وزارة الزراعة واستصلاح الأراضي
Ministry of Agriculture and Land Reclamation
مركز البحوث الزراعية
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute

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PLANT PROTECTION RESEARCH INSTITUTE
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Biochemical impacts and toxicity of Bis-(1,2-diphenyl-2-(p-tolylimino)-ethanone and its metal complexes against *Monacha obstructa* and *Eobania vermiculata* (Gastropoda: Hygromiidae: Helicidae)

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ARTICLE INFO

Article History
Received:16 /4 /2023
Accepted: 25/6/2023

Keywords

Schiff base, metal complexes, biochemical effects and land snails.

Abstract

The toxic effects of the Schiff base ligand **(L)**; bis-(1,2-diphenyl-2-(p-tolylimino) ethanone and its Co (II), Ni (II) and Cu (II) chloro complexes along with diaminodiphenylmethane (MDA) were screened against *Monacha obstructa* (Pfeiffer) (Gastropoda :Hygromiidae) and *Eobania vermiculata* Müller (Gastropoda: Helicidae) under laboratory conditions. A thin layer film technique was employed to assess the median lethal concentration for the investigated compounds. Five concentrations of the screened compounds were prepared using distilled water and DMSO. The obtained results emphasized that *E. vermiculata* was comparatively less susceptible to the Schiff base ligand, MDA and complexes (**1-3**) than *M. obstructa*. There were different sensitivity levels between the two tested snail species according to the type of tested compounds. The biochemical impact of the Schiff base ligand and its Cu (II) complex on the activity of alanine amino transaminase (ALT), aspartate amino transaminase (AST) and total proteins (TP) of *E. vermiculata* was reported. The data demonstrated that treatment with the ligand decreased the activities of ALT, AST and TP with mean values (4.67 ± 0.42), (67 ± 3.86) and (0.98 ± 0.14) less than control, respectively, whereas, ALT, AST activities and TP were increased after treated with complex (**3**) with mean values (7.44 ± 0.31), (16.34 ± 1.06) and (0.51 ± 0.08) more than the control, respectively.

Introduction

Land snails are molluscs, a group of invertebrate animals with soft unsegmented bodies that are enclosed in calcareous shells (Barker, 2001; Larramendy and Soloneski, 2012 and South, 1992).

Two common land snails, *Monacha obstructa* (Pfeiffer) (Gastropoda :Hygromiidae) and *Eobania vermiculata* Müller (Gastropoda: Helicidae) are important crop pests that cause considerable damage in agriculture, especially in areas where they find the conditions necessary for rapid multiplication. Damage caused by snails depends not only on their activity and population density, but also on their feeding habits, which differ from one species to another. Damage involving considerable financial loss is inflicted on cereal, vegetables, clover as well as other agricultural and field crops.

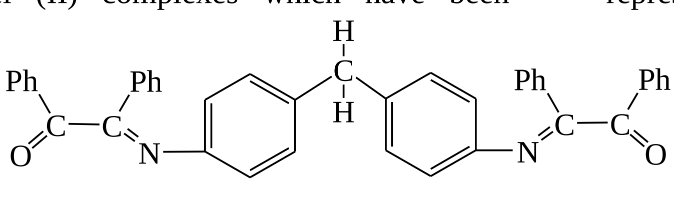
The land snails feed on leaves, roots, tubers, and ornamental plants (El-Okda, 1981). In addition, during movement, snails cause an undesirable smell which prevents men and animals from feeding on these contaminated plants (El-Okda, 1984). The chemistry of Schiff base ligands and their metal complexes is very important due to the wide range of applications as antibacterial, antifungal, anticancer and catalytic properties (Dhahagani *et al*., 2014; Jayabalakrishnan and Karvembu, 2002 and Balasubramanian *et al*., 2007). In addition, Schiff base complexes have many applications in biological and industrial systems (Uddin *et al.*, 2015). The Schiff base complexes can serve as models for some biomolecules, such as Schiff base nickel (II) complexes which have been regarded as models for enzymes such as urease (Asadi *et al*., 2013 and Carlsson *et al*., 2004).

In the present study, reported on the toxicity and some biochemical effects of the Schiff base ligand and its complexes against *E. vermiculata* and *M. obstructa* under laboratory conditions.

