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ORIGINAL ARTICLES

Evaluation of the biomarkers of the oxidative stress induced by a biopesticide: The Spinosad on an alternate model: *Helix aspersa*

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ABSTRACT

This work relates to the evaluation of the potential toxicity of a biopesticide Spinosad lately approved in Algeria on a gastropod *Helix apsresa.* The snails are exposed to increasing concentrations of Spinosad by topical application during 28 days. We sought to evaluate the oxydative stress on the level of digestive gland at *Helix aspersa* by the follow-up of activity GST, as well as the activity catalase, and the rate of reduced Glutathion. Moreover Malondialdehyde (MDA) considered as a marker of the cellular damage was also measured. The neurotoxicity of Spinosad was given by measuring the activity of the acetylcholine estérase (AChE). The results obtained show that the rate of reduced Glutathion (GSH) decreases in a manner proportions dependent accompanied by an induction on the activity Glutathion S transférase (GST) at the organizations treated compared to the witness. In in parallel, one attends an induction of the catalase activity in the presence of Spinosad. Concerning the contents of Malonedialdehyde (MDA), we note a stimulation in snails treated with the biopesticide compared to the control, one could highlight a neurotoxic effect of Spinosad translated by a progressive reduction in the Acetylcholinesterase activity (AchE) at the organisations treated compared to the control.

Key words: oxidative stress, CAT, GSH, GST, MDA, *Helix aspersa,* toxicity, biopesticide.

Introduction

The biopesticides correspond to a pesticide resulting from natural matters such as the animals, the plants, the bacteria and certain minerals. For example, the oil of canola and bicarbonate of soda have pesticides applications and are regarded as biopesticides. With the end of the year 2001, there were approximately 195 active ingredients registered voters biopesticides and 780 products. Among these pesticides, one site Spinosad .

Spinosad is the first member of the class Naturalyte Dow Agro science of insecticides and it is a mixture of neurotoxines will tétra macrolides cyclic, spinosyne A and D, produced during the fermentation of the actinomycète of the ground, *Saccaropolyspora spinosa* As such, it can be regarded as an bio-insecticide (Copping LG, Menn JJ. 2002), approved for use on more than 100 cultures in 24 countries (Thompson GD, Dutton R, Sparkes TC. 2000). Spinosad is highly toxic for the lépidoptères, dipterous and certain species of coleopters and has a single mode of action implying nicotinic acetylcholine post synaptic and receiver GABA (Watson GB 2001). This microbial insecticide acts like a poison of contact and stomach and is degraded quickly in the environment (Cisneros J, *et al*., 2002; Crouse GD, *et al*., 2001). Spinosad has a considerable toxicity of contact against the harmful organizations of the stored by-products (Toews MD, Subramanyam B. 2003).

The reactive species of oxygen (ERO), produced normal of the aerobic metabolism, are at the origin of cellular damage when they are produced in excessive quantity and that they cause an oxidizing stress. According to Storey (Storey KB 1996), the cytotoxicity of the reactive species of oxygen, three levels of protection are possible: prevention of the formation of ERO, the neutralization of the ERO by production of antioxydant defenses and compensation for the cellular damages generated after the oxidizing stress.

Consequently, the purpose of the present study is to evaluate if the answers related to the oxydative stresses measured in the snails *Helix aspersa* can be used as biomarqueurs of exposure relevant for the biopesticides.

Material And Methods

Chemical material:

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Spinosad is a mixture of two spynosynes A and D (85% of spynosineA, 15% of the spynosine D) is marketed under the name of Spinosad (To trace 480/l). The Solution mother and the dilution of Spinosad were prepared with distilled water.

Biological material:

The snails *Helix aspersa* weighing 6 ± 0,05 g, were collected during spring 2011 of a not polluted zone Seraidi located at Northeastern Algerian to 600 m of altitude. The snails are high under the controlled conditions of laboratory a photoperiod of 18h of light / 24h, a temperature of 20°C, a moisture from 80 to 95% and nourished with the flour of corn in limp of polystyrene transparent and perforated, with a wet sponge for the conservation.

