

Populational effect of a dithiocarbamate (thiram) fungicide on a freshwater cladocerus *Daphnia magna*

Chahrazad Belaid^{1*}, Ibtissem Sbartai¹, Mohammed Réda Djebar¹

¹Laboratory of Cellular Toxicology, Faculty of Sciences, Department of Biology, University of Badji Mokhtar, Annaba, B.P. 12, Annaba, 23000, Algeria

Abstract: Thiram is considered as a potential risk fungicide, which affects principally environment and aquatic organisms which are the most exposed victims. Literature studies have classified *Daphnia magna* from the key species as well as the water contamination's bioindicator. However, very limited studies have been published for treating the effects of thiram on daphnids. The current study aims to analyze the growth, reproduction, and neurotoxicity effects of thiram on *Daphnia magna* in order to obtain knowledge on the risk involved with the emission of these fungicide into the environment. The results obtained from the acute test after 24h-48h of thiram exposure (0.05- 0.10- 0.25- 0.50mg/L) showed an increase in the rate of immobilization. However, the chronic test of 21 days at lower concentration exposure (0.004- 0.008- 0.016- 0.032 mg/L) revealed a decrease in the different growth and reproductive parameters, this inhibition was confirmed by the results of the monitoring of the AchE activity, which decreases suggesting the thiram neurotoxic effect. thiram was found to be toxic to *Daphnia magna* as it is proved by standardized tests.

Keywords: Thiram, *Daphnia magna*, cytotoxicity test, acetylcholine esterase, biomonitoring

INTRODUCTION

The environment has been threatened for a long time by the excessive human activity. Generally, aquatic environment is the final receptacle of the chemical human activity, which gradually leads to deterioration and decomposition of water quality, aquatic balance and stability of ecosystems (Chevalier *et al.*, 2014). Among the most important contaminants, pesticides that represent a significant part of the current environmental pollution; although their main targets are plants, only a small proportion nearly to 0,1 reaches thereof; the rest enters the environment gratuitously, contaminating soil, water and air, where it can poison or otherwise adversely affect nontarget organisms (Arias-Estévez *et al.*, 2008). All these molecules, such as (thiram), are either acting directly on soils, contaminating food and affecting both biodiversity and human health, or indirectly by having an impact on the environment which could likewise poison organisms and cause rapid and harmful changes.

Physical, chemical, biological and microbial measurements are the basic approach for estimating freshwater environmental quality. Therefore, it is widely recognized that the use of indicators can greatly enhance the assessment and management of aquatic ecosystems. Bio-monitoring by using an indicator species *Daphnia magna* as predictive models and their responses, to determine the changes of water environment, is the typical technique in water quality assessment and ecosystem, due to the ability of representing the overall status of the environment, which permits the detection of trends through their sensitivity to a range of stressors, and to be measured and interpreted relatively easily. (Le *et al.*, 2016); (Li *et al.*, 2010); (Metcalf *et al.*, 1989); (Chen *et al.*, 2012) and (Neves *et al.*, 2015). However, according to the author's knowledge, there are relatively few published studies treating the long-term toxicity on crustaceans that have been found out in the literature, particularly in chronic effects of thiram on *Daphnia magna*. In the

Other hand, many researchers have used biomarkers as well as AChE, as practical and quick methods for evaluating in vivo cholinesterase-inhibiting compound. However, to the author's knowledge, no studies have been reported on *Daphnia magna* after thiram exposure using AChE. Therefore, we have focused in the current study on the AChE activity of *Daphnia magna* after acute thiram exposure in the sake of determining the observed effects concentrations inducing inhibition of AChE activities, and specific biomarker of pesticides neurotoxicity.

In view of the aforementioned background, this article contributes to establish data for the short and long-term toxicity of thiram, widely used in agriculture on freshwater bioindicator. The approach is based on analyzing the growth, reproduction, and neurotoxicity effects of thiram on *Daphnia magna* in order to obtain knowledge on the risk involved with the emission of these fungicide into the environment. Acute and chronic tests on *Daphnia magna* have been established to assess population sensitivity. Assessment endpoints were immobilization longevity, survival, number of cumulative molts, day to first brood, number of brood and number of viable juveniles per surviving female.

