EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 14(1):283-293(2022)
Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

The Toxic Effect of Some Newly Constructed Diaminodiphenylmethane Derivatives Applied *In Vitro* and *In Vivo* Trials against *Theba Pisana* in Relation to Mucus Secretion

Esam M. Emara[1*], Maher A. El-Sawaf[1] and Rasha F. Khalifa[2]
[1] Harmful Animals Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
[2] Econ. Entomology and Agric. Zoology Deptr., Faculty of Agric., Menoufia University.
[*] Corresponding author: esamemara@ymail.com

ARTICLE INFO
Article History
Received: 8/4/2022
Accepted: 28/6/2022
Available:30/6/2022

Keywords:
MDA derivatives, *Theba pisana*, Mucus secretion, *In vitro* assessment, *In vivo* evaluation.

ABSTRACT

The condensation reaction between diaminodiphenylmethane (MDA) and methomyl afforded the HL ligand. Cu (II) and Zn (II) chloro complexes of this ligand were prepared and their structures were elucidated by using some analytical and spectral methods. The thin-layer film method was used to assess the toxicity of the obtained compounds against *Theba pisana* species under laboratory conditions. The obtained data showed a remarkable toxicological activity of the achieved compounds. The tested compounds caused alternations in the quantity of the mucus secretions of the tested species after treatments leading to snail mortality. The achieved lab data authenticated the elevated toxicological impact of Zn (II) chelate on *Theba pisana* species (LC_{50} = 52.97 ppm). The white garden snail showed a considerable susceptibility to the HL ligand and Cu (II) complex (LC_{50} = 56.89 and 85.53 ppm, respectively). Field trials were conducted by spraying method and the population reduction percentage of the white garden snail was assessed. The obtained field trial results were compatible with those of laboratory tests. The tested compounds displayed significant reduction percentages of *Theba pisana* population after 7, 14, and 21 days of treatments. The HL ligand, Cu (II) and Zn(II) complexes recorded 94.47, 95.58 and 96.04%, respectively, as reduction percentages in *Theba pisana* population after 21 days of treatments under field conditions.

INTRODUCTION

The terrestrial gastropod mollusks (as snails and slugs) are serious pests attacking the vegetation including; vegetables, horticultural plants, and field crops in most areas of Egypt. The marketing of infected vegetables and fruits is significantly reduced (Glen *et al.*, 2000; El-Okda, 1980). Also, (Eshra, 2013) recorded that *Theba pisana* in Egyptian localities attacked various ornamental plants. The white garden snail; Theba *pisana* is one of the most serious land snails in Egypt, these snails have a destructive effect on an unusually broad range of plants, it makes irregular holes with smooth edges primarily on leaves or on the margins of leaves and on flowers, vegetables to citrus species and feed on the foliage of many gardens and ornamental plants. Therefore, the control of these snails became very important. Nowadays, the chemical control of snail populations using molluscicides is still the most efficient method, especially in large areas. (Eshra, 2014). Methomyl is a pesticide oxime that belongs to the carbamate group and is frequently employed as a broad-spectrum insecticide. The foliar treatment of tomatoes, crops, cotton, fruits, ornamentals and around poultry houses and dairies are all done with it (Bouzaida *et al.*, 2004; Pohanish, 2015). It is also employed to manage land snails and arthropods, including leafhoppers, ticks, spiders, flies, beetles, moths, aphids, and spider mites which are frequently found on a variety of field crops, from fruits to tobacco (Kidd *et al.,* 1991). MDA, known as diaminodiphenylmethane, is a hazardous substance that causes liver damage, skin and eye irritation (AbouEl-Enein *et al.*, 2015). Herein, this article reported on the synthesis of new MDA derivatives with promising toxicity. The LC_{50} values of prepared compounds were evaluated *in vitro* by thin layer film technique, while, field experiments were carried out to evaluate the activity of prepared compounds against *Theba pisana*.

MATERIALS AND METHODS

Diaminodiphenylmethane (MDA) was bought from Sigma-Aldrich Company. Methomyl (Copter 90% SP) with a chemical formula; ($C_5H_{10}N_2SO_2$) was provided by Egypt Chem International for Agrochemicals Company (Cairo, Egypt). Metal chloride salts were from (Merck). Employed reagents and solvents were possessed commercially. The required purity of (Copter) was accomplished via recrystallization, and then comparison with spectral data; (IR, NMR) of the standard samples before experimentation. Nicolet FT-IR spectrophotometer was used to detect FT-IR spectra in (4000–400 cm^{-1}) range. Elemental analyses; (C, H, N and S) were specified at The Regional Center for Mycology and Biology at Al-Azhar University by using PerkinElmer 2400 elemental analyzer. Mass spectra of the ligand and its synthesized metal complexes were recorded on the Direct Inlet part of the mass analyzer in the Thermo Scientific GCMS model ISQ at The Regional Center for Mycology and Biology, Al-Azhar University. Laboratory experiments were assessed at Plant Protection Research Institute, Giza, Egypt.

