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# TERRESTRIAL SNAILS AS BIOINDICATIVE ORGANISM FOR ASSESSING ECOTOXICOLOGICAL EFFECTS OF AGROCHEMICALS IN RURAL AREA.

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## ABSTRACT

Terrestrial snail, *Monacha cantiana* was used as a bioindicative organisms to assess the ecotoxicological effect in El Behira governorate. Five districts (El-Dalengat, Kom Hamada, Damanhour, Shoubrakhet and Etay El-Baroud) were selected for the animals collection during winter and spring seasons of 2008/2009. AChE activity was observed at a highest value (71.65 μmole/mg protein/min) in Damanhour district which considered a control zone. Lactic dehydrogenase (LDH) is an indicative criteria of exposure to chemical stress, was showed the lowest inhibition (13.7 IU/ mg protein) in Etay El-Baroud. On the other hand, from the family of enzymes with important roles in the biotransformation of xenobiotics substances, glutathione-*S*-transferase (GST) was chosen. It recorded significantly decreasing in activity of snails collected from El-Dalengat, Damanhour and Etay El-Baroud districts, respectively, with values (63.01, 73.54, and 87.99%) compared with control. The terrestrial snail was an efficient bioindicator that accumulate bioavailable contaminants as a diagnosis tools for toxicological responses.
**Keywords**: Land snails; Rural areas; Ecotoxicologic effect; Agrochemicals.

## INTRODUCTION

The use of nonhuman organisms as early warning systems for human health risk is not new. Sentinel animal models could involve mammalian or non mammalian species, domestic animals, or wildlife. Outcomes of interest included mortality and morbidity, developmental defects and reproductive effects, carcinogenicity, neurotoxicity, immunotoxicity, behavioral changes, and other. Sentinel animal populations could be exposed 1) to a single chemical or complex mixture, or 2) to different media in various locations (Stahl, 1997).

There are several potential advantages associated with using sentinel species as indicators of human health hazards. Potential applications identified for sentinel species included monitoring environmental media, identifying new exposures of potential concern as a result of observing changes in wild animal populations, and supporting risk assessment at several points in the process. The data could be useful for a weight of evidence approach in risk assessment decisions (Van der Schalie *et al.*, 1999).

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Among terrestrial invertebrates, the gastropods have the capability to accumulate different classes of chemicals and serve as pertinent species for monitoring trace metals, agrochemicals, urban pollution, and electromagnetic exposure (Beeby and Richmond, 2002, 2003; Berger and Dallinger 1993; Gomot de-Vaufleury and Pihan, 2000).

Biomarkers measure the interaction between a biological system and an environmental agent (WHO/IPCS, 1993). In *vivo* inhibition or induction of biomarkers is a good environmental tool to assess the exposure and the potential effects of xenobiotics on organisms (Dembele *et al.*, 1999; Ozmen *et al.*, 1999; Mc Loughlin *et al.*, 2000 and Varo *et al.*, 2001). Also, mosquitofish ChE activity seems to be a promising biomarker for use in biomonitoring programmes to diagnose the exposure of wild population of this species to anticholinesterase xenobiotics (Osten *et al.*, 2005). Thus, the aim of the study is assessing the ecotoxicological effects of agrochemicals in rural area on land snails as a biomarker for environmental pollution and human health hazards.

## MATERIALS AND METHODS

**Tested animals**

The terrestrial snail, *Monacha cantiana* was collected from the clover fields of El-Behira governorate during winter and spring seasons of 2008/2009 and transferred to the laboratory for biochemical analysis directly after transfer.

**Chemicals.**

All chemicals used in this study were obtained either from Sigma or BDH Companies and they were of the highest grade available.

**Description of the studied zones.**

Five districts of El-Behira governorate (El-Dalengat, Kom-Hamada, Damanhour, Shoubrakhet and Etay El-Baroud) were selected for land snails study. Where, they are in a difference in crop rotation and farmer's activities. During the sampling procedures, a screening questionnaire was conducted on the farmers.

**Biochemical studies.**

Snail tissues were dissected out directly after transfer. All tissues were homogenized in 0.1M phosphate buffer PH 7.4 (1:10 w/v) using a polytron homogenizer. The homogenates were centrifuged at 5000 rpm for 20 min at 4 °C and the supernatant was taken for determination the activities of acetycholinesterase (AChE), lacticdehydrogenase (LDH), glutathione-*S*-transferase (GST), and protein contents.