MATERIALS AND METHODS

Test chemical

A fungicide active substance of the dithiocarbamate family (Thiram) was used as the commercial preparation. It is mainly used as foliar surface treatment in orchards against e.g., apple scab and the peach leaf curl, *Botrytis spp.*, rust, monilia. Since drift is the main entry into surface waters of most pesticides used in orchards due to the height of treated trees. Solutions were prepared by dissolving the fungicide immediately before each experiment.

Test organisms

Daphnia magna was collected and transported to the laboratory. The culture was reared and maintained

in a 16-litre glass aquarium containing dechlorinated water (total hardness 250 ± 25 mg/L; pH= $7,9 \pm 0,2$) renewed twice a week at a controlled temperature and photoperiod ($20 \pm 2^\circ\text{C}$), with a light/dark cycle of 16/08h (Ferrando et al., 1995). Daphnids are daily fed with a mixture of algae (*Chlorella vulgaris*) and yeast (*Saccharomyces cerevisiae*), a parthenogenetic reproduction is triggered and gives rise to juveniles that are the subject of tests.

Acute toxicity test

Acute tests were performed according to the ISO 6341 (1996) procedure to determine the concentration that causes the immobility of young daphnids at the end of 24 and 48 hours. Three replicates of five neonates (<24-h old), were placed in 30 mL glass test tubes containing 10 mL for each test concentration (0.05 - 0.10 - 0.25 - 0.50 mg/L) dissolved in ISO medium, were tested, and the neonates were not fed during the test and were placed away from light. The assessment endpoint was immobilization, more precisely, juveniles that were unable to swim were considered immobile and those which still moved their antennae but did not swim within 15s after a gentle shaking were considered immobile. The test is validated only if the percentage of immobilization in controls is less than or equal to 10%. Immobile juveniles were visually counted, to assess the 50 % inhibitory concentration (IC₅₀) which inhibits 50% of the population growth.

Chronic toxicity test

Based on acute results, *Daphnia magna* (<24-h old) were then exposed during 21 days to low concentrations of thiram (0.004- 0.008- 0.016- 0.032 mg/L). The test includes two important life stages' animals, namely the juvenile phase and the reproductive phase, under defined conditions based on the international standard ISO 10706 (2000) procedure. 10 replicates/concentration of juvenile were exposed individually in 60-mL glass beakers, containing 40 mL of culture solution, of the food and pesticide at desired nominal concentrations. The newborn are separated from adult daphnids, and counted daily. The test solution (with food) was renewed every 2 days. The examined assessment endpoints were longevity, survival, number of cumulative molts, day to first brood, number of brood and number of viable juveniles per female.

Determination of AChE activity

In order to mimic acute test conditions, 20 animals were placed in test solution at the desired nominal thiram concentrations (0.05 - 0.10 - 0.25 - 0.50 mg/L). After 24 h, rare immobile organisms were removed, and just alive animals were pooled. Before analysis, animals were rinsed for three times to remove thiram adsorbed on their carapace. AChE activity determined according to the method of Ellman *et al.* (1961) The aim is to provide the enzyme (AChE) with an analogous artificial substrate, acetyl thiocholine, which will be hydrolyzed into acetic acid and thiocholine. The latter in the presence of DTNB gives a yellow product TNB. Absorbance at 412 nm was measured at 25°C every each 4 min during 20 min. The results are expressed in $\mu\text{mol} / \text{min} / \text{mg}$ proteins. The protein concentration of the homogenized animal sample was determined in triplicate by the Bradford method bovine serum Albumin (BSA) as a standard.

Statistical analysis

To characterize toxicity, the 50% inhibitory concentration (IC₅₀) which is under standard conditions inhibits 50% of population growth has been determined, immobility rates are corrected by the Abbott's (1925) formula; Transformed into Probits. Statistical analysis of the effects of the toxicant exposure on the different parameters tested has been compared by one-way ANOVA using GraphPad Prism 7 software.