Treatment :

Toxicity by way topics of Spinosad on the snails *Helix aspersa* is evaluated by using a method of topics application according to the method of (Hussein HI, *et al*., 1994; Radwan MA, *et al*., 2008).

For the preparation of the solutions mothers we took into account the active ingredient diluted in 1 ml of demineralized water. The increasing concentrations tested of Spinosad are: 25, 50,100 et150µg / snail.

For each treatment we chose 5 animals, the tests were repeated 3 times, and 60 µl of the prepared solutions are delicately applied once per day to the surface of the body of snail inside the shell by using a micropipette. The pilot animals receive 60 µl distilled water.

Preparation of the samples:

For the measurement of the biochemical biomarqueurs, after 7, 14 and 28 days of treatment, the snails are sacrificed after congelation.

•After dissection the hepatopancreas is withdrawn, and divided into four fragments, respectively, for the evaluation of the biochemical biomarqueurs (GSH, GST, CAT, MDA)

•For the proportioning of the acetylcholinesterase activity (AChE) we took the head of the animals

Measured parameters:

The activity Catalase (CAT) is directly analyzed by the method of Regoli and Principato, (Regoli F, Principato G 1995). The reduced glutathion is estimated by the method of Weckberker and Cory, (Weckberker G, Cory G 1988), the activity of glutathion-S-transférase (GST) is proportioned to 340 Nm by the process of Haibig and *et al.,* (Habig WH, 1974) And Malondialdéhyde MDA is determined by the method To drape & Hadley, (Draper HH, Hadley M. 1990), finally the activity of the acetylcholinesterase are measured by the method of Ellman and *et al.*(1961).

Stastical study of results:

The stastical analysis of the variance with two controlled factors (ANOVA) is used to consider the differences reported for the various studied parameters.

Results:

Figure (01) illustrates the variations of the rate of Glutathion reduced in the presence of increasing concentrations of Spinosad to the level of the hepatopancreas. Thus in the presence of xenobiotic, one notes that the rate of GSH in the hepatopancreas of snails decreases in a manner proportions dependent particularly for the strong concentrations, after 7 and 14 days of treatment the percentage of reduction reaches 7,29; 15,87% for snails treated respectively by concentrations 100 and 150 µg. after 28jours of treatment this rate pass to 57,12; 59,40% for the strong concentrations.

The analysis of variance ANOVA to two criteria of classification indicates differences very highly significant (P=0,001) for the factor treatment and the factor time and for the interaction time / treatment.

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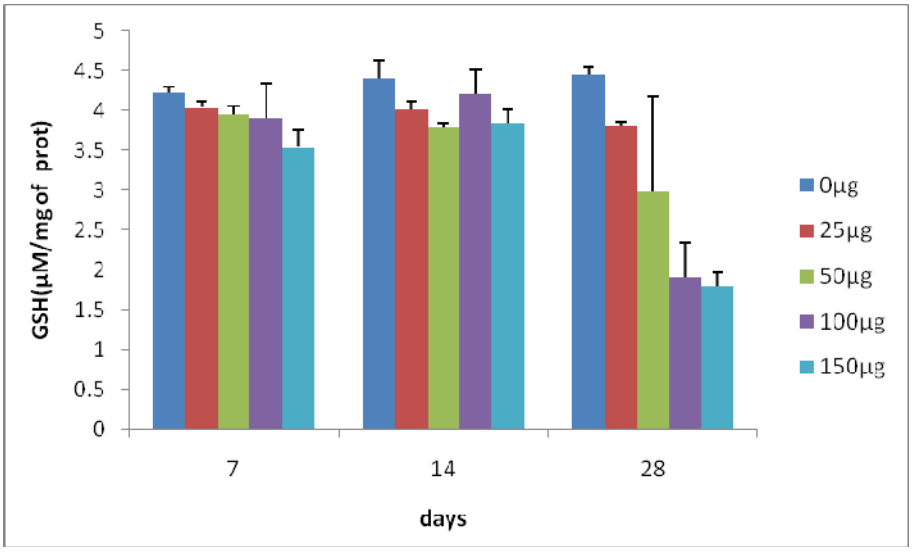

Fig. 1: Variations of the GSH in digestive gland of *Helix aspersa* after 7, 14 and 28 days of exposure to increasing concentrations of Spinosad.