AChE activity was determined by measuring the hydrolysis of acetylthiocholine iodide (ASChI)(Ellman *et al.*, 1961). An aliquote (0.02 ml) of 10% tissue homogenate in 0.1 mM phosphate buffer, PH 8.0 was added to a reaction mixture containing 0.075M ASCh, 0.01M of 5, 5-dithiodinitrobenzoic acid (DTNB) in a final volume of 3.0 ml. The mixture was incubated at 37 °C for 10 min and then the optical density was measured at 412 nm. AChE activity was expressed as $\mu$mole of ASCh hydrolyzed /min/ mg protein.

LDH activity was measured as freshly as possible in the homogenate according to the method of McComb (1983). The assay was done using Na pyruvate as a substrate and NADH as a cofactor. 50 µl of enzyme homogenate was preincubated with 1 ml of freshly prepared buffered substrate (0.075 mM) at 37 °C for 15 min. After few minutes, the reaction was initiated by addition of 100 µl of freshly prepared NADH (10 mg. $ml^{-1}$). Samples were incubated at 37 °C for 15 min. Colour was induced by adding 1 ml of 2,4-dinitrophenyl hydrazine (2 mM) and allowed at room temperature for 20 min. The reaction was terminated by addition of 10 ml NaOH (0.4M). After 10 min, the developed colour was measured at 510 nm. The activity of enzyme was expressed as unit/ mg protein.

Gultathion-*S*-transferase (GST) was determined according to the method of Vessey and Boyer (1984). The reaction mixture contained 0.2 ml of 4mM glutathione, 20 µl of enzyme supernatant and the volume was completed to 3 ml with 0.1M phosphate buffer, PH 7.0. The mixture was incubated for 20 min. The absorbance was measured at 340 nm using UV/Vis Spectrophotometer (Spectronic 21D, Bouch & Lomb). The enzyme activity was expressed as µmole/ mg protein/min.

The protein contents in snails were determined using bovine serum albumin as a standard (Lowry *et al.*, 1951).

**Statistical analysis.**

Data were calculated as mean± SE and analyzed using analysis of variance technique (ANOVA) followed by Least Significant Difference (LSD). Probability of 0.05 or less was considered significant. All statistical analysis was done with Costat Program (1986) on a personal computer.

## RESULTS AND DISCUSSION

Since about 30 years, the use of DDT in agriculture has been officially banned in Egypt. In 1962, toxaphene was cancelled from recommendations due to the development of resistance of cotton leafworm, followed DDT cancellation. The use of other pesticides (e.g., aldrin, dieldrin, chlordane, heptachlor, lindane, parathion, parathion-methyl, leptophos) were gradually restricted in the country. Most if not all, of the banned or severely restricted pesticides showed in Table 1 have been used in Egypt. Also, a number of "probable human carcinogenic" (group B) and possible human carcinogenic" (group C) pesticides were officially banned by Ministerial Act No. 874 for the year 1996.

During the season of 2001/2002, the number of pesticides registered in Egypt reached 330 formulations belonging to 175 active ingredients (a.i). Each of the insecticides and fungicides represent 34% of the total (a.i) number. Herbicides, acaricides, rodenticides and nematocides represent 21, 6, 3 and 2%, respectively (Mansour, 2004).

A surveyed questionnaire was conducted on the farmers of selected region. Over (85%) of them showed that, clover crop was the most infected with snails especially which cultivated after rice in crops rotation programme. The areas around or near irrigation or drainage canals were the most infected.

Table 1: List of some banned or severely restricted pesticides.

| Pesticide | pesticide |
|---|---|
| Aldicarb | Captafol |
| Heptachlor | Binapacryl |
| Lindane | Bromacil |
| Aldrin | Chlorobenzilate |
| Cyhexatine | Dinoseb |
| HCH (mix.) | Ethylene dichloride |
| DDT | Ethylene oxide |
| Mercury compounds | Hexachlorobenzene |
| Endrin | Maleic hydrazide |
| Paraquat | Methamidophos |
| Chlorodimeform | Monocrotophos |
| Ethylene dibromide | Parathion |
| Fluoroacetamide | Parathion methyl |
| Chlordane | Pentachlorophenol |
| 2,4,5-T | Phosphamidon |
| Dieldrin | Toxaphene |

Mansour, (2004).