Synthesis of HL ligand and its Cu (II), Zn (II) Complexes:

The **HL** ligand was prepared by mixing diaminodiphenylmethane (MDA) and methomyl in a methanolic solution with continuous stirring for 4 h at 60ºC in the presence of a few drops of H_2SO_4. The filtered-off solid ligand was washed several times with methanol and then dried under a vacuum over anhydrous $CaCl_2$.

HL: ($C_{18}H_{22}N_4SO$), Mass spectrum (m/z) Found/calc.: 343.21/342.47. Found (%): C 62.98; H 6.71; N 16.43; S 9.51. Calc. (%): C 63.13; H 6.48; N 16.36; S 9.36. IR (cm^{-1}): 3385, 3340 υ($NH)_2$), 1603, 1588 υ(C=N).

Cu (II) and Zn (II) complexes were prepared by adding copper and zinc chloride salts to a ligand methanolic solution with stirring for 5 h at 60ºC, after that, the formed complexes were filtered off and dried under vacuum over anhydrous $CaCl_2$.

Cu(HL)Cl$_2$: ($C_{18}H_{22}N_4SO.CuCl_2$), Mass spectrum (m/z) Found/calc.: 477.10/477.02. Found (%): C 45.67; H 4.39; N 11.92; S 6.79. Calc. (%): C 45.32; H 4.65; N 11.75; S 6.72. IR (cm^{-1}): 3384, 3338 υ($NH)_2$), 1615, 1578 υ(C=N).

Zn(HL)(H$_2$O)$_2$Cl$_2$: ($C_{18}H_{26}N_4SO_3.ZnCl_2$), Mass spectrum (m/z) Found/calc.: 515.16/514.88. Found (%): C 42.15; H 4.98; N 10.56; S 6.42. Calc. (%): C 41.99; H 5.09; N 10.88; S 6.23. IR (cm^{-1}): 3386, 3341 υ($NH)_2$), 1615, 1580 υ(C=N).

Investigated Animals:

Healthy adults of the white garden snail; *Theba pisana* adopting almost the same size and age were collected from an infested untreated ornamental farm located at Sirs Ellyan city, Menoufia governorate, Egypt during March. The assembled species were then transferred to the laboratory into plastic cups covered with cloth of muslin and maintained under laboratory conditions of 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 the trials start, allowing them to be adapted to laboratory conditions for two weeks. Dead snails were removed regularly. Snails with approximately the same shell diameter were starved for 24 h before treatments and then used in experiments.

Laboratory Tests:

Treatments were conducted using the thin-layer film method (Mourad, 2014). A series of five concentrations (25, 50, 100, 200, and 300 ppm) of the tested compounds were prepared by using DMSO. Two ml of each concentration was spread on the Petri-dish surface and moved in the circle for equal spreading. The solvent was then evaporated at room temperature, leaving a residual layer of the tested compound. Each treatment was conducted through three replicates along with control one, each with ten snails, along 72 h. The killed species were daily counted and removed. The corrected mortality was estimated by using Abbott's formula (Abbott, 1925).

Field Trials:

A nursery of ornamental plants in Menouf district, Menoufia governorate was utilized to perform field trials to assess the *in vivo* toxicity of the tested compounds against *Theba pisana*. The infested location was split into plots, each with four plants. Three plants out of them with $1m^2$ surroundings were considered to be three replicates for each examined compound and the rest plant represented the control. The tested compounds were applied as a spray on the infested plants with a hand sprayer. The treatment began 3 days after irrigation and was performed every 7 days from the first application. The reduction of snail population before and after (7, 14 and 21 days) of treatment was the base for detecting the efficacy of each compound. Moreover, the mortality percentage was estimated using the formula of (Henderson *et al.,* 1955).

Statistical Analysis:

LC_{50} values of the screened compounds with slope and fiducial limits of each treatment were assessed by employing the Probit analysis program (Finney, 1971). The results were subjected to analysis of variance method (ANOVA) and calculated as Mean ± SE. For significance, the means were compared by LSD method at the probability of 0.05 (Steel *et al.,* 1981).

RESULTS AND DISCUSSION

Construction of Achieved Compounds:

The IR spectrum of the HL ligand (Fig. 1) authenticated that the υ(C=O) of methomyl disappeared with the presence of a new band at 1588 cm^{-1}, assignable to υ(C=N), confirming the formation of the new HL ligand (El-Samanody *et al.* 2018). Moreover, the ligand spectrum displayed spectral bands at 3385 and 3340 cm^{-1}, attributed to $υ_{as}(NH_2)$ and $υ_{sy}(NH_2)$, respectively (El-Samanody *et al.*, 2017a; Emara *et al*., 2022), confirming that condensation reaction between methomyl and MDA took place at only one amino group of MDA. Upon chelation, the two υ(C=N) ligand bands (1603, 1588 cm^{-1}) underwent shift to higher and lower wavelengths by (12, 13) and (10, 8 cm^{-1}) for Cu(II) and Zn(II) complexes, respectively (Fig. 1). The aforementioned data concluded that complexation reaction of the bidentate ligand with copper and zinc ions afforded via the two azomethine nitrogen atoms.