The exposure of snails to increasing amounts of induced Spinosad of the modifications of the activity of Glutathione S transferase measured to the level of the hepatopancreas (figure 2). We note, indeed, that the activity of the GST tends to increase with there too an amount-answer effect.

Indeed, the percentages of increase passes from 9,68; 17,01% after 7 days with 54,12 84,73% after 14 days and reached a high rate of 86,66; 93,26% after 28 days for snails treated respectively by strongest concentrations 100 and 150 µg.

The statistical study indicates differences very highly significant (P=0,001) for the factor treatment and the factor times and highly significant with (P=0.01) for the interaction time / treatment.

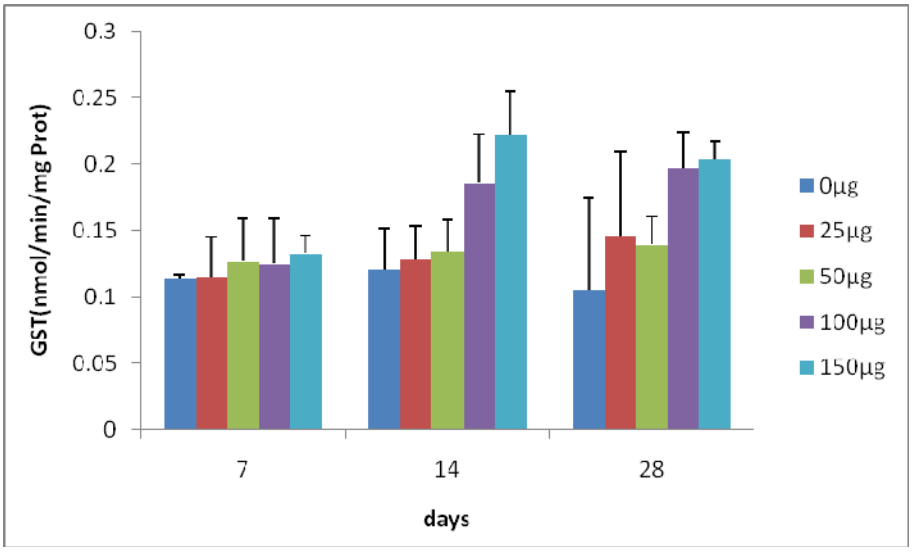

Fig. 2: Variation of activity GST in digestive gland of *Helix aspersa* after 7, 14 and 28 days of exposure to increasing concentrations of Spinosad.

Figure (03) illustrates the variations of the Catalase activity under the effect of the biopesticide on the level of the hepatopancreas. Our results show an increase proportions dependent. On the level of the hepatopancreas the percentages of increase passes from 22,03; 41,73;51,71% after 7 days with 53,77;49,59;84,82% after 14 days and reached after 28jours a high rate of 91,28;91,40;98,21% afterwards for snails treated respectively by the concentrations 50;100; 150 µg.

The statistical analysis reveals differences very highly significant (P=0,001) for the factor treatment.

