ORIGINAL ARTICLES

Response of paramecium sp. with respect to an insecticide (Proclaim): growth, content of MDA, AChE activity and respiratory metabolism

BOUARICHA Houda, BERREBBEH Houria, GRARA Nedjoud et DJEBAR Mohammed Reda.

Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba, 23000, B.P. 12. Algeria.

ABSTRACT

In this work, we were interested to evaluate the potential toxicity of a bio-pesticide based benzoate emamectin newly licensed in Algeria, on an alternative model, a freshwater ciliated protist, Paramecium sp. The effects of benzoate emamectin, commonly designated by the Proclaim, are evaluated in part on the growth and respiratory metabolism, and on the other hand, on the levels of total protein, the rate of Malondialdehyde (MDA) and Acetylcholinesterase activity (AChE) of paramecium. The results obtained demonstrate a violation of the general metabolism of protists, translated by disturbances in the levels of total protein, increased level of MDA which is a specific biomarker of toxicity in organisms treated with benzoate emamectin, and also inhibition of the AChE activity. It is the same for the respiratory metabolism, where there has been a strong stimulation of oxygen consumption. Finally inhibition of growth and mobility of paramecia treated with benzoate emamectin is observed.

Key words: Emamectine benzoate, toxicity, Acetylcholinesterase, MDA, respiratory metabolism, Paramecium sp.

Introduction

The pesticides of the family of Avermectines were largely used throughout the world since 1980 (Schweitzer N, et al., 2012) in the field of sciences veterinary surgeons and medical, because of their effectiveness and their large spectrum of activity against parasitic endosomates and ectos in the cattle and the domestic animals (Garric J, et al., 2007). The discovery of Avermectines led to a true upheaval in the fight against certain parasitoses and in particular the filarioses (Boussinesq M. 2005), the stongyloïdose and the human scale (Marigny K, et al., 2001). Their uses as pesticides in agriculture was born since 1985 (Lasota J.A, Dybas R.A. 1991) and more than five billion products marketed based on avermectines were sold in the monde (Shoop W and Soll M. 2002), of which Emamectine benzoate.

The proclaim, an insecticide obtained by chemical synthesis starting from the acaricide abamectine, by substitution of a aminomethyl (NH-CH 3 by a hydroxyl group (- OH) on the level of carbon C4 (Heasook K, et al., 2004). This insecticide of chemical name " ear-méthylamino-4-deoxyavermectineB1 4-benzoate " is a mixture of a maximum of 90% of ear-methylamino-4 benzoate ' deoxyavermectineB1a(MAB1a) and one minimum of 10% of ear-methylamini-4-deoxyavermetine 4benzoate B1b(MAB1b) (EMEA. 2003). This molecule is classified in the group of the activators of the channels chlorinates, it acts on the level neuromusculaire, while being fixed in an irreversible way at the level of receiver GABA and on the receiver glutamate H (Syngenta. 2005), it results from it a cellular hyperpolarisation which blocks any nervous activity leading to an inhibition of the muscular contraction of the insect thus a paralysie (Bloomquist J. 2003).

Our study consists of the evaluation of the toxicity of Proclaim, on a biological model alternatif: Paramecium sp.

The use of the paramécie like model of study in certain disciplines was reported by several authors, in Ecotoxicology the paramécie was used to study environmental qualities and the toxic effects of agricultural, industrial and domestic origin (Benbouzid H, et al., 2012; Madoni P. 2000; Venkateswara JR, et al., 2006; Amanchi NR. 2010; Azzouz Z, et al., 2011), in genetics because of sound séquençage of the genome which is well connu.Les researchers used Paramecium tetraurelia pour élaborer genetic, the form of gene and the change (Gao X, 2010) in physiology, the paramécie is used in general to study the role, the function and the cellular organization (Hemmersbach R et al., 2001; Monayez Z, et al., 2004). A recent study with made it possible to highlight a NADPH-diaphorase activity in the Pramecium primaurelia (Amaroli A, et al., 2006), it was shown as well as the paramécie primaurelia synthesizes functional molecules connected to system GABA.
erge and cholinergic (DelmonteCorradoM.U, et al., 2002), which makes of it an excellent model of study in neurotoxicity.

The objective of our work relates to the evaluation of the potential toxicity of the biopesticide based on Emamectine benzoate, and the follow-up of the biochemical parameters characteristic of the stress oxidizing, of which content of MDA, the neurotoxicity by the measurement of Acétylcholinestérase, and finally the respiratory metabolism of the protists used.

