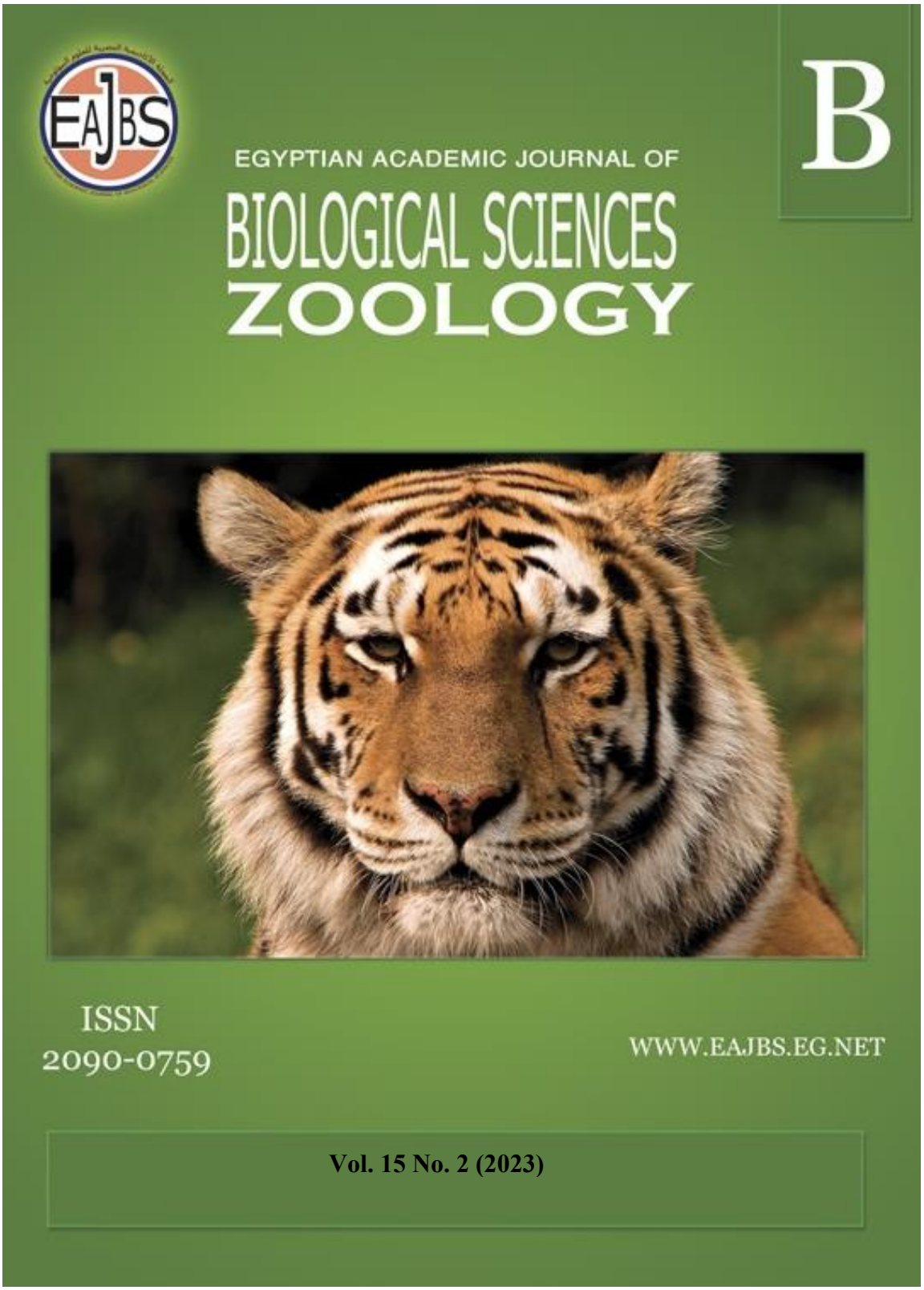

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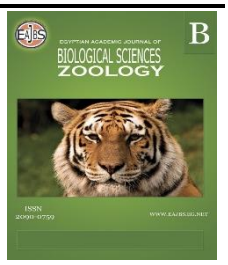

Biochemical and Toxicity Effects of Some 2-Benzoylpyridine Derivatives on *Eobania vermiculata* under Laboratory Conditions

