticides is reported as one of the most effective routes (Radwan *et al*., 2008). One of these chemical pesticides is the carbamates group (Abdel-Gawad *et al*., 2004). Methomyl is a monomethylcarbamate molluscicide that is used for controlling a number of pests that attack food crops, cotton and tobacco (Kidd and James, 1991). Low concentrations of methomyl and methiocarb molluscicides were used to control *E. vermiculata* snails and showed a high efficacy (Hamed *et al*., 2007). In this study, a series of divalent Lannate metal constructions were prepared. *In vitro* and *in vivo* anti-molluscicidal properties of the afforded coordinated constructions were screened.

MATERIALS AND METHODS

Used Chemicals:

Methomyl (Lannate 90%), chloride salts of divalent (cobalt, nickel, copper, zinc and cadmium), copper bromide, copper perchlorate salts and employed solvents and reagents were purchased from (Merck and Sigma-Aldrich companies) and commercial sources. The acquired purity of (Lannate) was achieved via recrystallization and then compared with spectral data of standard samples. The target metal complexes were prepared and identified according to the published method (Kaur *et al*., 2017).

Rearing of Tested Snails:

The obtained Lannate metal chelates were examined for their ability to kill the land snail; *Eobania vermiculata*. The herbivorous specimens were possessed throughout the spring and autumn from farms and untreated nursery plants in Sadat City, Menoufia governorate, Egypt. Land snails were collected and then taken right to the laboratory in muslin sacks. They were housed for two weeks prior to the studies in aerated plastic cages filled with moist ideal soil to acclimate and fed with green fresh lettuce leaves. Adults of *E. vermiculata* snails having roughly equal size were utilized in the experiments after removing any dead snails.

***In vitro* Investigations:**

The laboratory experiments were conducted at Sers Ellyan Agricultural Research Station. A contact technique was conducted to perform the treatments (Mourad, 2014). Dimethylformamide (DMF) solvent was employed in synthesizing metal construction stock solutions. Four concentrations of the tested metal complexes; (50, 100, 200 and 300 ppm) were prepared and a parallel control test was run. Three replicates of each experiment, each with ten snails, were run along 72 h. The corrected mortality was calculated according to (Abbott, 1925):

Corrected Mortality (%) = $\frac{Treatment\ mortality - Control\ mortality}{100 - Control\ mortality} * 100$

***In Vivo* Treatments:**

The synthesized cobalt, copper, and cadmium chloro complexes along with the copper bromide complex were screened in a field on brown garden snails at Citrus orchard (Orange trees), located in the district of Tala, Menoufia governorate. The area of this experiment was divided into plots, each with 4 trees. Three trees with 1m^2 surroundings represented three replicates for each chemical complex and one tree represented the control, the tested complexes were applied as a spray on orange trees using a hand sprayer (2 Liters for each). The application was started after 3 days of irrigation and was conducted every 7 days from the first application. Snail population reduction before and after (7, 14 and 21 days) of experiments was the base of determining the efficacy of each compound. The percentage of mortality was estimated depending on the (Henderson *et al.*, 1955) formula.

Analysis of Statistics:

The sub-lethal concentrations along with their slope and fiducial limits of each experiment were evaluated using (Finney, 1971). The achieved results were estimated as (Mean ± S.E.). The variance analysis (ANOVA) was then conducted. To test for significance, the LSD method was employed for the comparison of the means at a probability of 0.05 (Steel *et al.*, 1981).