Materiel And Methods

2.1 Biological material:

In our work, we used the paramécie, P aramecium sp. It is fresh and stagnant water a micro-organism, a well-known kind of the cilia.

2.2 Chemical material:

The insecticide used in our experiments is Proclaim, composed based on Emamectine benzoate, of which the chemical structure is represented in figure 01.

![Chemical structure of Emamectine benzoate](image)

Fig. 1: Chemical structure of Emamectine benzoate.

2.3 Méthode of treatment

The aliquot ones of 100ml of paramécies are treated with increasing concentrations of insecticide, 0,5 \( \mu \text{M/L} \); 1,5 \( \mu \text{M/L} \); 3\( \mu \text{M/L} \) and 4,5 \( \mu \text{M/L} \).

The kinetics of growth of the paramécies is carried out by counting of the cells under optical microscope of type LEICA DM 1000, by using a magnetic meter, and this après fixing with Lugol's solution (Beisson J, et al., 2010).

2.4 Measure biochemical and enzymatic parameters:

The proportioning of total proteins is carried out according to the method of Bradford (1976). Le proportioning of malondialdehyde is carried out according to the method To drape et Hadley (Draper H.H, Hadley M. 1990) and the proportioning of the acétylcholinestérase activity is carried out according to the method of Ellman et al. (1961) modified by Trielli stall (2006)

The respiratory activity is measured thanks an electrode to oxygen, of type HANSATECH (2000).

The statistical analysis of the variance to a controlled factor (Test T of Student) is used to consider the differences reported (Dagnelie P. 1999).

3.1 Effects of Emamectine benzoate on the cell multiplication:

Figure 02 gathers the variations of the cell multiplication of the paramécies treated by the concentrations of Proclaim.

It is noted that the number of paramécies temoins is practically constant during all the duration of the experimentation. The treatment Proclaim causes a strong inhibition (50%) with the weakest concentration and ½ H of traitement,il in is the same for all the other concentrations of proclaim. With the strongest concentration of this last one notes an inhibition of 75%, always after ½ H of treatment, it is significant to note on this figure which Proclaim causes a significant fall (75%) and rapid (½ H) of paramecium sp.
Fig. 2: Kinetics of growth of the paramecium (Contol and treated) with the concentrations of Proclaim.

3.2 Effects of Proclaim on contents of total proteins:

Figure 3 represents the variations of total proteins according to the various concentrations of Proclaim on Paramecium sp. The treatment by Proclaim causes a strong stimulation of the content of total proteins directly related to the concentration of this last, this saturation of total proteins according to the time and reached with the strongest concentration of Proclaim, an increase of almost 40% compared with the control cells.

Fig. 3: Effects of the various concentrations of Proclaim on total proteins of the paramecium.

3.3 Effects of Proclaim on the respiratory metabolism:

Figure 04 illustrates the effects of the various concentrations of Proclaim on the respiratory metabolism of the paramécies. On notes that the treatment by Proclaim tends to strongly stimulate the respiratory activity of the treated cells, particularly with the strongest concentration, where it reached the 80%. Ainsi, the treated cells present a rather significant respiratory activity, translated by a significant oxygen uptake.
Fig. 4: Effects of concentrations from Proclaim on the metabolism respiratoire

Table 1: Effects of the various concentrations of Proclaim on the contents of Malondialdehyde (MDA) at the paramecium:

Table 1: gathers the variations of the rate of MDA obtained at the paramecies after treatment by the concentrations of Proclaim at times 24, 48, and 72h.

<table>
<thead>
<tr>
<th></th>
<th>0µM/L</th>
<th>0,5 µM</th>
<th>1,5 µM</th>
<th>3µM</th>
<th>4,5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>0,0249±0,00015</td>
<td>0,0264±0,000556</td>
<td>0,0306±0,000153</td>
<td>0,0339±0,0001</td>
<td>0,0497±0,0001</td>
</tr>
<tr>
<td></td>
<td>P ≤0,05 *</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
</tr>
<tr>
<td>48h</td>
<td>0,0362±0,00025</td>
<td>0,0354±0,00001</td>
<td>0,0378±0,000152</td>
<td>0,0573±0,000152</td>
<td>0,0877±0,0001</td>
</tr>
<tr>
<td></td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≥0,05</td>
<td>P ≤0,001 ***</td>
</tr>
<tr>
<td>72h</td>
<td>0,0451±0,0002</td>
<td>0,0644±0,0002</td>
<td>0,057±0,00770</td>
<td>0,0728±0,0002</td>
<td>0,0864±0,00152</td>
</tr>
<tr>
<td></td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
<td>P ≤0,001 ***</td>
</tr>
</tbody>
</table>

The treatment by Proclaim tends to increase the contents of MDA at the three processing times (24, 48, 72h). At the strongest concentration of xenobiotic, the contents of MDA obtained are practically twice higher than at the IL witnesses than the rate of MDA obtained with 0,5 is however necessary to note and 1,5 µM remain close to that recorded at the control cells.