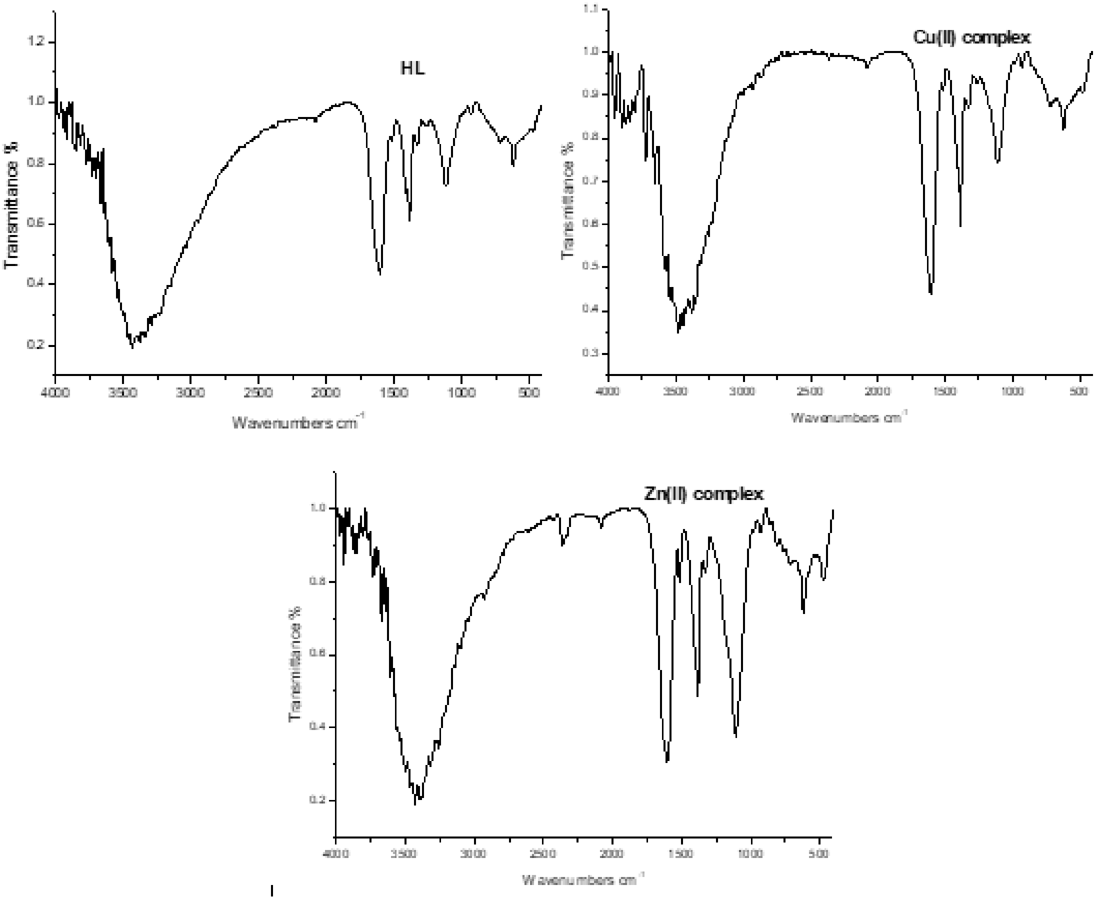


Fig. 1. IR spectra of prepared compounds.

Mass spectra (Fig. 2) of HL, Cu (II) and Zn (II) complexes provided another evidence for their formation through the appearance of characteristic molecular ion peaks at m/z = 343.21, 477.10 and 515.16 amu, respectively, in agreement with their calculated theoretical molecular weights and elemental analyses.

Upon the aforementioned elemental analyses, IR and mass spectral data, the proposed structures for synthesized compounds are displayed in Figure (3).

