Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(12):274-279

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Use of electrochemical technics as a tool for the evaluation of the in vitro mutagenic potential of abamectin pesticide

I. Ghenabzia[a], T. Lanez*[b], M. Didi Ould Elhadj[a] and R. Ahmedi[b]

[a]*University of Ouargla, ECOSYS Laboratory, Faculty of Natural and Life Sciences, Ouargla, Algeria*
[b]*University of El Oued, VTRS Laboratory, Faculty of Sciences and Technology, El Oued, Algeria*

ABSTRACT

The aim of this study isto evaluate the mutagenic and/or the carcinogenic potential of the pesticide "abamectin" which largely used in agriculture in the region of El-Oued (South Algeria), the mutagenic and/or the carcinogenic potential was evaluated by the detection of the adduct formed from the reaction of the pesticide and nucleosides. The products of the reaction of abamectin with nucleosides at physiological conditions were detected and quantified by cyclic voltammetric measurements and confirmed using reverse-phase HPLC. Also, the obtained results showed that only thymidine (T) and deoxyadenosine (dA) nucleosides can form adducts with abamectin which confirm its mutagenic potential.

Keywords: pesticides; carcinogen; mutagen, nucleosides; abamectin.

INTRODUCTION

Pesticides, which include insecticides, acaricides, fungicides, algaecides, herbicides, weedicides and bactericides, are among the most potentially hazardous compounds to human, animals, and the environment[1-3], they can damage the environment and accumulate in ecosystems which cause serious disturbances[4-8]. In fact these chemicals which are toxic by nature, and hence can contaminate the water we drink, the air we breathe, the food we eat and the earth on which we walk.Some pesticides are capable of interacting with cellular structures directly or after processing by metabolic enzymes, and form a covalent bond with DNA nucleosides to produce DNA adducts [9].

As demonstrated by various epidemiological studies [10-14], some types of cancer are increasing particularly rapidly if the environment (water, air, food, earth ...) is contaminated with pesticides.

The permeation of the active ingredient of the pesticide in the human body increases the development of the cancers cellules. This active material is fixed by covalent bond to nucleophilic sites present in the DNA and form adducts which can be mutagenic in a first time, then carcinogenic [15]. Hence, the detection of adducts can be a very useful tool for evaluating mutagenic and/or carcinogenic potential of pesticides. [16].

Till now, frequent methods were conducted to quantify DNA nucleosides using electrochemical technics at various electrodes materials (e.g., glassy carbon, carbon paste, and chemically modified electrodes) have been reported [17-22]. Moreover, the combination of chromatographic or electrophoretic methods with spectroscopic and electrochemical detections has been developed for the detection and quantification of nucleosides adducts [23-27].

This paper presents the use of cyclic voltammetry measurements to detect and quantified adducts obtained from the reaction of the pesticide abamectin and the nucleosides thymidine and deoxyadenosine respectively, the results was confirmed by HPLC analysis.

EXPERIMENTAL SECTION

Chemical

HPLC-grade acetonitrile, dipotassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Fisher Scientific Co. and used as received. Thymidine and Deoxyadenosine were purchased from Aldrich Chemical Co. and used as received. High purity water was used in all experiments. All other reagents used were of analytical grade.

Pesticide

Among several commonly used pesticides in the El Oued region, one is chosen for evaluation of its mutagenic potential,

Common name: abamectin
Commercial name: Avermectin
Molecular Structure:

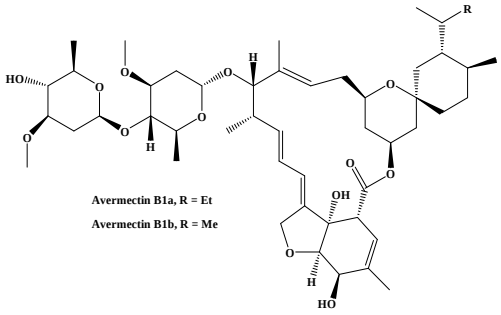

Figure 1. Molecular Structure of abamectin

Formula: $C_{48}H_{72}O_{14}$ (B1a), $C_{47}H_{70}O_{14}$ (B1b)
Active ingredient: abamectin
Concentration: 18 g/l
Dosage: 50-75ml/hl

This pesticide was supplied from Arysta lifscience SAA (France).