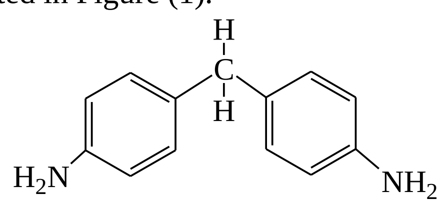
Materials and methods

1. Experimental:

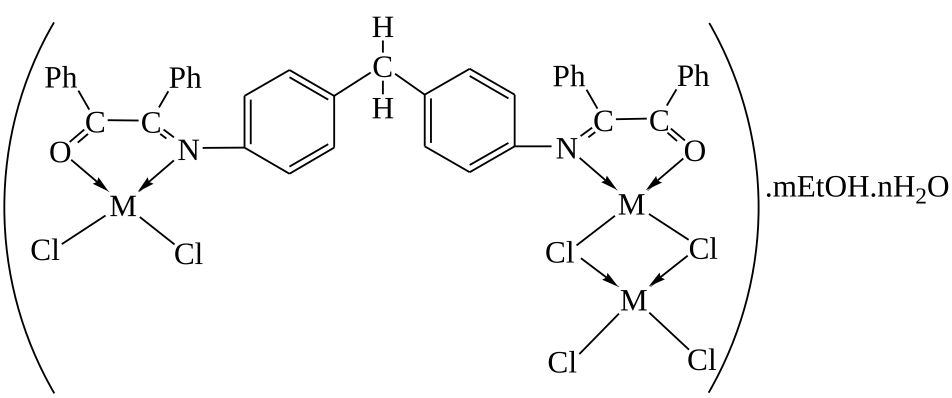
Benzil and diaminodiphenylmethane (MDA), metal chloride salts, reagents and solvents were of analytical grade (Merck, Sigma and Aldrich) and purchased from commercial suppliers. The Schiff base ligand (L) along with its metal complexes were prepared and identified as reported in the literature (Emam *et al*., 2017). The chemical structure of the tested compounds is represented in Figure (1).



L



MDA



Complex	m	n
1	0.5	7.5
2	0	11
3	0.5	5.25

Figure (1): Structure the Schiff base ligand (L), MDA and its complexes.

3. Laboratory tests:

The thin layer film technique was used to estimate the median lethal concentration (LC_{50}) of the investigated compounds according to Ascher and Mirian (1981) (El Gohary and Genena, 2011 and Mourad, 2014). Five concentrations of the tested compounds were prepared: (50, 150, 250, 350, 450 ppm) using distilled water and DMSO. Two mL of each concentration were deposited and distributed on the bottom of a petri dish by moving the dish gently in circles. Solvents were evaporated under room conditions leaving a thin layer film of the applied concentration of the tested compounds. Five healthy adult snails of the tested species were placed and exposed to the candidate concentration of the tested compound for 72 hrs. A parallel control test was carried out using water and DMSO. Dead snails were counted and removed from the boxes every 24 hrs. Probit analysis was used to compute LC_{50} values (Finney, 1971). Data were calculated as Mean±SD and analyzed using the Analysis of Variance Technique (ANOVA) followed by Least Significant Differences (LSD). The corrected mortality was calculated according to (Abbott, 1925):

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

4. Biochemical tests on *Eobania vermiculata*:

The experiments were carried out under laboratory conditions 24±1ºC and 57±1 RH. The treated snails were transferred from stock culture to plastic cups filled with moist soil and covered with muslin cloth. Biochemical studies were made after three days of treatment. After that, shells of the tested snails were removed by making a cut around the whorls in a continuous manner. Snail tissues were dissected out and all tissues of treatments were homogenized in distilled water and then centrifuged at 8000 rpm for 15 minutes at 5ºC in a refrigerated centrifuge. The supernatants were kept in a freezer till used to determine the activities of alanine amino transaminase (ALT), aspartate amino transaminase (AST) and total proteins (TP). The activities of ALT, AST and TP were determined (Young, 1995 and 2001).