Effects of Proclaim on the acetylcholinesterase activity (AChE) at the paramecium:

The results obtained relating to the effects of the treatment by the proclaim on the AChE activity at the paramecium are gathered in table 2.

Table 2: Results obtained relating to the effects of the treatment by the proclaim on the AChE activity at the paramecium.

<table>
<thead>
<tr>
<th></th>
<th>0µM/L</th>
<th>0,5µM/L</th>
<th>1,5µM/L</th>
<th>3µM/L</th>
<th>4,5µM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>3,65±0,0408</td>
<td>3,19±0,0405</td>
<td>2,09±0,0439</td>
<td>1,29±0,0445</td>
<td>0,76±0,0451</td>
</tr>
<tr>
<td></td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
</tr>
<tr>
<td>48h</td>
<td>4,49±0,621</td>
<td>3±0,5</td>
<td>1,29±0,005</td>
<td>0,61±0,05</td>
<td>0,75±0,0048</td>
</tr>
<tr>
<td></td>
<td>P &lt;0,01 **</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
</tr>
<tr>
<td>72h</td>
<td>4,53±0,05</td>
<td>2,32±0,043</td>
<td>0,81±0,047</td>
<td>0,62±0,053</td>
<td>0,46±0,059</td>
</tr>
<tr>
<td></td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
<td>P &lt;0,001 ***</td>
</tr>
</tbody>
</table>

According to this table, one notes that the treatment by Proclaim with tendency to inhibit the AChE activity in a significant and significant way, since it reached here nearly 80% with 24h, 85% ±48h and nearly 90% with 72h, and this with the strongest concentration of Proclaim.

It should be noted however that the AChE activity remains very sensitive to the treatment by Proclaim, since we record an inhibition of this one as of the weakest concentration used.
Discussion:

The pesticides have specific characteristics which distinguish them from the other chemical agents used by the Man. Those relating to mainly their intentional introduction into the environment, their inevitable circulation in the biosphere, their possible exposure has very broad populations like their high biological reactivity.

Up to what point do the answers of the cellular biomarkers studied in this work make it possible to highlight the presence or the effect of a toxicity?

The results obtained in our work show that the toxicity of Proclaim is expressed initially by an inhibition of the cell multiplication to the strongest concentrations and this following the penetration of the poison inside the cell (Beaumont, 1998). Our results are in agreement with those of Einicker-Spangled and et al., (2002) which studied the toxicity of zinc and copper on Euglena Gracilis and those of Rouabhi, (Rouabhi R. 2006) which highlighted an inhibition of the growth of the paramécies following a treatment by two insecticides, and Sbartai and et al. (2009), which studied the effect of Bifenzate on the ciliés protists. The study of Garric and et al.,(2007) confirms the toxicity of a pesticide the ivermectine on Daphnia magna and an green alga Pseudokirchneriella subcapitata and that by the inhibition of the growth from these two espèces. Une study undertaken by Jawich (2006) concernant the toxicity of pesticides with respect to two kinds of yeasts, revealed an inhibition and a death of these micro-organisms.

In our study we highlighted an increase in the rate of the total proteines among protists proportions dependent in the presence of Proclaim, and time. These results go in the same direction as those of Piccini and et al., (1994). Massaya and et al., (2002) highlighted a significant increase of total proteins under the effect of a chemical stress in the cilia protists and rabbits. In the study of Sbartai and et al., (2009). The toxic demonstrations of the bifenzate on the paramécium, we note an induction proportions dependent on the synthesis total proteins. Following a treatment carbamate on marine organizations, Radwan and et al., (2010) record strong contents of proteines. According to Zaouani (2010), an induction of the carbonyl proteines in the rat Wistar treated by ingestion of Proclaim is observed.