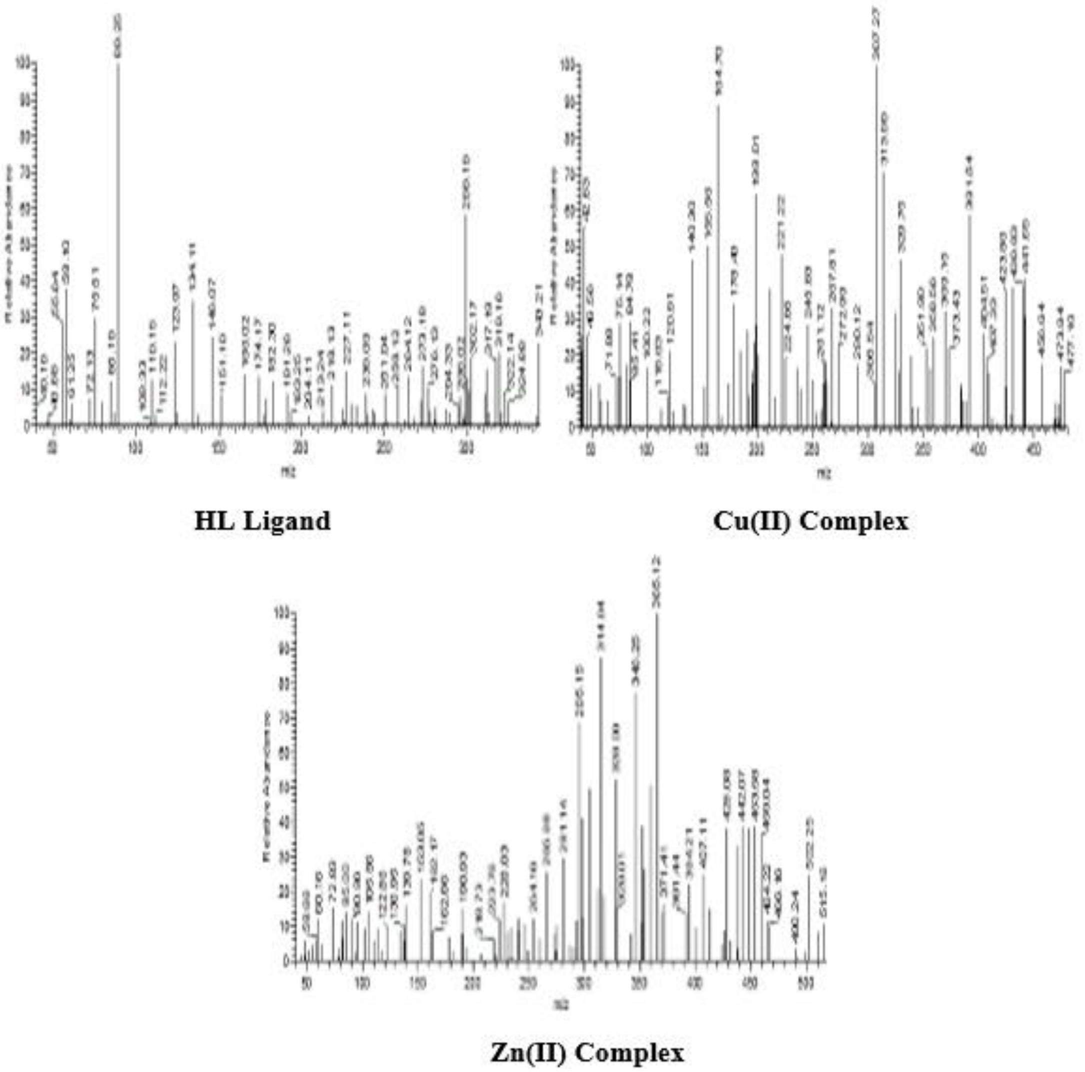


Fig. 2. Mass spectra of prepared compounds.