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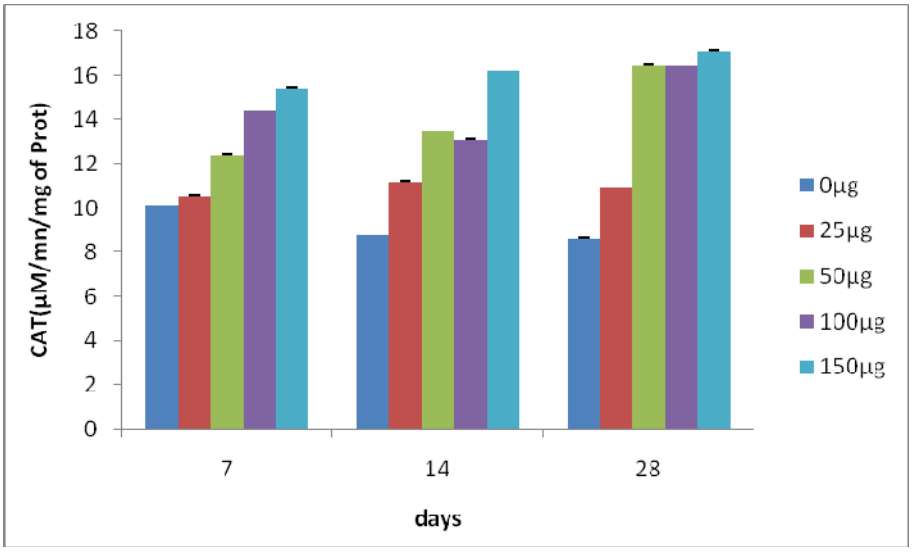

Fig. 3: Variations of the Catalase activity in the digestive gland of *Helix aspersa* after 7, 14 and 28 exposure days to increasing concentrations of Spinosad.

Table (1) represents the variations of the rate of MDA on the level of the hepatopancreas in the presence of Spinosad. We note that this rate tends to increase in a manner proportions – dependent. The percentages of increase passes from 28,67; 59,47% after 7 days with 33,20; 74,73% after 14 days and reached after 28jours a rate of 49,35; 82,36% afterwards for snails treated respectively by concentrations 100 and 150 µg.

The statistical study indicates differences very highly significant (P=0,001) for the factor treatment and significant with (P=0,05) for the factor times and highly significant with (P=0.01) for the interaction time / treatment.

Table 01: Variation of the MDA in the digestive gland of *Helix aspersa* after 7, 14 and 28 of exposure to increasing concentrations of Spinosad

parameter	days	Concentration of Spinosad (µg/escargot)				
		0	25	50	100	150
MDA (µmol /mn /mg Prot)	7	133,04 ± 1,71	133,98±9,6	120,60±1,3	171,20±28,71	212,16±32,51
	14	132,52 ±0,6	137,48±11,81	150,59±26,57	176,52±23,77	231,57±26,2
	28	134,59 ± 2,93	121,35±2,63	122,62±1,44	201,02±1,82	245,45±2,1

Table (2) represents the variations of the AchE activity measured on the level of the head of snails at various durations to Spinosad.

We note that in the presence of xenobiotic, the acetylcholine esterase rate tends to decrease in a manner proportions – dependent, and the percentage of this reduction corresponds to 30,95; 30,88; 50,09% for snails treated respectively by concentrations 50, 100 and 150 µg.

The results of the analysis of the variance indicates differences very highly significant (P=0,001) for the factor treatment and the factor time and for the interaction time / treatment.

Table 02: variation of the acetyl cholinesterase activity on the level of the head of snails after 7, 14 and 28 days of exposure increasing concentrations of Spinosad.

Parameter	days	Concentration of Spinosad (µg/snail)				
		0	25	50	100	150
AChE (µmol /mn /mg Prot)	7	40,04 ± 0,09	40,08±0,02	40,01±0,03	30,97±0,14	30,94±0,08
	14	40,02±0,1	40,03±11,81	40,02±26,57	30,82±23,77	30,72±26,2
	28	40,07 ± 2,93	30,75±0,1	20,47±0,03	20,49±0,04	20,00±0,06

Discussion:

The xenobiotics ones are one of the major causes of oxidizing stress. In the invertebrates, the marine molluscs were largely used like model of study to highlight biomarqueurs of environmental pollution. Thus, Borcovíc and *et al*., (2005). showed that the production of antioxydant enzymes in the mussel Mediterranean *Mytilus galloprovincialis* was influenced by the presence of industrial pollutants in the environment.

The measurement of the activity of the antioxydant enzymes thus constitutes a marker of the disordered state of the biological systems which makes it possible to indirectly evaluate the antioxydant statute of an

individual. Measurements of the nonenzymatic activities but also of form of sensitive genes are also markers of this antioxydant statute.