The toxicity of insecticide studied at the paramecium is at the origin of an increase in the rate of (MDA), principal actor of the peroxidation of the fatty acid poly unsaturated in the membranes. Il is also under product of the biosynthesis of the prostaglandin (Coeurdassier M, et al., 2001). ROS can oxidize lipids (Ercal N, et al., 2001; Tweeddale H.J., et al., 2007), and the lipidic peroxydation is followed of a structural change of the biological membranes as well as other elements containing of lipids (Bebianno M.J, et al., 2005; Al-Mutairi D.A, et al., 2007). Il follows a loss of the permeability and potential of the membrane, as well as an inactivation of the membrane receivers (Pamparin D.M, et al., 2005), being able to lead to dead of the cells. Thus, the lipidic peroxidation is an endogenous source of the damage of the ADN (Marnett L.J, 2002).

Results obtained in our work and those of Djekoun and et al., (Djekoun M. 2012) which studied the toxic effects of environmental industrial pollutants on micro-organisms: sacharomyces cervisiae. Are convergent,

These results suggest an accumulation of the xenobiotics leading to a disorganization of the membran systems probably in relation to the installation of a state of oxdative stress. It noted a simultaneous fall of the lipid level with an increase in the rate of MDA which could be due to a significant reduction in the polyninsaturés fatty acids and to the peroxidation of the acids linoleic and linolenic. Similar results were reported by Ouariti and et al., (Ouariti O, et al., 1997) in tomato plants after exposure to cadmium.

The exposure of youthful Daphnia magna to the menadione and the endosulfan show a high level of the lipidic peroxydation and weak enzymatic activities antioxidant (Barata C, 2004).

Proclaim exerts a neurotoxicity by the inhibition of the neurotransmission of the nervous system peripheral (McKellar Q.A 1996; Willis K.J,Ling N. 2003; Syngenta, 2007).

It was shown that Paramecium primaurelia synthesized molecules related to system GABA ergic and cholinergic (DelmonteCorrado M.U, 2002). Following pharmacological experiments undertaken by using drugs cholinomimetic and the inhibitors of the acetylcholinésterase, it was shown that the molecule acetylcholinesterase could be implied in the modulation of the actions inter cellular leading to the process of sexual conjugation (DelmonteCorrado M.U, et al., 1999; Delmonte Corrado M.U, et al., 2001; Delmonte Corrado M.U, 2005; Trielli F, 1997).

The results obtained from the rate of AChE in our treatment during the various experiments on the paramécies, highlight a reduction amount-dependent on the activity of this one as of the weakest concentrations of Proclaim, which constitutes an indicating signal of oxidizing stress.

The irreversible effect of Proclaim in this work would be with its mode of action because it interferes with the operation of the nervous system like antagonists of a neurotransmitter (synapses GABA) and of a receiver (Glutamate H) on the muscular surface entrainant an irreversible relaxation (Syngenta 2005).

The study of Bucci, (2005) bearing on the participation of the GABAA complex in the control of the stroke of the ciliés Protozoa, showed that the GABAA complexes were pharmacologiquement activated by a selective agonist the muscimole, and effects on the stroke of Paramecium primaurelia. are then analyzed. The
paramecium stroke normally ahead, but the activation of the GABAa receiver induces a particular answer, characterized by alternate periods of tourbillant. These effects are inhibited by the antagonists of GABAa the bicuculline and picrotoxine.

The treatment of the paramécie by Proclaim induces a disturbance in the locomotor behavior of the protists, and that while acting on the GABAa system. Knowing that the proclaim is an antagonist of receptor GABA, it will be fixed in a irreversible way at this site.

The EMB is a depressor which inhibits the neurotransmission in the peripheral nervous system by increasing the permeability of the membranes of the nervous and muscular cells to the ions chloride, involving a constant hyperpolarisation and an elimination of the nerve impulse resulting in the paralysis and death (McKellar Q.A et Benachaoui H.A 1996).

Trielli and et al. (2006) studied the impact of compounds organophosphorés (basudin, cidial and fenix) on terrestrial protists Colpoda will inflata (Ciliophora, Colpodiae) by using the spectrophotometric analysis (Trielli F, et al., 2006). These researchers highlighted the presence of three activities of the cholinestérases type, and the BChE activity misses practically. This result is in agreement with the duality between AChE and BChE probably originating in the gnatostomes (Massoulé J, et al., 1993). The cholinergic activity present in the terrestrial protist Colpoda inflata is very weak, it east can sensitive to the inhibitors used during the experimentation, and it could not reduce by organophosphates not tested, in other words, the têtés pesticides could not act on the cholinergic system which represents the alternative target of the organophosphates (Casida J.E and Quistad G.B. 2004).