Instruments

PGP301 potentiostat with voltamaster 4 version 7.08 software (radiometer analytical SAS), rotary evaporator (IKA Evaporator RV 06-ML), high performance liquid chromatograph (LC 20 AL, (Shimadzu Scientific Instruments, Kyoto, Japan), UV-Visible spectrophotometer (PRIM Advanced SCHOTT Instruments Gmbh), Incubator (Heidolph Instruments, type Heizmodul, Germany).

Reaction of abamectin with nucleosides

Reactions of abamectin with nucleosides were carried out using phosphate buffer 0.05 M solution at pH 7.2 as a solvent. The general procedure was as follows: An excess of newly supplied abamectin (5 mM/L) was added to 25 ml of phosphate buffer containing the nucleosides: thymidine (1 mM/L) or deoxyadenosine (1 mM/L). The solutions were shaken until the reaction mixture become homogenous, then incubated at 37°C under agitation for 72 h. The reaction mixture was then analyzed using cyclic voltammetry and liquid chromatography technics.

Cyclic voltammetry

Cyclic voltammetric measurements were carried out using voltalab40 PGZ301 potentiostat/galvanostat (radiometer analytical SAS). Experimentations were made in a double walled electrochemical cell of 25 mL and conventional three electrode system was employed. Glassy carbon working electrode (radiometer analytical SAS), having area 0.013 cm^2, a platinum wire counter electrode, and an Hg/Hg_2Cl reference electrode (3.0 M KCl). Data acquisitions were accomplished with a Pentium IV (CPU 3.0 GHz and RAM 1 Gb) microcomputer using VoltaMaster4 software version 7.08 (radiometer analytical SAS). Graphs plot were carried out using OriginLab software version 2.0 (Integral Software, France).

Cyclic voltammetric measurements were run from 0.8 to 2.5 V. All measurements were carried out at room temperature (25 ± 1 °C).

Chromatography

This has been done through high performance liquid chromatographic (HPLC) technics to detect the formation of nucleosides adduct using chromatograms. Analyses were performed utilizing a high performance liquid chromatograph (LC 20 AL equipped with universal injector (Hamilton 25 µl) UV-VIS detector SPD 20A (Shimadzu). After incubation of the reaction mixture of nucleoside and pesticide for 72 hours at 37° C it was then analyzed under the following conditions: stationary phase, Shim-pack VP-ODS C18 (250 mm L. x 4.6 mm I.D., 5 µm). Flow rate: 1 mL/min. Oven temperature: 25 °C. UV detector: detection wavelength 260 nm. Injection volume: 20 µL. Run time: 50 min. A linear gradient was used for mobile phase as follows: linear gradient from 0% B to 12% B, 0-30 min; linear gradient from 12% B to 50% B, 30-50 min.

RESULTS AND DISCUSSION

Electrochemical results

Typical cyclic voltammograms for thymidine, deoxyadenosine, and their reaction mixture with abamectin using a potassium phosphate buffer system are shown in Figure 2 and 3. Both figures show an electrochemically irreversible oxidation at positive potentials. Voltammograms registered after 72 hours of incubation of the reaction mixture at 37 °C indicate clearly the decrease of the anodic current density of both thymidine and deoxyadenosine indicating the decrease in their concentration in the reaction mixture.