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ABSTRACT

Two derivatives of 2-Benzoylpyridine namely; (E)-4-((E)-4-((E)-phenyl(pyridin-2-yl)methyleneamino)benzyl)-N-(phenyl(pyridin-2-yl)methylene)benzenamine **(L)** and its Cu(II) complex were prepared and screened *in vitro* on *Eobania vermiculata* species. The assessment of LC_{50} values of screened chemicals after 3 days of treatments was performed via the contact method. A series of four concentrations of the investigated compounds; (50, 150, 250, and 350 ppm) was prepared by using a mixture of distilled water and DMSO (3:1) by volume. The obtained results demonstrated that the tested snails were strongly affected after treatments with the prepared chemical compounds, which reflected the high toxicity of those compounds. LC_{50} data of (E)-4-((E)-4-((E)-phenyl(pyridin-2-yl) methyleneamino) benzyl)-N-(phenyl(pyridin-2-yl) methylene) benzenamine and copper chelate are 54.81 and 91.29 ppm, respectively. Biochemical investigations for the treated snails were carried out to estimate the total protein content (TP) as well as the activity of the liver (ALT) and (AST) enzymes after 72 h from starting the exposure. The data authenticated that the **(L)** and Cu (II) complex reduced the AST activity to (53.97 ± 1.06 U/L) and (42.93 ± 0.96 U/L), respectively, compared to (56.97 ± 0.72 U/L) for control. While a high increase in ALT activity was noticed after treatment with the two tested compounds. The (TP) content of the tested snails decreased from (22.11 ± 0.22 mg/g) in control to (21.11 ± 0.80 mg/g) and (18.43 ± 0.71 mg/g) in the **(L)** and Cu (II) complex, respectively.

INTRODUCTION

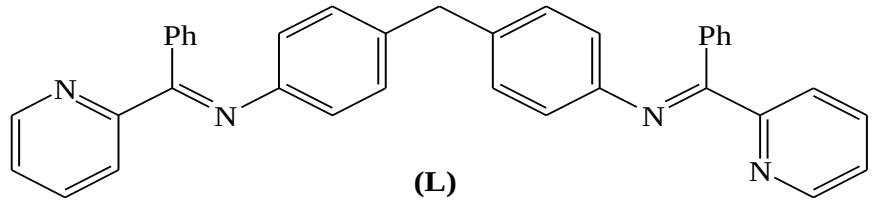
Eobania vermiculata is widespread in Egypt and considered one of the most detrimental stylommatophora pests (Heiba *et al*., 2002; Ali *et al.*, 2020). They are widely distributed and exist in fields, orchards, vegetables, ornamental plants and crops of fruits (Shetaia *et al*., 2009; Heikal, 2015; Abou Senna *et al*., 2016; Rady *et al*., 2019; Kadry *et al*., 2018 and Shahawy, 2018). Controlling these species chemically displayed a high efficiency. AST and ALT are vital enzymes with importance in the gluconeogenisis route through the linkage of carbohydrate metabolism and the amino acids along with indication for lesions of tissue (Abdel-Hamid, 2008). Also, biological operations occurring in snails are

comparatively affected by total protein content as well as transaminase enzymes (Abd El-Aal, 2004). The alteration in the physiological parameters (e.g. ALT, AST enzymes and total proteins) of the investigated snails reflects obvious changes in the protein metabolism (Mahmoud *et al.*, 2002). This current study aimed to investigate the synthesized chemicals' toxicity under laboratory conditions and spotlight their efficacy on some important biochemical parameters of *Eobania vermiculata*.

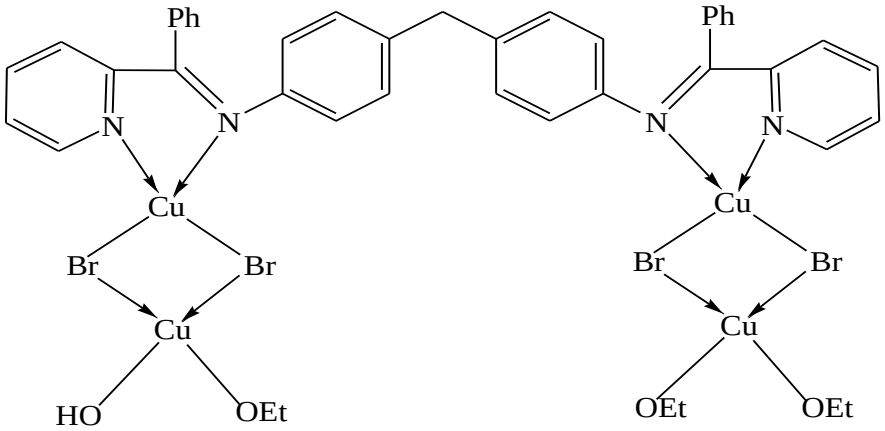
MATERIALS AND METHODS

Utilized Chemical Compounds:

2-Benzoylpyridine was purchased from Sigma Company and 4,4'-methylene-dianiline was bought from BDH Company. Copper bromide along with the used reagents and solvents were from commercial suppliers. (E)-4-((E)-4-((E)-phenyl(pyridin-2-yl)methyleneamino)benzyl)-N-(phenyl(pyridin-2-yl)methylene)benzenamine **(L)** and its Cu(II) complex were synthesized and elucidated according to literature (Emam *et al*., 2017). The chemical structure of synthesized compounds is shown in Figure 1.