RESULTS AND DISCUSSION

Acute toxicity test

Exposure juveniles to thiram has a negative effect on survival/mobilization as is shown in Figure (1). Immobilization rate increases in a dose-dependent manner for organisms treated by increasing thiram concentrations for 24h exposure; it is nil (0.05 mg/L) but having 53.33% (0.5 mg/L). Statistical analysis showed highly significant differences. After 48h exposure, a significant increase immobilization rate of treated daphnids compared to controls. Inhibition was observed at (0.05 mg/L), there it is 26.67%, more than half of the population is inhibited 60% at (0.5 mg/L). Killing effects accentuated with time of exposure. This was reflected by the EC₅₀ values, which decreased from 0.35 mg/L after 24h to approximately the half 0.19 mg/L after 48h.

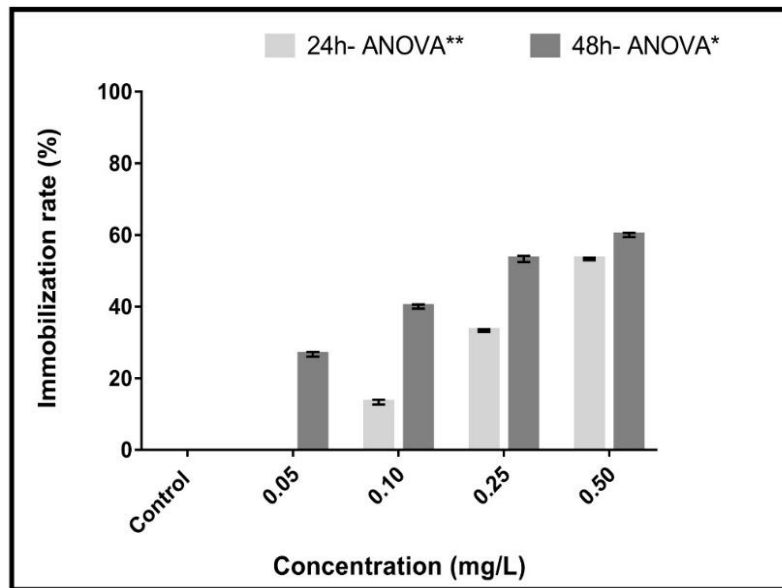


Fig. 1. Immobilization rate of *Daphnia magna* after 24h-48h of thiram exposure.

For instance, 48h-LC50 value of thiram was reported to be 0.21 mg/L, as signaled in 2017 regulation (EC) No. 1907/2006 and as it is found by Van Leeuwen *et al.* (1985) in short-term toxicity tests of dithiocarbamates. 48h-EC50 for thiram reported in the present study was near to that reported by regulation. Our results are in the same line with acute toxicity assessment of deltamethrin. (Toumi and al., 2013) and Chlordane (Manar *et al.*, 2009) on *D. magna*, it is confirmed in both cases the dose-dependent on the concentrations of xenobiotics tested and the effect on daphnia mortality. Moreover, several tested fungicides indicated that the most toxic formulation for all tested freshwater species was the thiram 80% WG formulation, the most sensitive organism was *D. magna* (24-EC50=0.03 mg/L) which was 3-fold higher for (48-EC50 with 0.01 mg/L) (Kyriakopoulou *et al.*, 2009). In comparison to previous studies, *D. magna* used in the present study was more sensitive to thiram. Moreover, the 48-h EC50 in our study was lower than the previously reported values. The EC50 value in *D. magna* varied under different conditions, such as temperature, population, body size, and pre-exposure, which indicated that different processes (i.e. intrinsic tolerances level and capability of detoxification system) contribute to thiram toxicity under certain conditions. In addition, media and culture conditions, including pH, hardness, light and alkalinity are also probably causes of different toxicity to xenobiotics demonstrating the sensitivity of daphnids to the commercial molecule. These findings imply that further studies are required to assess the acute toxic effect of xenobiotics on this species, under different conditions. Okamoto *et al.*, 2015); (Friberg-Jensen *et al.*, 2010) and (Lee *et al.*, 2010). Chemical exposure time and body size are responsible for these differences in the toxicity of xenobiotics; in particular, the smaller the body size is, the higher the metabolic and accumulation rate are, which could render these sensitive species to

environmental stressors (Offem and Ayotunde, 2008). Interclonal acute variation in *D. magna* was extensively reviewed as factors ranging (Baird *et al.*, 1991). Hence, it appears that clonal sensitivity is chemical-specific. Although environmental factors such as diet and culture conditions, remain the major cause of inter-laboratory variation.