In physiological condition, oxygen, element essential to the life, produce permanently on the level of mitochondrion of the activated oxygenated species (EOA) to which belong the free radicals; these last are equipped with oxidizing properties which lead them to react, in the environment where they are produced, with a whole series of biological substrates.

To protect itself from this toxic effect of oxygen, the organization develops systems of defense which make it possible to control the production of the EOA. Among these antioxydant systems of defense one finds the glutathion (an intracellular tripeptide representing the major part of nonproteinic thiols), the CAT, which reduces hydrogen peroxide out of water, of the enzymes related to the GSH, GPx, GST and GR. In addition, the MDA is a breakdown product of the reactions of lipidic peroxidation It is used like indicator of oxydative stress at the vertebrate ones and the invertebrates (Pampanin DM, *et al*., 2005). La majority of the biomarqueurs was developed and validated at like model of study of the various compartments (Livingston DR 1991; Ribera D. 1998).

The glutathion is the major nonenzymatic antioxydant in the animal cells, it is the most abundant cellular thiol, implied in the metabolism and the processes of transport and in the protection of the cells against the toxic effects of the endogenous and exogenic compounds, including the reactive species of oxygen and heavy metals (Taniguchi M, *et al*., 1989).

The GST are enzymes of biotransformation of phase II, whose function is to combine with a molecule of glutathion a large variety of substrates to allow their elimination. These substrates can be molecules endogenous, but also the xénobiotiques ones like the HAP, the PCB and the pesticides. They indeed have an interesting activity as a biomarquor of contamination, in particular by the lipophilic organic contaminants of types HAP, PCB and pesticides (Narbonne JF *et al*., 1991).

Our results highlight a reduction in the rate of the GSH, and an increase in activity GST this increase is a response to the oxidative stress caused by the presence of Spinosad in the body (Farombi E, *et al*., 2007).

The strong reduction in the rate of the GSH could be explained by a direct réaction/liaison of the biopesticide with the glutathion, indeed the groupings carboxyls of the glutathion (amine group, sulfhydrile group (- GH) as two peptides) are combined with the xénobiotique one (Galaris D, Evangelou A. 2002). This interaction glutathion-Spinosad takes place thanks to the intervention of the GST which allows the conjugation of xenobiotic or its metabolites with the GSH during phase II of the metabolisation.

Of the another with dimensions reduction of the rate of GSH can be also explained by the increase in the use of this last by the GST in the reaction of conjugation, this is confirmed by our results which indicate an induction of the GST in the presence of Spinosad, these results are in agreement with work of (Canesi and *et al.* (Canesi L, *et al*., 1999); Regoli and *et al*. (1998).

Our results are in agreement also with work from (Radwan *et al.* 1992), which highlighted an induction of activity GST after exposure of the gastéropode terrestre(*Theba pisana* to carbamates or those of (Pandey *et al.* 2005) at the gastéropode *C.penctatus* Selon Grara, (2011) the increase in the rate of GSH in the snail *Helix aspersa* subjected to a stress by the ETM would be with the interlocking of the process of defense of the organisation.

The Catalase is an enzyme of the phase II which plays a significant role in the mechanisms of detoxification. It catalyses the reduction of hydrogen peroxide out of water and molecular oxygen.The change of activity CAT is explained by cellular lesions caused by the exposure to contaminants (Shijin W, *et al*., 2011).

In our study we highlighted an increase amount depend on the Catalase activity at the batches treated by Spinosad, this increase could be due A the intensification of the antioxidant activity (Grara N. 2011) the catalase is sensitive to certain inductive contaminants of oxydative stresses on the level of the cellular membranes like the HAP, the PCB, certain pesticides (Livingston DR. 1991), the increase in the catalase activity could be explained by an adaptive mechanism to prevent the accumulation of the ROS resulting from the presence of our biopesticide, and our result are in perfect agreement with those of El –Gendy *et al.* (2009) which showed that in the presence of the snail *Theba pisana*

Malonedialdehyde (MDA) is a breakdown product of the reactions of lipidic peroxidation which is formed at the time of the attack of the lipids polyinsaturés by reactive species of oxygen generated by contaminants.