Nitrogen and trace elements were highly use in their fertilization where, rice, bean, wheat, tomato, onion, potatoes and sugermellon were the highly cultivated in El-Behira region. Pesticides were used to control all the pests on the crops except the snails.Where, 10% only was recorded for snails control. The surveyed farmers convinced that, Benthiocarb was used at least (70%), followed by carbofuran (25%), while methamidophos, mancozeb, profenofos, chlorpyrifos, and chlorpyrifos-m (10%). Most of the farmers work in their fields especially in pest control (90%) as a foliar treatment (90%), but in case of soil application was (70%). On the other hand, crops harvesting were handled (100%) in all districts. But mechanical programs were accounted for (70%) in another cases. Registered pesticides by Ministry of agriculture and crops application were presented in Table 2.

**Biochemical studies.**

The percentages of AChE activity were showed in Table 3. The results showed that, inhibition of enzyme activity of snails collected from El-Dalengat district was observed at the lowest value (32.82 μmole/mg protein/ min). In contrast, Damanhour district was considered as a control zone at the highest value (71.65 μmole/mg protein/ min). Inhibition of cholinesterase (ChE) activity has been frequently used in wildlife toxicology to diagnose the exposure to anticholinestrase chemicals such as organophosphate (OP) and carbamate (CB) pesticides (Fossi *et al.,* 2001; Fulton and Key, 2001 and Sanchez, 2001).

Lacticdehydrogenase (LDH) activities in homogenate tissues of terrestrial snail were presented in Table 4. The data were varied in the examined zones of El-Behira governorate. Samples collected from Etay El-Baroud district showed the lowest inhibition of LDH enzyme as a mean 13.7 IU/ mg p7rotein.

**Table 2: Registered pesticides in Egypt, crop application, and special groups.**

| Pesticides | Crops | Group |
|---|---|---|
| B.t. | Clover, potatoes | Insecticide |
| Benthiocarb | rice | Herbicide |
| Butachlor | rice | ≈ ≈ |
| Carbaryl | cotton | Insecticide |
| Carbofuran | Tomato, rice | Nematocide/insecticide |
| Carbosulfan | Onion, cotton, maize, tomato | Insecticde |
| Carboxin+Thiram | Sugermillon, maize | Fungicide |
| Chlorfenapyrohos | Potatoes, tomato | insecticide |
| Chlorpyrifos | Cotton, tomato, potatoes | ≈ ≈ |
| Chlorpyrifos-methyl | Soyabean, tomato, potatoes | ≈ ≈ |
| Diazinon | Sugerbeet, rice | ≈ ≈ |
| Diniconazole | wheat | Fungicide |
| Edifenphos | rice | ≈ ≈ |
| Fenamiphos | tomato | Nematocide |
| Fenpropathrin | cotton | Insecticide |
| Fentrothion | Wheat, rice, maize, potatoes | ≈ ≈ |
| Fluazifop-p-butyl | Potatoes, cotton, soyabean | Herbicide |
| Glyphosate | bean | ≈ ≈ |
| IGR's | cotton | Insecticide |
| Imidacloprid | Potatoes, cotton, tomato | ≈ ≈ |
| Isoproturon | wheat | Herbicide |
| Ivermectin | Cotton, soyabean, potatoes, sugermellon | Inesecticide |
| Linuron | potatoes | Herbicide |
| Malathion | Wheat, bean, maize | Insecticide |
| Mancozeb | Tomato, bean | Fungicide |
| Metaloxyl | Potatoes, tomato, onion | ≈ ≈ |
| Methomyl | Potatoes, maize, soyabean, tomato, clover, cotton | Insecticide |
| Metribuzim | Potatoes, tomato, wheat | Herbicide |
| Mineral oils | Tomato, potatoes | Insecticide |
| Oxadiazon | rice | Herbicide |
| Oxamyl | tomato | Nematocide |
| Oxyfluorfen | Onion | Herbicide |
| Pendimethalin | Cotton, onion | ≈ ≈ |
| Pirimiphos-methyl | Onion, maize, tomato, potatoes | insecticide |
| Profenofos | Sugerbeet, cotton, tomato, potatoes | ≈ ≈ |
| Prothiofos | Bean, potatoes, sugermillon | ≈ ≈ |
| Thiodicarb | Cotton | ≈ ≈ |
| Tolclofos methyl | Cotton, potatoes | Fungicide |
| Triazophos | Bean, cotton, maize, tomato, potatoes | insecticide |

Source: Ministry of agriculture(2001), Egypt.