RESULTS AND DISCUSSION

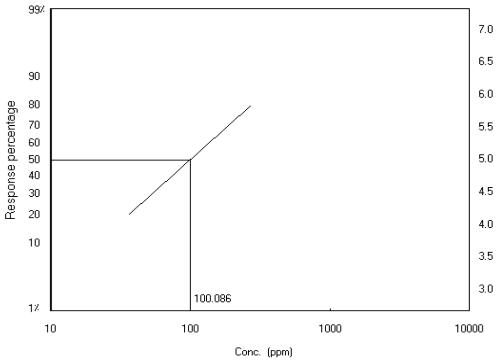
Laboratory Experiments:

The *In vitro* LC_{50} data of the tested compounds are shown in Figure (1) and represented in Table (1). The high toxicity of Lannate (methomyl) is attributed to the presence of free nitrogen, oxygen and sulfur atoms in its skeleton. The elevated toxic influence of examined chelates is attributed to the joining between metallic cations and enzymes (El-Samanody *et al.*, 2017a). The data shown in (Fig. 1) emphasized the comparatively elevated copper chelates **(**3-5**)** toxic impact ascribed to the presence of copper cations with high toxicological influence (El-Samanody *et al.*, 2017b). These cations have a strong tendency towards free thiol groups (cysteine) in proteins, causing ALP enzyme inactivation. Furthermore, competition may occur between Cu(II) cations with Mg(II) and Zn(II) cations on binding sides in enzymes which indeed causes distortion for active centers of these enzymes (Alnuaimi *et al.*, 2012; El-Samanody *et al.*, 2017b). The reason for the high toxicity of Co(II) chelate (100.09 ppm) may be arisen from the activity of cobalt ions towards (ACP and ALP enzymes), causing immobilization for these enzymes and keeping them in an unfavorable conformation (Alnuaimi *et al.*, 2012). Ni(II) complex has an LC_{50} value of 148.62 ppm. This relatively high cytotoxicity may be due to the interference with the metabolism of iron, manganese, calcium, zinc, copper, or magnesium essential metals, which leads to suppression or modification of Ni(II) cations toxicity. Nickel ions are immunotoxic and the activity of all cell types participating in the immunological response is highly affected by Ni(II) cations (Cempel *et al*., 2006).

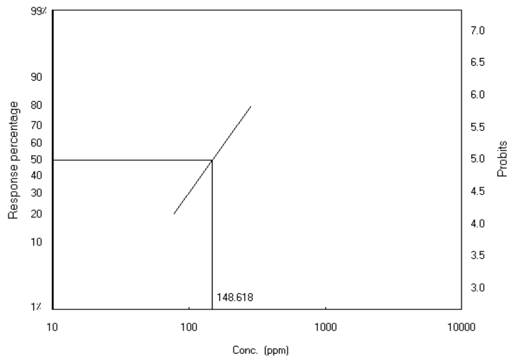
It is probable that the nickel (II) toxic impacts resulted from its ability to replace other cations in proteins and enzymes, or binding with sulfur, oxygen and nitrogen atoms of cellular components, causing its inhibition (Cempel *et al*., 2006). Cd(II) complex **(7)** exhibits the highest cytotoxic effect among tested compounds, while, Zn(II) complex **(6)** has the lowest one (Fig. 1). The sensitivity of *E. vermiculata* towards cadmium chelate is ascribed to its bonding with enzyme's cation binding centers (Alnuaimi *et al*., 2012). When cadmium ions combine with a protein like metallothionein, their concentration is increased by 3,000 times. Before cysteine-metallothionein reaches the kidney, where it accumulates in the renal tissue producing nephrotoxicity, it causes hepatotoxicity. Cadmium can lead to an iron deficiency through its interactions with the ligands cysteine, glutamate, histidine and aspartate. Due to the fact that cadmium and zinc cations have the same valence, cadmium can substitute zinc in metallothionein and prevent its working as a cell's free radical scavenger (Jaishanker *et al*., 2014).