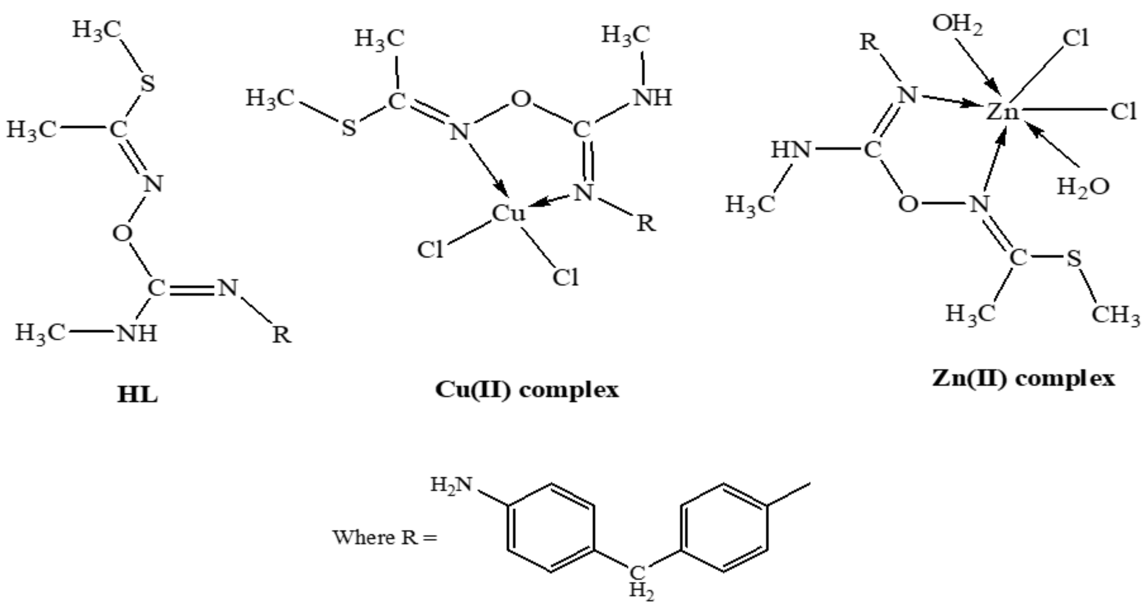


Fig. 3. Proposed structures for prepared compounds

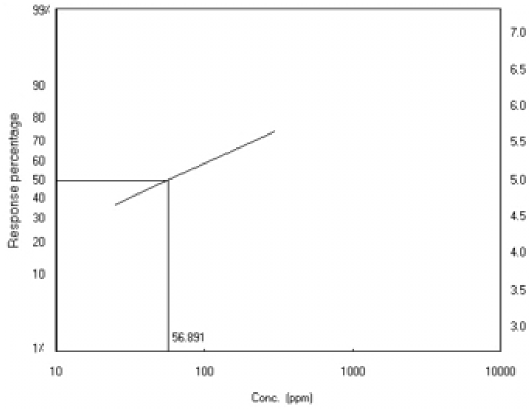
Efficacy of Tested Compounds on Mucus Secretion:

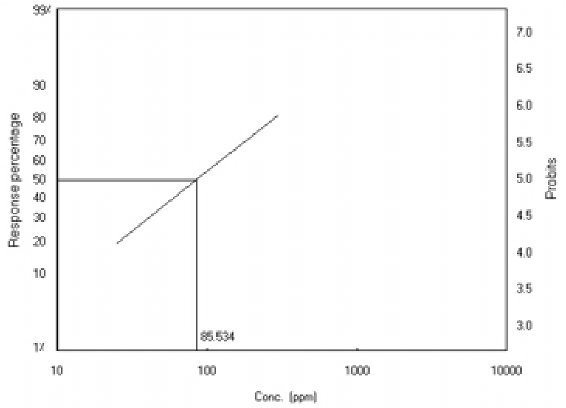
In order to investigate and compare the quantity of the mucus secretion before and after treatments, a light microscope was used, relying on the fact that mucus shows a high affinity to FR-amide antibody and thus it was easily to be detected. The laboratory experiments showed a significant excess of mucus secretion after treatments with the HL ligand, Cu (II) and Zn(II) complexes. The quantity of the secreted mucus increased as the exposure period increased till the complete dryness of snails and death. By comparing the quantity of mucus secretion after treatments with tested compounds, *Theba pisana* recorded the highest sensitivity for mucus secretion upon treatment with Zn (II) complex. Also, the HL ligand showed a higher efficacy on *Theba pisana* mucus secretion than Cu (II) complex. These results may be due to the efficacy of the tested compounds on some vital enzymes such as alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes (having a critical role in mucus secretion, catabolism, phagocytosis, protein synthesis and snails shell formation). This mode of action is ascribed to the inhibition of certain enzymes that detoxify the active molluscicidal component in the snail body. Thus, a higher titre of the active molluscicidal component caused more inhibition of those enzymes critical to the snail (Khalil, 2016; Rao *et al.,* 2003). It is concluded that the tested compounds caused alternations in the quantity of the mucus secretions of the tested species after treatments leading to its mortality.

Laboratory Experiments:

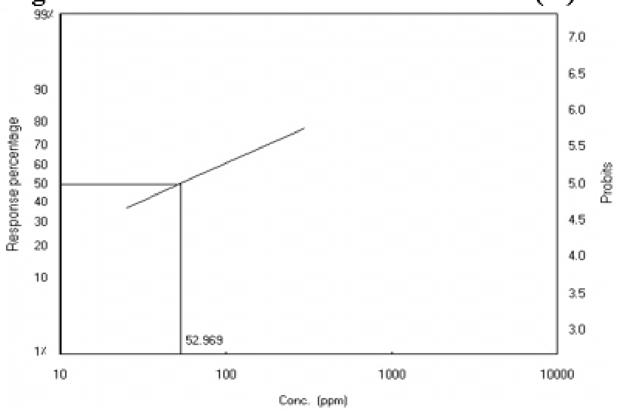
The *in vitro* treatment of *Theba pisana* with the investigated compounds for 72 h with the thin-layer film method established their promising toxicity. An elevation in the level of *Theba pisana* mortality is observed as concentration of investigated chemicals increased. Zn (II) exhibited the highest toxicity (LC_{50} = 52.97 ppm with a slope ± S.E. = 1.496 ± 0.161) compared to the HL ligand and Cu(II) complex (Fig. 4). Also, the results proved that *Theba pisana* was more susceptible to HL ligand than Cu (II) complex. The LC_{50} values of HL ligand and Cu (II) complex are 56.89 and 85.53 ppm with slopes ± S.E. = (1.547 ± 0.161) and (1.759 ± 0.164), respectively. The *in vitro* LC_{50} values data of the tested compounds are tabulated in Table (1).