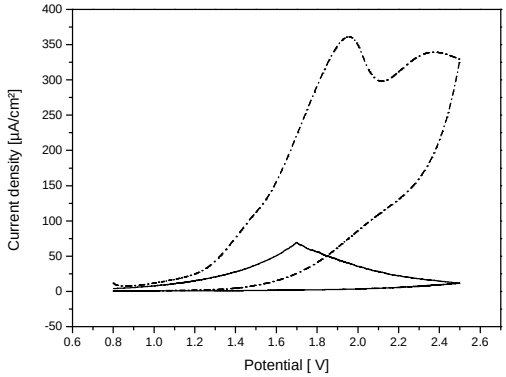

Figure 2. Cyclic voltammograms of 1 mM solutions of thymidine (dotted line) and thymidine + abamectin (full line) in 0.05 M phosphate buffer solution on a glassy-carbon electrode, pH= 7.2 at scan rate 100 mV/s

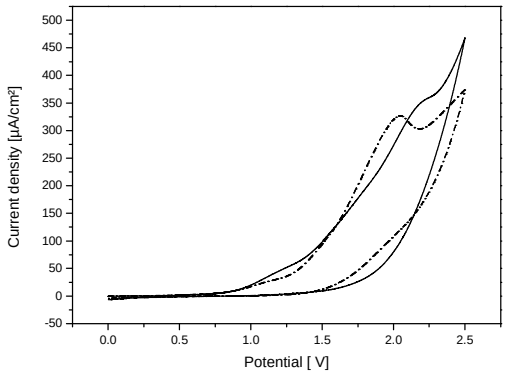

Figure 3. Cyclic voltammograms of 1 mM solutions of deoxyadenosine (dotted line) and thymidine + abamectin (full line) in 0.05 M phosphate buffer solution on a glassy-carbon electrode, pH = 7.2 at scan rate 100 mV/s

Chromatography results

Chromatograms obtained from HPLC analysis of the reaction mixture of abamectin and thymidine after 72 hours of incubation under agitation at 37°C yielded one major product together with a number of minor products (Figure 5). Comparison of the UV absorption spectrum of the major product to that of thymidine (figure 4) clearly identified this substance as a thymidine adduct. A second major peak was often present in the chromatograms of reaction mixtures. This compound was also found in control.

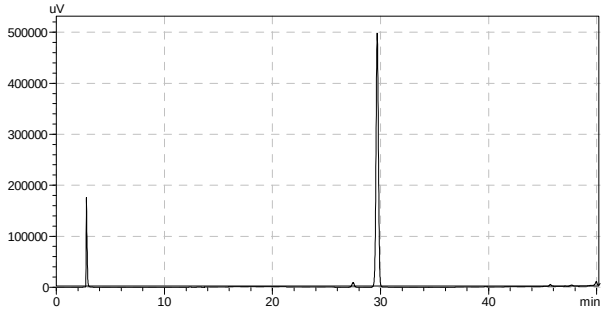

Figure 4: HPLC chromatogram of thymidine

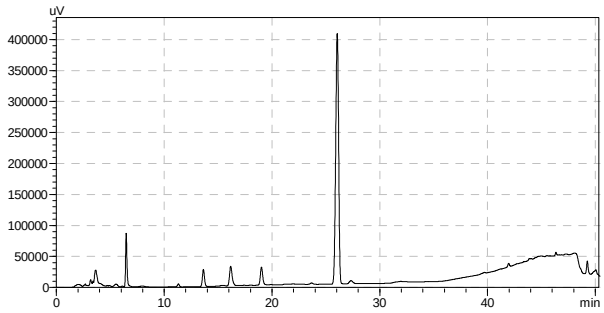

Figure 5. HPLC chromatogram obtained from the analysis of the reaction mixture of abamectin and thymidine after 72 h of incubation under agitation at 37°C

The same results were obtained from HPLC analysis of the reaction mixture of abamectin and deoxyadenosine. Comparison of chromatogram of the reaction mixture (figure 7) to that of deoxyadenosine (figure 6) indicate clearly the formation of a product which could be identified as deoxyadenosine adduct. The peak of deoxyadenosine was also present in the chromatogram of the reaction mixtures which can be explain by the low yield of the adduct formation as confirmed by electrochemical assays.