Our results are in agreement with those of Barata and et al., (2004), which Daphnia magna exposed with two organ phors molecules and a carbamate, the malathion, the chlorpyrifs and the carbofuran. The work relates to the evolution of the acetyl cholinestérase activity and carboxyl esterase, with inhibition of the enzymes: the carboxyl esterase which more sensitive to are organophosphates than the acetyl cholinesterase, while these two enzymatic activities showed sensitivities equivalent to the carbofuran (carbamate). Moreover work of Falugi et al. (2002), revealed that the treatment of Dicyostelium discoideum with a neurotoxic organophosphate molecule the basudin, causes a diminution amount dependent on the activity of the propinocholinesteras.

Trielli and et al., (2007), highlighted a reduction in the cholinestérase activity to a significant degree at Euplotes crassus, in the Eukaryotes micro-organisms treated with the xenobiotic neurotoxin ones (basudine, HgCl2 AFD 25) (Trielli F, et al., 2007).

The micro-organisms is avid of oxygen, it is the essential element for their life, thus at the time of the elimination of xenobiotic, the electrons produced at the time of the detoxification of the organization by the cytochromes P450 (CYP450), will react with oxygen (Guengerich.; 1991). Oxygen can also react with the electrons which escape the respiratory chain (Lock and et al., 1977). The direct combination of the oxygen brought to the cells with these electrons implies the formation of an anion superoxyde which is at the origin of the radicalizing phenomena, this last then can dismuter either spontaneously, or in an enzymatic way to give hydrogen peroxide (H2O2, which can in its turn be transformed into radical hydroxyl °OH, most reactive of the free oxygenated radicals (ROS).

In our work, the results indicate a fall of cellular breathing at the paramécies treated with the strongest concentrations of Proclaim, and this as of the weakest concentration and after 48H of treatment insecticide.

Our results go in the same direction of those (Druez and et al., 1989) which tested the effect of Gossypol on morphology, the mobility and the metabolism of Dunaliella bioculata (whipped Protiste) considered as a cellular model. An identical result is reported in the study of Rouabhi, (2006) which studied the effect of Diflubenzuron and Flucycloxuron on the morphology and the physiology of Paramecium sp (Druez D , et al., 1989).

Certain effects of Chromium (Cr) on the energy metabolism of Protist heterotrophic, Euglena gracilis have summer brought back by Jasso-Chavez and et al., (2010), in these cells chromium accumulated, the synthesis of cystein and the glutathione was induced, as well as the inhibition of the breathing of the insulated mitochondries.

Addition of Cr (VI) or Cr (III) with mitochondries isolated or directly in the induced cytosol of the cultivated cells an inhibition from breathing. Protein Lesteneurs of the mitochondriaux complexes I, III, IV and V, and the receivers ANT(Adénosine nucleotide translocase) decreased in the presence of Cr (VI) in the culturescellulaires. The authors suggest that the cytotoxicity of Cr (VI) would have entrainé the reduction in breathing and glycolysis and consequently, a fall of the cellular ATP.

It was highlighted which the extremely toxic cadmium at several Protists with des concentrations as weak as 0.5 mM at the algae and 2.5 mM at protozoairesciellés (Kaplan et al., 1995). At the watery organizations Ivanina and Sokolova, (2008), At the watery organizations Ivanina and Sokolova, (2008) studied the exposure of the oysters Crassostrea virginica has 50 ig L -1 Cd for one period going from 30 to 40 days, leresultats show that Cd involved a significant rise in the rate insulated deconsommation of oxygen on the level of the gills of oysters (46%) (Ivanina A.V, Sokolova I. M. 2008).
The transfer of the electrons between complexes I (NADH FMN deshydrogenase, ND) and II (succinate deshydrogenase, SD), on the one hand, and complexes III and IV (cytochromes has, B and c) of the other, called upon the coenzyme Q mitochondrial (ubiquinone, UQ), reduced in ubiquinol (UQH$_2$) at the level of the complexes I and II and oxidized in its radicalizing form ubisemiquinone, UQH$^\circ$, then in form ubiquinone during the transfer of the electrons to the complexe III. At the time of this cycle, part of the electrons escapes starting from the radicalizing intermediate form, UQH$^\circ$, of the coenzyme Q, to react directly with the oxygen dissolved in the cytoplasm and to form the anions radicalizing superoxydes, O$_2^\circ$ (Lock and Al, 1977).

References


Barata, C., S. Arun, P. Cinta, 2004. Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to Daphnia magna. Aquatic Toxicology, 66: 125-139.

Beaumont et Cassier., 1998. Travaux Pratique de Biologie Animale, Zoologie,Embrylogie, Histologie, 3 ème édition DUNOD.


Syngenta. Proclaim productsheet. 10: 2-6.