Table 1. *In vitro* treatment results of investigated chemicals against *E. vermiculata*

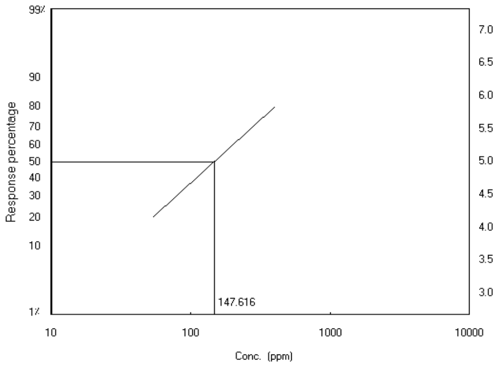
No.	Complexes	Concentrations (ppm)	Corrected Mortality %	LC_{50} (ppm)	Slope ± S.E.
1	$CoCl_2$–Methomyl complex	50	36.40	100.09	1.504 ± 0.224
		100	43.00		
		200	68.56		
		300	78.05		
2	$NiCl_2$–Methomyl complex	50	12.67	148.62	2.516 ± 0.251
		100	28.75		
		200	69.89		
		300	74.33		
3	$CuCl_2$–Methomyl complex	50	15.85	147.61	2.201 ± 0.241
		100	32.88		
		200	64.27		
		300	73.99		
4	$CuBr_2$–Methomyl complex	50	11.32	142.06	2.650 ± 0.255
		100	33.15		
		200	69.21		
		300	78.16		
5	$Cu(ClO_4)_2$–Methomyl complex	50	11.11	149.16	2.490 ± 0.251
		100	33.58		
		200	65.38		
		300	75.28		
6	$ZnCl_2$–Methomyl complex	50	11.11	181.69	2.401 ± 0.256
		100	22.80		
		200	52.76		
		300	72.39		
7	$CdCl_2$–Methomyl complex	50	29.10	82.72	1.839 ± 0.233
		100	64.29		
		200	77.22		
		300	81.15		



Chelate (1)



Chelate (2)



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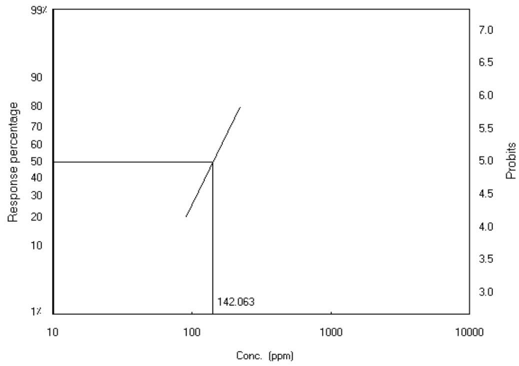

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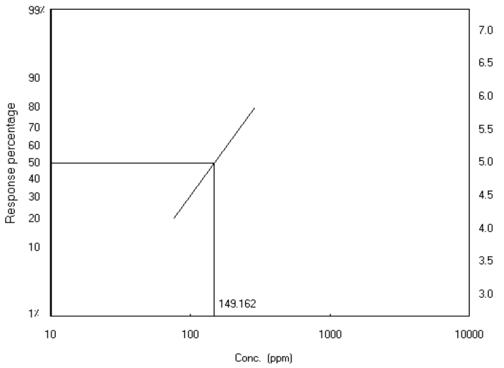
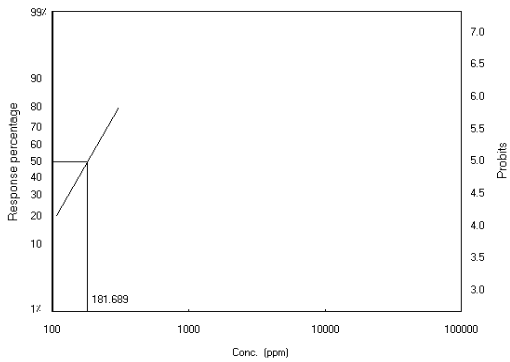
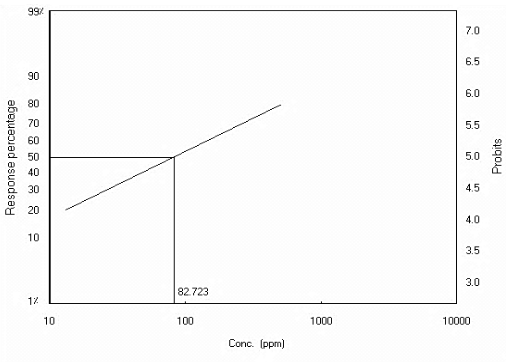