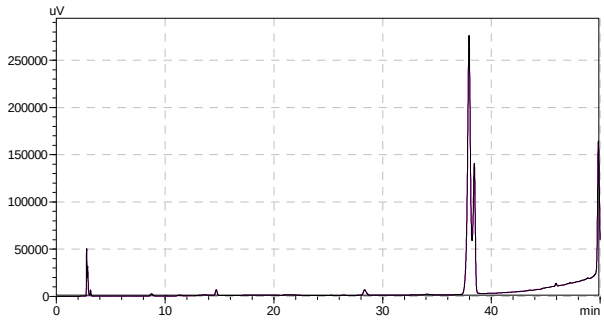

Figure 6. HPLC chromatogram of pure deoxyadenosine

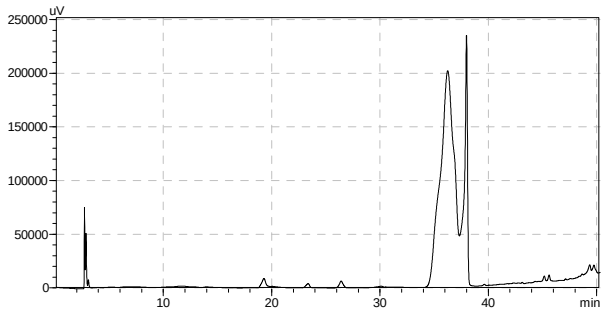

Figure 7. HPLC chromatogram obtained from the analysis of the reaction mixture of abamectin and deoxyadenosine after 72 h of incubation under agitation at 37°C

Both data obtained from chromatographic and electrochemical studies demonstrate the interaction between abamectin pesticide and nucleosides. Cyclic voltammograms presented in figure 2 and 3 indicate clearly the decrease in the anodic current density of nucleosides, this decrease is assigned to the diminution in nucleosides concentration in the reaction mixture. Further analysis of the reaction mixture by HPLC chromatography show clearly the formation of nucleosides adducts (figures 5 and 7).

The yield of the formation of the adduct obtained from nucleosides and pesticide can be calculated from electrochemical data using the following equation 1.

$$\text{Yield } \% = \left(1 - \frac{\mathrm{i}_a(t=0)}{\mathrm{i}_a(t=72h)}\right) \times 100 \qquad (1)$$

$\mathrm{i}_a(t=0)$, anodic pic current desity of pure nucleoside.

$\mathrm{i}_a(t=72h)$, anodic pic current desity of nucleoside in the reaction mixture after 72 hours of incubition at 37°C.

$$\text{Yield } \% = \left(1 - \frac{67.93}{359.93}\right) \times 100 = 81.13\ \%$$

The yield of the deoxyadenosine adduct formed by abamectin can also be calculated using formula of equation 1 as follows:

$$\text{Yield } \% = \left(1 - \frac{325.52}{353.63}\right) \times 100 = 7.95\ \%$$

CONCLUSION

In this paper, we have studied and evaluated the mutagenic potential of pesticide "abamectin"most used in agriculture in the region of El-Oued (South Algeria). The evaluation was achieved by the detection and analysis of the nucleosides adducts formed by abamectin using chromatographic and cyclic voltammetric analysis. Both technics revealed the interaction between nucleosides and pesticide. The yield of thymidine and deoxyadenosine adduct formed by abamectin, calculated using the decrease in the anodic pic current density was 81.13 and 7.95 % respectively.

Acknowledgements
This research was supported by the Algerian ministry of higher education and was administrated by VTRS laboratory under faculty of sciences and technology. The authors are grateful to Dr. Ahmed Chenna for many valuable discussions and insights provided during the course of this work. We are also indebted to Abdelkirm Rebiai and Ali Tliba for their help.