This region was considered as a control. On the other hand, agrochemicals applied in Damanhour induced the highest cells damage effects of snails to observe highest activity of enzyme level 18.06 IU/ mg protein followed by Kom Hamada and El-Dalengat, respectively.

Table 3: Activity of acetylcholinesterase and protein contents in terrestrial snail tissues, *Monacha cantiana*.

| Location | Activity ± S.E | | | | Mean ± S.E | LSD 0.05 | % of control | Protein content |
|---|---|---|---|---|---|---|---|---|
| | Zone 1 | Zone 2 | Zone 3 | Zone 4 | | | | |
| El-Dalengate | 42.21±4.59 | 27.42±3.70 | 34.07±4.13 | 27.57±3.65 | 32.82±3.31 | 6.8 | 45.81 | 3.26±1.04 |
| Kom-Hamada | 27.39±3.56 | 42.41±4.60 | 50.49±5.02 | 85.82±6.55 | 51.53±4.14 | 7.9 | 71.92 | 3.13±1.02 |
| Damanhour | 95.39±6.91 | 69.55±5.88 | 51.00±5.05 | 70.67±5.94 | *71.65±4.91 | 10.4 | 100 | 3.60±1.10 |
| Shoubrakhet | 41.68±4.57 | 57.61±5.37 | 43.75±4.68 | 53.75±5.13 | 49.21±4.05 | 9.7 | 68.68 | 3.19±1.03 |
| Etay El-Baroud | 56.99±5.34 | 60.38±10.06 | 90.69±6.73 | 61.58±5.55 | 67.41±4.74 | 12.0 | 94.08 | 3.36±1.06 |
| LSD0.05 Between the cities | 11.6 | | | | | | | |

Cholinesterase activity is expressed as µmole acetylthiocholine hydrolyzed /mg/min.
Each value is the mean of three samples ±SE

Table 4: Lacticdehydrogenase (LDH) activity in the terrestrial snail tissues, *Monacha cantiana*.

| Location | Activity ± S.E | | | | Mean ± S.E | LSD 0.05 | % of control |
|---|---|---|---|---|---|---|---|
| | Zone 1 | Zone 2 | Zone 3 | Zone 4 | | | |
| El-Dalengate | 16.43±2.87 | 13.61±2.61 | 17.22±2.93 | 16.55±2.88 | 15.95±2.31 | 2.7 | 116.42 |
| Kom-Hamada | 11.42±2.39 | 18.43±3.04 | 18.63±3.05 | 17.76±2.98 | 16.56±2.35 | 3.2 | 120.88 |
| Damanhour | 18.43±3.04 | 17.95±3.00 | 16.95±2.91 | 18.91±3.07 | 18.06±2.45 | 4.1 | 131.82 |
| Shoubrakhet | 14.73±2.71 | 13.69±2.62 | 15.69±2.80 | 14.59±2.70 | 14.68±2.21 | 6.8 | 107.15 |
| Etay El-Baroud | 14.60±2.70 | 16.64±2.88 | 13.69±2.62 | 9.87±2.22 | *13.70±2.12 | 5.3 | 100 |
| LSD0.05 Between the cities | 5.3 | | | | | | |

LDH activity is expressed as IU /mg protein.
Each value is the mean of three samples ±SE.

Glutathione-*S*-transferases (GST) are a family of enzymes with important roles in the biotransformation of both xenobiotics and endogenous substances. Therefore, induction of GST activity has been used as a biomarker of exposure to xenobiotics with electrophillic centers (Falkner and Clark, 1992; Gallagher *et al.*, 1992). The results of enzyme activity which presented in Table 5 showed that, significantly decrease in GST levels in the snail compared with control were observed in El-Dalengat, Damanhour and Etay El-Baroud districts, respectively, with a values (63.01, 73.54 and 87.99%). While, Shoubrakhet district is considered a control zone in this assay.