Chronic toxicity test

All the studied parameters during the chronic experiments were influenced by thiram to which the organisms were exposed. To our Knowledge, there is no data concerning thiram using chronic test variation were found in the literature. Long-term thiram exposure affects survival, growth and different fecundity parameters on *D. magna* registered at the end of 21 days of time exposure. Barring the day to first brood, all other assessment endpoints generally decreased in a concentration dependent manner.

Figure (2a) shows concentration-response for population longevity of daphnids which declined with the increase of thiram concentrations, that were decreases significantly ($P \leq 0.001$), compared to controls a score of 21 days was observed. It reduces from 20.8 days at 0.004mg/L, and it was very affected by the highest concentration 0.032mg/L which decreases by almost half (12.2 days) justified by thiram exposure stress. Longevity linked to stressors, and Metal concentrations of Zinc copper caused a significant reduction in longevity (Winner, 1981).

Survival rate at the 21st day decreases significantly ($P \leq 0.001$) with increasing exposure concentration and time figure (2b), thiram showed a clear effect on survival during the test period, control survival was 100%, however no organism survives to the 21st day in (0.032 mg/L), it was approximately the half (60%) survived in 0.008 mg/L. Several researchers have suggested that survival in chronic toxicity tests is the best index of toxicity because it is more sensitive and

less variable than reproductive parameters (Day and Kaushik, 1997).

Average number of molts, was decreased significantly ($P \leq 0.001$) with increasing exposure

concentration and time (Fig. 2c), compared to the control daphnids 6,7 mean, it was approximately the half 3.8 and 3.2 for 0.016 mg/L and 0.032 mg/L respectively.

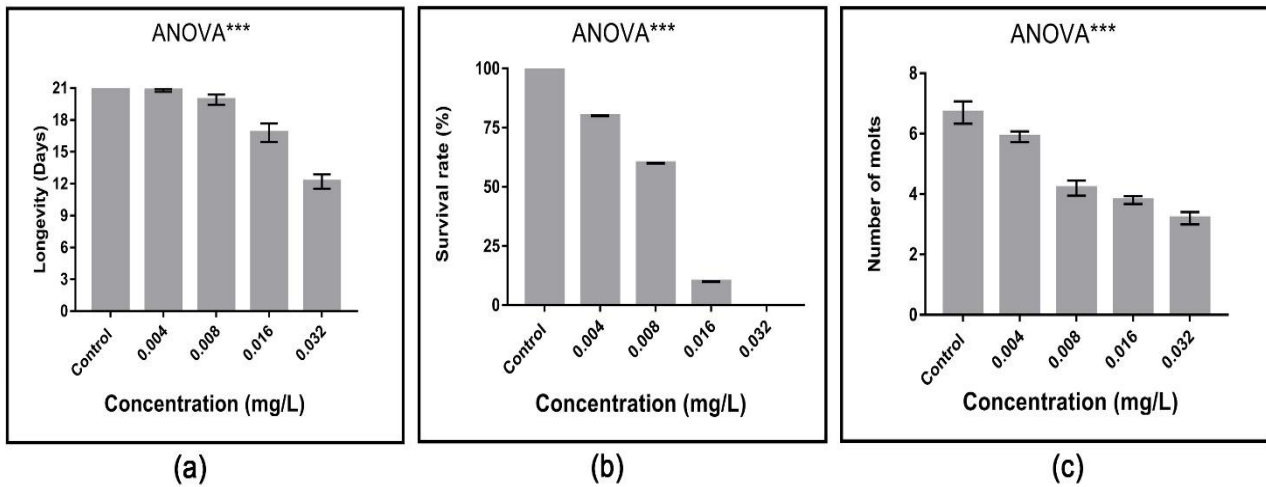


Fig. 2. Effects of thiram on longevity (a), on survival rate (b) and on number of molts (c) of *Daphnia magna* during 21 days of exposure.

Reproduction of the surviving adults exposed to low-dose and chronic exposure of thiram was significantly affected. The required time to each reproductive maturity increased progressively compared with control, but the differences were only statistically significant ($p < 0.05$). The age at first reproduction (Fig. 3a) was increased from 10,8 days for control might develop faster to 11,3 (0,004 mg/L), 11,75 (0,008 mg/L), 11,87 (0,016 mg/L) and delay to 12 days (0,032 mg/L).