(L)



Cu complex

Fig. 1. Structure of prepared compounds.

Tested Snails:

Adult snails of *Eobania vermiculata* with shell size (12–15 mm) weighing (2.5–4 g) were assembled from the infected ornamental nurseries in spring at Shebin El-Kom district, Menoufia Governorate, Egypt. The obtained species were moved to the lab at Sers Ellyan Agricultural Research Station in a bag of muslin and then kept in rearing housing of plastic boxes with suitable moist soil feeding on fresh lettuce leaves for two weeks to achieve acclimation. Healthy-reared species were used in treatments.

Determination of the Median Lethal Concentration:

The contact method was carried out to detect the median lethal concentration of the investigated compounds (Mourad, 2014). Stock solutions of the examined chemicals were achieved by employing a mixture of distilled water and DMSO (3:1) by volume. Four different concentrations of the tested compounds; (50, 150, 250, and 350 ppm) were prepared and a parallel control test was performed. Three replicates of each treatment, each

with ten snails, were conducted for 72 hrs.

Analysis of Statistics:

Statistical package for the social sciences (SPSS), 20th Version was performed to carry out analysis of statistics. Variance analysis in one-way was conducted to evaluate sub-lethal concentrations efficacy in comparison with control and their influence on some biochemical parameters levels within the same conditions. Results were calculated as (mean ± S.E.) of three replicates. A probability of 0.05 was considered for significance.

Biochemical Tests:

Preparation of Homogenate Samples:

Three replicates were done over three days for individuals of the brown garden snail upon treatment with LC_{50} concentrations of examined chemicals. The dead snails were removed after treatments. Distilled H_2O was employed to homogenize live snails which were then subjected to centrifuging by utilizing (BECKMAN GS-6R Centrifuge) for ten minutes at 5°C and 6000 rpm. The obtained supernatant was then subdivided into small parts (0.5 ml and stored at –20°C). Each biochemical estimation was conducted via 3 replicates.

Total Protein Content Estimation:

Total protein content was estimated via conducting the Bradford (1976) method, using a Bovine serum albumin standard. TP content was estimated as (mg/g) body weight.

Determination of Transaminase Enzymes:

The activity of transaminase enzymes was determined by the Reitman and Frankel (1957) technique, using DiaSys kits; Diagnostic system at Plant Protection Research Institute. The transaminases enzymes activities were expressed as U/L.

RESULTS AND DISCUSSION

Laboratory Tests:

The *in vitro* experiment data (Table 1) authenticated the high toxicological influence of the two tested chemical compounds. The sensitivity of the brown garden snails toward tested compounds increased as the exposure period and concentration increased. The ligand **(L)** exhibited promising high toxicity against *E. vermiculata* (LC_{50} = 54.81 ppm with a slope of 0.942 ± 0.203). This relatively high toxic effect may be ascribed to the increased number of nitrogen atoms present in its chemical structure (El-Samanody *et al*., 2017). The presence of these nitrogen atoms may lead to the initiation of a carcinogenic process (Garrigós *et al.,* 2002). The noticed high sensitivity of *Eobania vermiculata* toward Cu(II) complex (LC_{50} = 91.29 ppm with a slope of 1.895 ± 0.218) is attributed to the existence of the divalent copper ions that are able to react with enzymes (El-Samanody *et al*., 2017). The enhanced toxicological effects of these Cu(II) ions are related to the enhanced affinity of copper ions for cysteine (free thiol groups of protein). Cu(II) ions cause ALP enzyme inactivation by binding to its free thiol group. Moreover, copper ions have the ability to bind with the enzymes' active sides and caused its distortion, competing for enzyme binding sites of magnesium and zinc. Whereas, some redox processes encompassing the enzyme may also be involved ascribed to the cycling redox nature of copper ions (Alnuaimi *et al*., 2012).