Table 5: Glutathione-*S*- transferase (GST) activity in the terrestrial snail tissues, *Monacha cantiana*.

| Location | Activity ± S.E | | | | Mean ± S.E | LSD 0.05 | % of control |
|---|---|---|---|---|---|---|---|
| | Zone 1 | Zone 2 | Zone 3 | Zone 4 | | | |
| El-Dalengate | 21.51±3.28 | 0.41±0.04 | 9.10±2.13 | 20.41±3.19 | 12.86±2.07 | 1.9 | 63.01 |
| Kom-Hamada | 17.11±2.92 | 16.05±2.83 | 22.29±3.34 | 20.54±3.20 | 19.00±2.52 | 2.8 | 93.09 |
| Damanhour | 13.65±2.89 | 15.73±2.80 | 15.94±2.82 | 14.71±2.71 | 15.01±2.24 | 3.8 | 73.54 |
| Shoubrakhet | 22.19±3.33 | 20.46±3.20 | 18.93±3.08 | 20.04±3.21 | *20.41±2.61 | 4.7 | 100 |
| Etay El-Baroud | 23.50±3.43 | 22.25±3.33 | 14.48±2.69 | 11.62±2.41 | 17.96±2.46 | 2.0 | 87.99 |
| LSD0.05 Between the cities | 4.6 | | | | | | |

The activity is expressed as µmole /mg protein/min.
Each value is the mean of three samples ±SE.

The biochemical parameters which examined were a good indicators for agrochemicals used in the studied region (El-Behira governorate) as observed from surveyed questionnaire. In the urban area, Regoli *et al.* (2006) used the terrestrial snail *Helix aspersa* as sentinel organism for ecotoxicologic effect in some regions of Italy. While, In Egypt, the terrestrial snails *T pisama* were used as a quantitative indicator of environmental metals pollution in Alexandria city which induce oxidative stress (Mohamed, 2008).

**Conclusion**

In our study we noticed the relationship between residents exposure in rural areas in Egypt to agrochemicals associated with wildlife response in agreement with previously showed by (Van der Schalie *et al.*, 1999 and Regoli *et al.*, 2006). However, the ecotoxicologic approach appears to be a valuable tool for monitoring environmental quality in rural areas. Finally, the terrestrial snail was an efficient bioindicator that accumulate bioavailable contaminants and allowed the integration of these data with toxicologic responses (Laskowski and Hopkin, 1996b; Pihan and de Vafliury, 2000 and Swaileh *et al.*, 2001a).

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## استخدام القواقع الأرضية كدليل بيولوجي لتقييم السمية البيئية للكيماويات الزراعية في المناطق الريفية


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**المعمل المركزي للمبيدات-مركز البحوث الزراعية- وزارة الزراعة-مصر**



استخدمت القواقع الأرضية (قوقع البرسيم) كدليل بيولوجي لتقييم السمية البيئية في محافظة البحيرة. خمسة مراكز (الدلنجات- كوم حماده- دمنهور- شبراخيت- إيتاي البارود) تم اختيارها لهذه الدراسة خلال فصلي الشتاء والربيع لعام ٢٠٠٨/٢٠٠٩ . إنزيم الأستيل كولين أستريز أظهر أعلى نشاط (٧١،٦٥ ميكرومول/مجم بروتين/دقيقة) في القواقع المجمعة من مركز دمنهور. بينما إنزيم لاكتيك دي هيدروجيناز وصل لأقل قيمة له (١٣،٧ وحدة إنزيم/مجم بروتين) في مركز إيتاي البارود. على الجانب الأخر إنزيم الجلوتاثيون إس ترانسفيراز أظهر انخفاض ملحوظ في القواقع المجمعة من مراكز الدلنجات- دمنهور- إيتاي البارود على التوالي وكانت القيم كالتالي (٦٣،٠١- ٧٣،٥٤- ٨٧،٩٩ %) مقارنة بالكنترول. وهذا يبين أن القواقع تعتبر وسيلة تشخيصية لتقييم التلوث والسمية البيئية.