The total average production of young per female (Fig. 3b) was also affected significantly ($P \leq 0.001$) at

all the thiram tested levels. Cumulative number brood control was 3,2 decreased 30 times for the highest concentration (0,032 mg/L). The lowest thiram concentration (0,004 mg/L) had also effected number of broods 2,9 at mean.

Reproduction was significantly reduced, as a result, the total number of neonates per living *D. magna* in 21 days decreased significantly ($P \leq 0.001$) and declined progressively from 22,7 young (control) to 0,5 young (0,032 mg/L), thiram significantly was reduced the total number of neonates per surviving female (Fig. 3c).

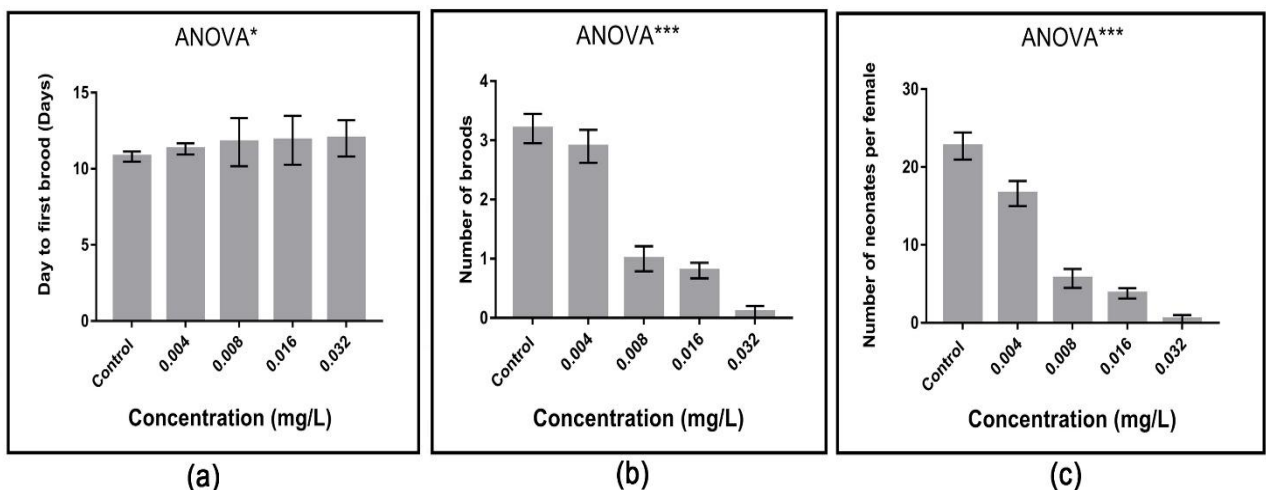


Fig. 3. Effects of thiram on reproductive maturity (a), on number of brood (b) and on total neonates produced by *D. magna* after 21 days of exposure.

Clear concentration-response relationships for the total number of neonates per surviving female were observed. A significant decrease has been observed in mean number of young per female and mean brood

size of *D. magna* exposed to concentration higher than 0.011 mg/L fenitrothion (Ferrando *et al.*, 1995). Similar effects on reproduction were described for *D. magna* exposed to sublethal levels of endosulfan and

methylparathion, a reduction in total young per female, number of broods and number of days to the first reproduction (Fernandez-Casalderrey et al., 1995). A decline in the total number of young per female, mean brood size and number of broods was also demonstrated when *D. galeata mendotae* was exposed to fenvalerate, but no observed effect in the number of days to the first reproduction (Day and Kaushik, 1997). Similar effects on reproduction were observed on *D. magna* exposed to sublethal levels of DCA, when observing an increase in the day of the first production of young (Crossland and Hillaby, 1985). It is well known that fecundity is the most sensitive trait to toxicity in the *D. similis* organism, which may be triggered to lower fertility rate under unfavorable environmental conditions (Toumi et al., 2013); (Crossland and Hillaby, 1985) and (Ebert, 2011). Villarroel et al. (2003) also found that the number of neonates per female was the most responsive parameter of the effect of propanil on daphnids when IC₅₀ was calculated. However, sodium bromide, 3,4-dichloroaniline and propanil affect the eggs in the brood pouch (Baird et al., 1991); (Pereira et al., 2007). A direct poisoning of eggs in the brood pouch phenomena could explain our results. Moreover, a significant energy proportion is allocated to the detoxification process set up by the organism and to