The variation in the sensitivity level of *Theba pisana* toward the investigated compounds can be related to the chemical structure of these compounds which contains nitrogen, oxygen and sulfur atoms that enhance their toxic effect (El-Samanody *et al*., 2017b). By comparing the chemical structures of the parent compounds (MDA and methomyl) with the newly synthesized HL ligand, it could be concluded that the increased number of nitrogen atoms in the HL ligand structure is responsible for its higher toxicity. Also, the free unchelated amino group in the tested compounds skeleton can react in cells leading to the initiation of a carcinogenic process (Garrigós *et al.,* 2002). Moreover, the increased toxicity of Cu (II) and Zn (II) complexes is attributed to the presence of metal ions that are able to react with enzymes (El-Samanody *et al*., 2017b). The promoted toxicological effect of copper ions may be due to their very high affinity for protein-free thiol groups (cysteine).





HL Ligand **Cu(II) Complex**



Zn(II) Complex

Fig. 4. *In vitro* LC_{50} values of the tested compounds.

Cu (II) ions are more likely to bind to the free thiol group of ALP enzyme and hence causing enzyme inactivation. Meanwhile, Cu (II) ions can bind and distort the active site of enzymes, or even compete for Mg (II) and Zn (II) binding sites in these enzymes; however, due to the redox cycling nature of Cu (II), it is possible that some redox reactions involving the enzyme may be involved as well (Alnuaimi *et al*., 2012). Furthermore, zinc and cadmium have comparatively the same oxidation states and biological activity, and hence cadmium can replace zinc present in metallothionein, thus preventing its scavenging of free radical involved in the cell (Jaishankar *et al.*, 2014). Furthermore, Zn (II) ions can bind with the sites of metal-ions in enzymes (Alnuaimi *et al*., 2012). Its concentration increases 3,000-fold when it binds to cysteine-rich proteins such as metallothionein. The cysteine-metallothionein complex causes hepatotoxicity in the liver and then it circulates to the kidney and gets accumulated in the renal tissue causing nephrotoxicity. Zinc ions have the capability to bind with cysteine, glutamate, histidine and aspartate ligands and can lead to the deficiency of iron.

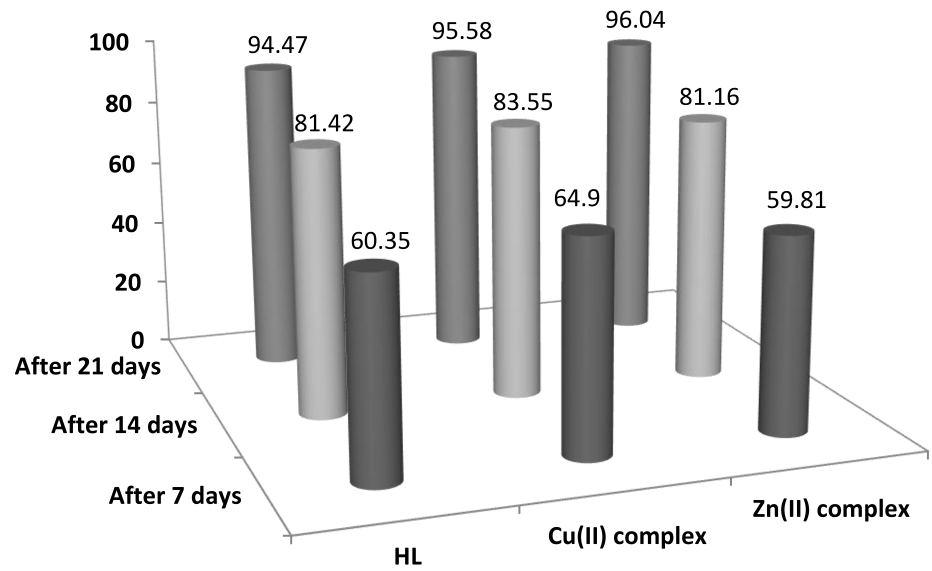
Table 1. *In vitro* LC_{50} data of the tested compounds.