Results and discussion

1. Laboratory experiments:

The obtained data (Table 1) authenticated that *E. vermiculata* and *M. obstructa* species were more susceptible to MDA than the free Schiff base ligand. The toxicity of the free ligand decreases relative to the two land snails upon complexation. The present results showed that *E. vermiculata* was comparatively less susceptible to the Schiff base ligand (L), MDA and complexes (1-3) than *M. obstructa*. Reviewing the above mentioned results, it is obvious that there are different susceptibility levels between the two tested snail species according to the type of tested compound. These differences in the sensitivity levels may be due to the physiological state of the snail which changes from one species to another (Godan, 1983 and El Gohary and Genena, 2011). It is noticeable that MDA has a higher toxicity than the free Schiff base ligand against the two land snail species. This may be attributed to the presence of free amino groups in the former which can react in cells leading to the initiation of carcinogenic process (Garrigós *et al*., 2002). The relatively high toxicity of the Schiff base ligand is ascribed to the free oxygen and nitrogen atom located in its chemical formula. The three tested metal complexes displayed considerable toxicity against the two land snail species, ascribed to the binding between metal cations and enzymes (El-Samanody *et al.*, 2017a). The results displayed in (Figure 2) demonstrated the relatively high toxicity of copper chelate which was attributed to the high toxic influence of copper cations (El-Samanody *et al.,* 2017b). These cations inactivate ALP enzyme because of its strong tendency towards free thiol groups (cysteine) in proteins. Moreover, competition occurred by Cu (II) cations for binding with Mg(II) and Zn (II) sites in enzymes, which distort the active centers of these enzymes as a result (El-Samanody *et al.*, 2017b and Alnuaimi *et al.*, 2012). The activity of cobalt ions to ALP and ACP enzymes may be the reason for the high toxicity of the Co (II) chelate (Alnuaimi *et al*., 2012). Co (II) ions immobilize ALP and ACP enzymes and keep them in an unfavorable conformation. Ni (II) complex has a relatively high cytotoxicity, attributed to its interference with the metabolism of divalent iron, manganese, calcium, zinc, copper, and magnesium metals, leading to suppression or modification of the toxic effects of Ni (II) cations. It is probable that the toxic impacts of nickel result from its capability to substitute other cations in enzymes and proteins or bind with sulfur, oxygen, and nitrogen atoms of cellular components (enzymes and nucleic acids), which are then inhibited (Cempel and Nikel, 2006). It has been demonstrated that nickel is immunotoxic, affecting the activity of every individual cell type involved in the immunological response (Cempel and Nikel, 2006).

Table (1): LC_{50} values of MDA, Schiff base ligand (L) and prepared metal complexes against the two land snails.

No.	Compound	LC_{50} (ppm)	
		Eobania vermiculata	***Monacha obstructa***
	L	177.83	144.88
	MDA	62.73	49.58
1	$[Co_3(L)Cl_6].0.5EtOH.7.5H_2O$	432.15	423.12
2	$[Ni_3(L)Cl_6].11H_2O$	444.72	434.16
3	$[Cu_3(L)Cl_6].0.5EtOH.5.25H_2O$	281.83	261.87

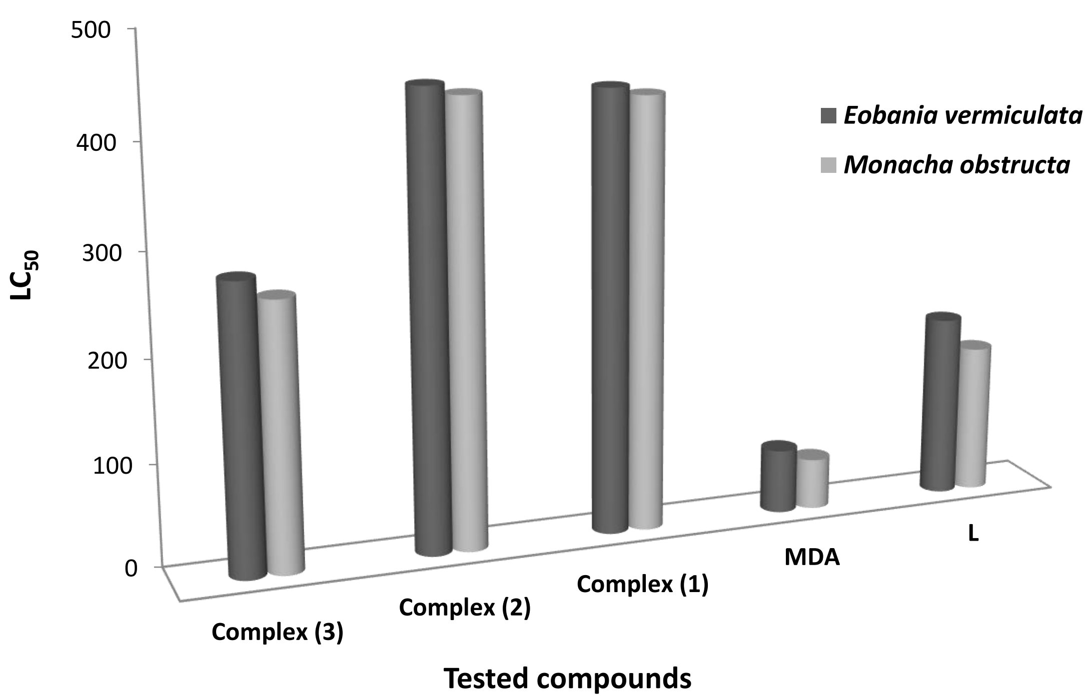


Figure (2): LC_{50} values of the tested compounds against the two land snail species.