Chelate (5)



Chelate (6)



Chelate (7)

Fig. 1. Estimated *In vitro* LC_{50} values of target chelates **(1-7)**

Field Trials:

Results of field trials of the investigated chemicals on *E. vermiculata* are tabulated in Table (2). Data authenticated that complex (1, 3 and 4) displayed the highest *E. vermiculata* population reduction (94.22, 94.19 and 92.86%, respectively), while, complex **(7)** had the lowest toxic efficacy; it displayed only a 3.27% reduction of *Eobania vermiculata* population. The obtained results concluded that similar effects were observed by Soha Abdalla. M. (2008) revealed that methomyl compounds caused a severe decrease in enzyme activity compared with control. Also, methomyl insecticide low concentrations can reduce AChE (acetylcholinesterase), provoking the accumulation of excess acetylcholine at neuromuscular junctions (Xuereb *et al*., 2009; Laguerre *et al*., 2009; Rakhi *et al*., 2013 and, Varo *et al*., 2008).

It is concluded that the effect of most tested compounds under field conditions was nearly similar on *E. vermiculata*. The obtained field trial data of cadmium complex **(7)** are contrary to those achieved by lab experiments. This result may be related to the weather conditions and surrounding factors such as; temperature, humidity, light……etc., that induce degradation and resulted in inhibiting its toxicological effects.

Table 2. Percentage of population reduction of *E. vermiculata* after field trials

No.	Complexes	Population Reduction (%)		
		After 7 days	After 14 days	After 21 days
1	$CoCl_2$–Methomyl complex	61.24	81.83	94.22
3	$CuCl_2$–Methomyl complex	70.09	85.12	94.19
4	$CuBr_2$–Methomyl complex	71.79	86.78	92.86
7	$CdCl_2$–Methomyl complex	2.53	2.89	3.27

The concluded results gained from this study assert that screened chemicals possess a considerable molluscicidal activity against *Eobania vermiculata*. So, these complexes may be helpful to control land snail species. It is necessary to perform further studies on these compounds to achieve their most probable action mode against snails.

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ARABIC SUMMARY

تقييم السُمية المعملية والحقلية لبعض المتراكبات المعدنية المشتقة من مبيد اللانيت على قوقع الحدائق البني الذي يصيب أشجار الموالح

عصام محمد عمارة(1)، ماهر عبد العزيز الصواف(1)، رشا فؤاد خليفة(2)

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تم تحضير مجموعة من متراكبات الكوبلت، النيكل، النحاس، الزنك والكادميوم المشتقة من مبيد اللانيت. تم تقييم فاعلية المتراكبات المُحضرة على قوقع الحدائق البني معمليًا وحقليًا بطريقة الملامسة وطريقة الرش على التوالي . أثبتت النتائج أن كل متراكب تم اختباره له درجات متفاوتة من الحساسية مع نشاط كبير ضد القواقع المُختبرة .أظهرت بيانات الاختبارات المعملية أن متراكب الكادميوم الثنائي كان الأكثر سُمية تجاه القواقع المُختبرة. كما أكدت التجارب الحقلية أن المتراكبات المُحضرة من (كلوريد الكوبلت، كلوريد النحاس، بروميد النحاس) حققت نسبًا عالية في تقليل أعداد قوقع الحدائق البني (94.22 و94.19 و92.86 ٪ على التوالي)، بينما كان متراكب الكادميوم هو الأقل فعالية حيث قلل أعداد القوقع بنسبة 3.27 ٪ فقط.