REFERENCES

[1] JJ Casarette; GC Freyer; WL Yauger; HW Klemner. *Hawaii Archives of Environmental Health*., **1968**, 17, 306-311.
[2] B Krauthacker; AK Tanja; M Kralj; B Thalcevic; E Reiner. *International Archives of Occupational and Environmental Health.*, **1980**,45, 217-220.
[3] P Anna; P Nandoor; F IIdiko. *Science of the total environment.*, **1988**, 73, 229-244.
[4] KP Cantor;A Blair;G Everett;R Gilsor;LF Brumeister;LM Brown. *Cancer Research.*, **1992**, 52, 2447-2455.
[5] A Ferrer;AM Bona;M Castellano;J ToFigueras;M Brunet. *Bulletin of Environmental Contamination.*, **1992**, 48, 561-566.
[6] YK Matuo;JNC Lopes;IC Carsanova;T Matuo;JLC Lopes. *State of Sao Pauto, Brazil Arch Environ Contam Toxicol*., **1992**, 22, 167-175.
[7] JJ Addy;LF Robert;P Alphouse. *Journal of environmental science and health part A: environmental science and engineering*., **1992**, 27, 967-981.
[8] GMH Swaen;C Varvliet;JJM Slangen; F Sturmans. *Scandinavian Journal of Work, Environment and Health*., **1992**, 18, 201-204.
[9] GS Garbellini;CV Uliana;H Yamanaka. *Journal of the Brazilian Chemical Society*.,**2013**,24(12), 1942-1949.
[10] SK Yadav. *Journal of Human Ecology*., **2010**, 32(1), 37-45.
[11] D Belpomme; P Irigaray; L Hardell;R Clapp;L Montagnier;S Epstein;AJ Sasco. *Environmental Research.*, **2007**, 105, 414-429.
[12] MB Schenker;SA McCurdy. *American Journal of Industrial Medicine.*, **1990**, 18, 345-351.

[13] BL Waddell;SH Zahm;D Baris;DD Weisenburger;F Holmes;LF Burmeister;KP Cantor;A Blair. *Cancer Causes Control*., **2001**, 12, 509-517.
[14] T Zheng;SH Zahm;KP Cantor;DD Weisenburger;Y Zhang;A Blair. *Journal of Occupational and Environmental Medicine*., **2001**, 43, 641-649.
[15] PA Oliveira;A Colaço;R Chaves;H Guedes-Pinto; PLF De-La-Cruz;C Lopes. *Annals of the Brazilian Academy of Sciences*., **2007**, 79(4), 593-616.
[16] TÇavaş;SKönen. *Mutagenesis*., **2007**, 22(4), 263-268.
[17] RN Sheng;F Cotton. *Analytical Chemistry*., **1991**, 63, 437-442.
[18] HS Wang;HX Ju;HY Chen. *Analytica Chimica Acta*., **2002**, 461, 243-250.
[19] P Wang;J Ren. *J Pharm Biomed Anal*., **2004**, 34, 277-283.
[20] DK Xu;L Hua;HY Chen. *Analytica Chimica Acta*., **1996**, 335, 95-101.
[21] WR Jin;HY Wei;X Zhao. *Electroanalysis*., **1997**, 9, 770-774.
[22] G Chen;QC Chu;LY Zhang;JN Ye. *Analytica Chimica Acta*., **2002**, 457, 225-233.
[23] S Zhao. *J Chromatogr B*., **2003**, 797(1), 63-90.
[24] B Kenneth;N Beckman;N Bruce. *J Biol Chem*., **1997**, 272, 19633-19636.
[25] A Chenna;A Perry;B Singer. *Chemical Research In Toxicology*., **2000**, 13, 208-213.
[26] A Chenna;CR Iden. *Chemical Research In Toxicology*., **1993**, 6, 261-268.
[27] A Chenna;RA Rieger;CR Iden. *Carcinogenesis*., **1992**, 13(12), 2361-2365.