Compound	Concentrations	Corrected Mortality %	LC_{50} (ppm)	Slope ± S.E.
HL	25	25.83	56.89	1.547 ± 0.161
	50	48.22		
	100	69.55		
	200	79.82		
	300	84.32		
$Cu(HL)Cl_2$	25	13.20	85.53	1.759 ± 0.164
	50	38.22		
	100	58.92		
	200	72.66		
	300	81.25		
$Zn(HL)(H_2O)_2Cl_2$	25	28.33	52.97	1.496 ± 0.161
	50	49.11		
	100	71.18		
	200	82.09		
	300	83.24		

Field Trials:

The field trial data of the tested compounds against *Theba pisana* is represented in Figure (5). It is obviously clear that all tested compounds exhibited nearly the same toxic effect toward *Theba pisana* under field conditions. The afforded results were in agreement with those of laboratory tests. The obtained data showed that the HL ligand exhibited a significant population reduction percentage after 7 days of treatment (60.35%) compared with (64.90 and 59.81%) for copper and zinc complexes, respectively. Whereas, the highest population reduction percentages of *Theba pisana* occurred after 21 days of treatments with the HL ligand, Cu(II) and Zn(II) complexes (94.47, 95.58 and 96.04%, respectively). It is noteworthy to mention that Zn(II) complex had the highest *in vitro* and *in vivo* toxicological activity. The aforementioned results established that the toxicity of the prepared methomyl derivatives is concentration-dependent, in agreement with those of (Eshra *et al.*, 2014), who reported that the inhibition of *Theba pisana* acetylcholinesterase (AChE) activity by methomyl was concentration-dependent. The aforementioned data are in agree with the results of Singh *et al.*, (2003) and Adrian *et al.*, (1947), who demonstrated that organophosphate and carbamate pesticides especially (methomyl) reduced the activity of AChE, and this level of reduction was time and concentration-dependent. Salama *et al.*, (2005) stated that the mode of action of methomyl and its derivatives against land snails could be due to the induction of oxidative stress in addition to their anticholinesterase potencies.

The present study demonstrates that the newly synthesized compounds have an effective toxic effect either *in vitro* or *in vivo* against *Theba pisana* species, and so they may be used as a useful molluscicidal.



Tested compounds

Fig. 5. Population reduction % of *Theba pisana* under field conditions

REFERENCES

Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology,* 18: 265–267.

AbouEl-Enein, S. A.; Emam, S. M.; Polis, M. W., and Emara, E. M. (2015): Synthesis and characterization of some metal complexes derived from azo compound of 4,4`-methylenedianiline and antipyrine: Evaluation of their biological activity on some land snail species. *Journal of Molecular Structure,* 1099: 567–578.

Adrian, E. D.; Feldberg, W., and Kirby, B. A. (1947): The cholinesterase action of gluorophosphonates. *British. Journal of Pharmacy Cmemother,* 2: 56–58.

Alnuaimi, M. M.; Saeed, I. A., and Ashraf, S. S. (2012): Effect of various heavy metals on the enzymatic activity of E. coli alkaline phosphatase. *International Journal of Biotechnology and Biochemistry,* 8: 47–59.

Bouzaida, I.; Ferronato, C.; Chovelon, J. M.; Rammah, M. E., and Herrmann, J. M. (2004): Photocatalytic degradation of the anthraquinonic dye, Acid Blue (AB25): a kinetic approach. *Journal of Photochemistry and Photobiology A: Chemistry,* 168: 23–30.

El-Okda, M. M. K. (1980): Land snails of economic importance on Vegetable crops at Alexandria and neighboring regions. *Journal of Agricultural Research Review,* 58: 79–86.

El-Samanody, E. A.; AbouEl-Enein, S. A., and Emara, E. M. (2018): Molecular modeling, spectral investigation and thermal studies of the new asymmetric Schiff base ligand;(E)-N'-(1-(4-((E)-2-hydroxybenzylideneamino) phenyl)ethylidene) morpholine-4-carbothiohydrazide and its metal complexes: Evaluation of their antibacterial and anti-molluscicidal activity. *Applied Organometallic Chemistry,* 32: 42–62.

El-Samanody, E. A.; Emam, S. M., and Emara, E. M. (2017a): Synthesis, characterization, molecular modeling and biological activity of metal complexes derived from (E)-N'-(furan-2-ylmethylene) morpholine-4-carbothiohydrazide. *Journal of Molecular Structure,* 1146: 868–880.

El-Samanody, E. A.; Polis, M. W., and Emara, E. M. (2017b): Spectral studies, thermal investigation and biological activity of some metal complexes derived from (E)-N'-(1-(4-aminophenyl) ethylidene) morpholine-4-carbothiohydrazide. *Journal of Molecular Structure,* 1144: 300–312.

Emara, E. M.; El-Sayed, W. A.; Khalaf-Allah, A. S. A.; Alminderej, F. M.; Abdel-Monem, Y. K., and Abd-Rabou, A. A. (2022): Spectral studies, thermal investigations, and anticancer activity of some divalent metal complexes derived from 2-(4-bromophenylamino) acetohydrazide ligand. *Applied Organometallic Chemistry,* 36: e6657.

Eshra, E. H. (2014): Toxicity of methomyl, copper hydroxide and urea fertilizer on some land snails. *Annals of Agricultural Science,* 59: 281–284.