The MDA is a powerful agent alkylant able to react with the biological macromolecules. It is used as indicator of oxidative stress induced by contaminants at the bivalves sailors (Pellerin-Massicote J. 1994). When the oxidative stress submerges the protective forces like the Catalase activity, a noxious effect on the membranes can be observed via an increase in the contents of MDA, since this one is a product of the lipidic peroxidation.

In present study, levels very highly significant of MDA in gland digestive of molluscs *Helix aspersa* treated by Spinosad are noted and our results are in agreement with those of El –Gendy and *et al.,* (2009) which studied the toxic effects of a pesticide containing copper on the snail *Theba pisana* they highlighted a significant level of (MDA) after treatment of the animals. Moreover Shwela and *et al.* (2010) highlighted a

significant increase in the rate of (MDA) in the marine snail *Lymnaea natalensis* exposed to environmental pollutants it is the same for work for Salama and *et al.*,(2005) which highlighted an induction in the rats after exposure to certain pesticides.

The AchE represents a biomarker of neurotoxicity usually employed to reveal the exposure to the chemicals such as the organochlorinated ones and the carbamate (Fulton MH, Key PB. 2001)' inhibition of AchE was frequently employed in toxicology, to diagnose certain environmental contaminants such as the complex mixtures of pollutants, detergents and heavy metals. (Diamantino TC, *et al*., 2003).

AchE is implied in the transmission systems of the nerve impulse through the organization. The inhibition of the enzyme by the many neurotoxic ones involves an accumulation of the transmitter substance, the acetylcholine (AchE), in the synaptic space, which maintains of this fact a permanent transmission of the nerve impulse, leading to died of the individual (Bocquene G, *et al*., 1996).

In the invertebrates, the existence of motoneurones cholinergic as that of receivers specific to acetylcholine was highlighted at molluscs and the gastropoda (Weiss Y, *et al*., 1993). The AChE activity is used as marker of exposure to the inhibiting pesticides in molluscs.

Our results seem to be in adequacy with those quoted in the literature, indeed, the activity of AchE decreases gradually following the exposure of snails to the various concentrations of Spinosad. This inhibition continues until the end of the duration. We could show that the treatment by increasing concentrations of Spinosad inhibits in a amount-dependent way the Acetyl cholinesterase activity

Work supports our results, in particular those of Radwan and *et al*., (2008), Coeurdassier and *et al.* (2001), Salama and *et al.*,(2005) which highlighted an inhibition of the AchE activity after exposure of the terrestrial gastropoda to the pesticides.

The snail is a herbivore and detritivore, this mollusc gastéropode pulmoné is exposed to pollution of the grounds, the plants and the atmosphere and represents of this fact an integrating model complementary to the underground organizations like the annelids or the organizations to strictly herbivorous mode or detritivore, (Grara N, *et al*., 2012) It enters the food mode of the man, and can cause significant concentrations of residues in pesticide within its organization. It is regarded as one of the trophic chain links, (Grara N, *et al*., 2012) It is the prey the many predatory ones such as the mammals, the birds, etc and can thus be at the origin of transfers of the pollutants (contaminant) (Hussein HI, et aol., 1994; Radwan MA, *et al*., 2008).

The pollutants organic most persistent and toxic, likely to be transferred in the food chains and to cause health risks human and animal are due to the strong toxicity of certain pesticides. They can occur following a direct exposure (industrial workmen producing the pesticides and operators in charge of their use) or indirect (consumers and people present on the spot). The effects chronic of the exposure to the pesticides and likely to compromise public health exposed are in particular those dependent on the bio-accumulation and the persistence of the substances, their irreversible purposes such as the cancerogenicity, the mutagenicity, the genotoxicity, or for their harmful purposes on the immune system or endocrine of the mammals, fish or the birds.

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