Table 1. Mortality and lethal concentrations of tested compounds**.**

Compounds	Concentrations (ppm)	Corrected Mortality (%)	LC_{50} (ppm)	Slope ± S.E.
L	50	50	54.81	0.942±0.203
	150	65.32		
	250	66.67		
	350	83.33		
$[Cu_4(L)Br_4(OH)(OEt)_3].2.5EtOH$	50	29.99	91.29	1.895±0.218
	150	66.67		
	250	83.33		
	350	83.33		

Biochemical Investigations:

The obtained data concerned with AST, ALT activity and TP content of *Eobania vermiculata* after treatments with the tested compounds are displayed in (Fig. 2). The results in (Table 2) illustrate the efficacy of the investigated chemicals on *E. vermiculata* AST activity. The data emphasized that the (L) and its Cu(II) complex inhibited the AST activity to (53.97 ± 1.06 U/L, S.D. = 1.95) and (42.93 ± 0.96 U/L, S.D. = 1.60), respectively, compared to (56.97 ± 0.72 U/L, S.D. = 0.90) for control (Fig. 2).

Table 2. Efficacy of the investigated chemicals on *E. vermiculata* AST activity

Compounds	AST (U/L)			Mean ± S.E.	S.D.
	R_1	R_2	R_3		
L	55.9	52.0	54.0	53.97 ± 1.06	1.95
$[Cu_4(L)Br_4(OH)(OEt)_3].2.5EtOH$	44.6	41.4	42.8	42.93 ± 0.96	1.60
Control	57.9	56.1	56.9	56.97 ± 0.72	0.90

On the contrary, a noticeable elevation in the ALT activity of *Eobania vermiculata* was observed after treatments with the (L) and its Cu (II) complex (Table 3). The ALT activity was raised from (5.07 ± 0.51 U/L, S.D. = 0.45) in control to (27.37 ± 0.18 U/L, S.D. = 0.06) and (27 ± 0.72 U/L, S.D. = 0.90) for the (L) and Cu (II) complex, respectively (Fig. 2).

Table 3. Efficacy of the investigated chemicals on *E. vermiculata* ALT activity.

Compounds	ALT (U/L)			Mean ± S.E.	S.D.
	R_1	R_2	R_3		
L	27.3	27.4	27.4	27.37 ± 0.18	0.06
$[Cu_4(L)Br_4(OH)(OEt)_3].2.5EtOH$	27.9	26.1	27.0	27 ± 0.72	0.90
Control	5.5	4.6	5.1	5.07 ± 0.51	0.45

Moreover, the total protein content (TP) of the tested snails decreased from (22.11 ± 0.22 mg/g) in control to (21.11 ± 0.80 mg/g) and (18.43 ± 0.71 mg/g) in the **(L)** and Cu (II) complex, respectively (Table 4).

Table 4. Efficacy of the investigated chemicals on *E. vermiculata* TP content.

Compounds	TP (mg/g)			Mean ± S.E.	S.D.
	R_1	R_2	R_3		
L	27.3	27.4	27.4	21.11 ± 0.80	1.12
$[Cu_4(L)Br_4(OH)(OEt)_3].2.5EtOH$	27.9	26.1	27.0	18.43 ± 0.71	0.87
Control	22.1	22.2	22.0	22.11 ± 0.22	0.80

Cell necrosis and digestive gland damage occurred by the action of insecticides leading to leakage of cell enzymes and hence causing an alternation in transaminase enzyme activity (Gaber *et al*., 2022; Kammon *et al*., 2010). Changes in biochemical signs are considered an indicator of the surrounding environmental conditions and risk measurements of snails (Khalil, 2016). Enzymes activity enhancement occurred by the action of pesticides is related to necrotic effects and liver degeneration by which the leakage of enzymes out of the cells takes place (Arfat *et al*., 2014). The outcome data are in agreement with those of (Al-Attar, 2005) who deduced that; ALP, ALT and AST enzyme activity was enhanced significantly in the cadmium-exposed fish. Enzymes of ALP, AST and ALT are found in liver cells and muscle, intestine, heart and gill tissues. So, animals require suitable energy to overcome the toxic effects after direct exposure. During breaking down the free amino acid to overcome the required energy, toxic effects are occurred through gluconeogenesis, producing an elevated level of the activity of transaminase enzymes (Samanta *et al*., 2014; Neelima *et al.*, 2013). The efficacy of this toxic stress is reflected in cell permeability which in turn caused enhancement in enzyme activity because of their leakage out of the infected cell (Meenakshi *et al*., 2020). These data are nearly the same emphasized by Banaee *et al*., (2016). The current study established that total protein content was comparatively reduced in snails after exposure to the **(L)** and Cu(II) complex than the control one. El-Shenawy *et al*., (2012) emphasized that the TP content was reduced in the digestive gland of *Eobania vermiculata* species. The reduction in total protein content may have resulted from the inequivalence between synthesis and degradation rates of body tissue total protein.