Eshra, E. H.; EL-Shahaat, M. S., and Abdel-Nabi, S. S. (2014): Interaction of Methomyl with Brown Garden Snail, *Eobania vermiculata* (Müller), and White Snail, *Theba pisana* (Müller) Acetylcholinesterase Activity. *Alexandria Science Exchange Journal*, 35: 16–22.

Finney, D. J. (1971): Probit Analysis, 3rd ed., Cambridge University Press, London. 318.

Garrigós, M. C.; Reche, F.; Marín, M. L., and Jiménez, A. (2002): Determination of aromatic amines formed from azo coourants in toy products. *Journal of Chromatography A*, 976: 309–317.

Glen, D. M.; Wilson, M. J.; Brain, P., and Stroud, G. (2000): Feeding activity and survival of slugs, *Deroceras reticulatum* exposed to the rhabditid nematode *Phasmarhabditis hermaphrodita*: a model of dose response. *Biological Control*, 17: 73–81.

Henderson, C. F., and Tilton, E. W. (1955): Tests with acaricides against the brown wheat mite. *Journal of Economic Entomology,* 48: 157–161.

Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B. B., and Beeregowda, K. N. (2014): Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology,* 7: 60–72.

Khalil, A. M. (2016): Impact of methomyl Lannate on physiological parameters of the land snail *Eobania vermiculata*. *Journal of Basic and Applied Zoology,* 74: 1–7.

Kidd, H., and James, D. R. (1991): The Agrochemicals Handbook, third ed. Royal Society of Chemistry Information Services, Cambridge, England.

Mourad, A. A. (2014): Molluscicidal effect of some plant extracts against two land snail species, *Monacha obstructa* and *Eobania vermiculata*. *Egyptian Academic Journal of Biological Science (F.Toxicology and pest control),* 6: 11–16.

Pohanish, R. P. (2015): Sittig's Handbook of Pesticides and Agricultural Chemicals 2nd ed.

Rao, I. G.; Singh, A.; Singh, V. K., and Singh, D. K. (2003): Effect of single and binary combinations of plant-derived molluscicides on different enzyme activities in the nervous tissue of *Achatina fulica*. *Journal of Applied Toxicology,* 23: 19–22.

Salama, A. K.; Osman, K. A.; Saber, N. A., and Soliman, S. A. (2005): Oxidative stress induced by different pesticides in the land snail, *Helix aspersa*. *Pakistan Journal of Biological Science*, 8: 92–96.

Singh, S. K., and Singh, A. (2003): Effect of the plants *Thevetia peruviana* and *Alstonia scholaris* (Family: Apocynaceae) on acetylcholinesterase activity of *Lymnaea acuminat* snails. *Egyptian Journal of Schistosomaiasis and Infectious and Endemic Diseases,* 25: 31–40.

Steel, R. G. D., and Torrie, J. H. (1981): Principle and Procedure of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill Book Company, New York.

ARABIC SUMMARY

سُمية بعض مُشتقات داي أمينو فينيل ميثان المُشيدة حديثًا والمُطبقة معمليًا وحقليًا على قوقع الحدائق الأبيض وعلاقتها بإفراز المُخاط

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

[1] قسم الحيوانات الضارة – معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيز ة
[2] قسم الحشرات الإقتصادية و الحيوان الزراعي – كلية الزراعة –جامعة المنوفية

تم تحضير عامل الترابط **(HL)** ومتراكباته المشتقة من كلوريدات النحاس والزنك ثنائية التكافؤ من خلال تفاعلات التكثيف بين داي أمينو فينيل ميثان والميثوميل. تم التوصل للتركيب الكيميائي للمركبات المُحضرة باستخدام بعض التحاليل الفيزيائية والطيفية. تم إستخدام طريقة التلامس لتحديد درجة سُمية المركبات معمليًا علي قوقع الحدائق الأبيض. أثبتت النتائج التي تم الحصول عليها وجود نشاط سُمي ملحوظ للمركبات المختبرة. كما تسببت المركبات المُختبرة في حدوث إضطرابات وتغييرات في كمية الإفرازات المخاطية للقواقع بعد المعاملات والتي أدت بدورها إلى نفوق الحلزون. أظهرت النتائج المختبرية أن متراكب الزنك ثنائي التكافؤ لديه التأثير الأكثر سُمية تجاه القواقع الأرضية المُختبرة (LC_{50} = 52،97 جزء في المليون). كما أشارت النتائج إلي الحساسية الكبيرة لحلزون الحدائق الأبيض تجاه عامل الترابط **(HL)** ومتراكب النحاس ثنائي التكافؤ. تم إجراء التجارب الحقلية باستخدام طريقة الرش، وأكدت النتائج التي تم الحصول عليها حقليًا التوافق مع نتائج الاختبارات المعملية. وأظهرت المركبات المُختبرة نسب انخفاض كبيرة في عدد قوقع الحدائق الأبيض بعد 7 و14 و 21 يومًا من المُعاملات.