2. Biochemical studies:

The results of biochemical tests of the Schiff base ligand (L) and Cu(II) complex (3) on *E. vermiculata* (Figure 3), as model examples, reveal that, treatment with the ligand decreased the activities of ALT, AST and TP with mean values (4.67 ± 0.42), (67 ± 3.86) and (0.98 ± 0.14) less than control, respectively, whereas, ALT, AST activities and TP were increased after treated with complex (3) with mean values (7.44 ± 0.31), (16.34 ± 1.06) and (0.51 ± 0.08) more than control, respectively. These significant changes in AST and ALT activities in *Eobania vermiculata* pointed out the functional disorder of the liver (Choudhary *et al*., 2003; Kalender *et al*., 2005; Kammon *et al*., 2010 and Arfat *et al*., 2014). However, it is conceivable that the tested compounds might be interacting primarily with the liver and muscle tissue cell membranes. These results in structure damage and changes in enzyme leakage and in metabolism of the constituents. So, tested compounds may pressurize this enzyme into plasma because of enzyme leakage breakdown. It is known that these enzymes are principally localized in the cytoplasm, and they will be secreted into the blood after hepatocellular injury, resulting in an increase in their levels in the serum. Consequently, this study may suggest that any damage in hepatopancreatic cells of land snails may result in alteration in the serum (Hemolymph) AST and ALT levels.

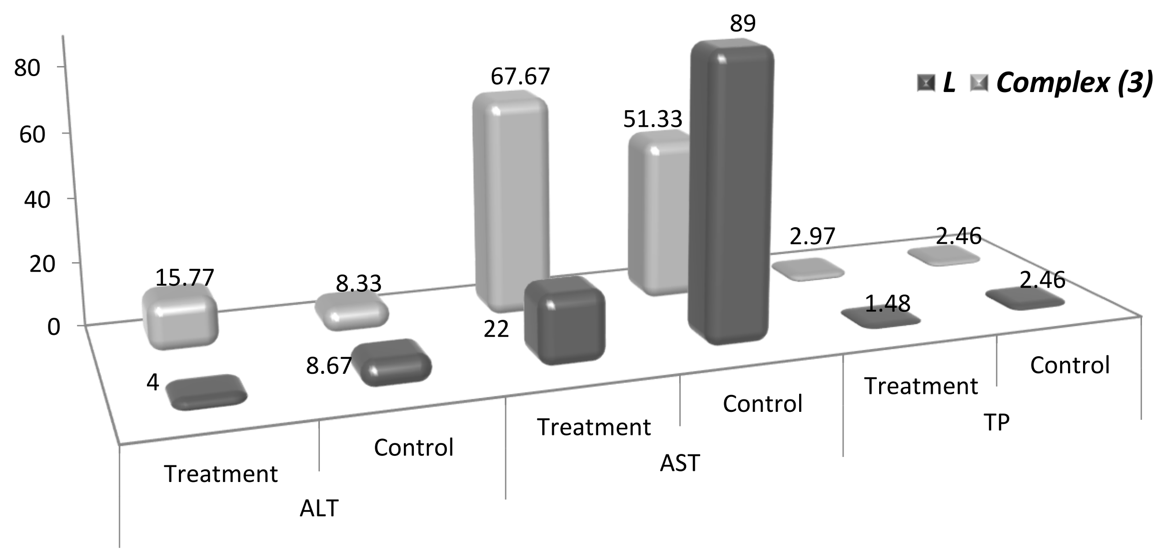


Figure (3): ALT, AST and TP activities of *Eobania vermiculata* after 72 hrs. treatments with the Schiff base ligand (L) and complex (3).

The data presented in this study reveal that the tested chemical compounds caused an alternation in some biochemical targets which could lead to serious metabolic and cellular damage in *E. vermiculata* and *M. obstructa* compared to other chemical traditional pesticides or molluscicides (Mahal *et al*., 2015). Thus, these compounds may be helpful to control land snails. Further studies are still needed to provide the most probable mode of action of these compounds on land snails.

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