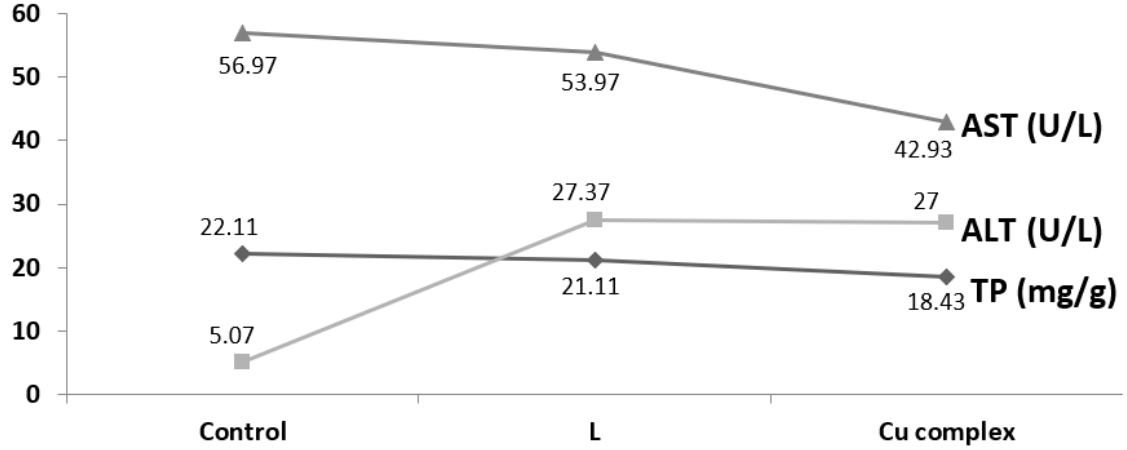


Fig. 2. AST, ALT activity and TP content of *E. vermiculata* after treatment with the tested compounds

Conclusion

The present *in vitro* study evaluated the toxicological effects of some 2-Benzoylpyridine derivatives against *Eobania vermiculata* by contact method. The results concluded promising toxicity of the tested compounds. Also, the tested compounds were screened for their efficacy on some biochemical parameters; AST, ALT and TP content. The achieved results emphasized the significant high effects of the tested compounds on the target parameters. So, these compounds may be used for controlling *Eobania vermiculata* after studying their effects on human and animal health.

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ARABIC SUMMARY

التأثيرات البيوكيميائية والسُمية لبعض مشتقات 2-بنزويل بريدين علي قوقع الحدائق البني تحت الظروف المعملية

عصام محمد عمارة[1]، ماهر عبد العزيز الصواف[1]، رشا فؤاد خليفة[2]

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تم تحضير عامل ترابط مشتق من 2-بنزويل بريدين ومتراكبه مع النحاس، وتقييم سُميتهم المعملية علي قوقع الحدائق البني بطريقة التلامس لمدة 3 أيام. أُجريت المُعاملات باستخدام سلسلة من 4 تركيزات للمركبات المُختبرة التي تم تحضيرها باستخدام مخلوط من الماء المُقطر و DMSO. أظهرت النتائج التي تم الحصول عليها أن القواقع المُختبرة تأثرت بشدة بعد المُعاملات مما يعكس السُمية العالية لتلك المركبات. تم إجراء الفحوصات البيوكيميائية للحلزونات المُعالجة لتقدير محتوى البروتين الكلي (TP) بالإضافة إلى نشاط إنزيمات الكبد (AST و ALT) بعد 72 ساعة من بدء التعرض. أكدت البيانات أن عامل الترابط (L) و متراكب النحاس قللوا نشاط AST إلى (53،97 ± 1،06 U/L) و (42،93 ± 0،96 U/L) على التوالي، مقارنة بـ (56،97 ± 0،72 U/L) للكنترول. بينما لُوحظ إرتفاع كبير في نشاط ALT بعد المعاملة بالمركبين المختبرين. إنخفض محتوى (TP) للحلزونات المُختبرة من (22،11 ± 0،22 مجم/جم) للكنترول إلى (21،11 ± 0،80 مجم/جم) و (18،43 ± 0،71 مجم/جم) لعامل الترابط (L) و متراكب النحاس على التوالى.