the physiological damage caused by the pesticides presence, thus leading to a reduction in the available energy for growth, reproduction and acquisition of food resources, which could potentially influence the survival chances (Leblanc and McLachlan, 1999). It is not surprising that parameters related to reproduction (i.e. number of neonates per live adult and the number of cumulative molts) were associated because neonates are liberated from the brood pouch few hours before molting (Toumi et al., 2013).

Reduction, in body length could explain our results, cause a decrease in fecundity of the daphnids because the reduced body size leads to the decrease of brood chamber and consequently limits the accommodation of the eggs.^[31] According to our results, thiram could cause lethal and sub-lethal effects on daphnids.

Acetylcholinesterase activity (AChE)

Figure 4 illustrates the effects of different thiram concentrations on acetylcholinesterase activity (AChE). We note a significant dose-dependent AChE activity decrease for treated daphnids compared to controls. In fact, the highest enzymatic activity 0.570 $\mu\text{mol}/\text{min}/\text{mgP}$ in control of organisms decrease by half 0.217 $\mu\text{mol}/\text{min}/\text{mgP}$ (0.05mg/L) reaches 0.118 $\mu\text{mol}/\text{min}/\text{mgP}$ for 0.5mg/L.

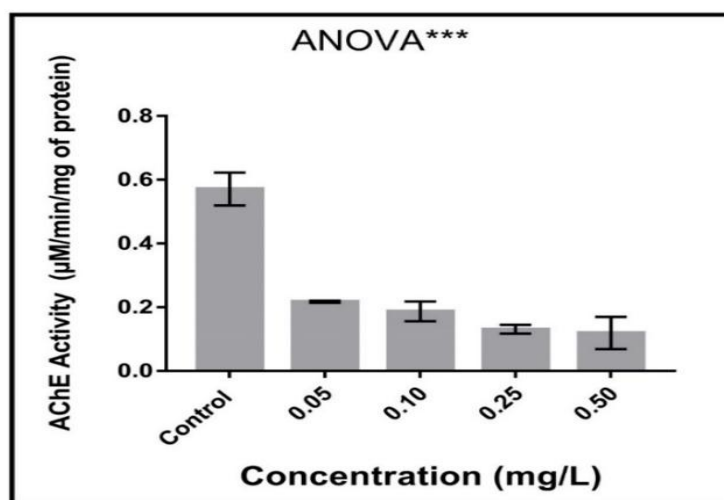


Fig. 4. Variation in acetylcholinesterase activity of *Daphnia magna* after 24h of thiram exposure.

Cladocerans have a primitive neural system and/or neurological responsive mechanism, including AChE that seems to be responsive inhibited to a multiplicity of contaminants. Pesticides especially organophosphate pesticides and carbamates are known to be the main contaminants in aquatic environments and are mostly neurotoxic inhibiting acetylcholinesterase (AChE) activity.

To our knowledge, there are no studies that tested the effect of thiram on *Daphnia magna* AChE activity. In our study, a decrease of AChE was observed suggesting a neurotoxic action of thiram on non-target organisms such as daphnids in our case. This neurotoxicity could be directly responsible for the

individuals immobility exposed to different xenobiotics concentrations. This relationship between the AChE inhibitions of *D. magna* was shown after an exposure to Parathion and Dichloroos (Sturm, and Hansen, 1999). Intermediate effects were found for the thiram to fish and are often considered to be neurotoxic, which were only mildly inhibiting to AChE (Olson and Christensen, 1980). In addition, female rats fed the high level of thiram developed hind limb ataxia or paralysis (Lee and Peters, 1980). AChE activity inhibition resulted in unregulated nerve ending activation and paralysis in organisms (Casida, 1964) which could induce the loss of nerve conduction ability, and then cause hyperactivity, loss of

coordination, convulsions, paralysis and other kinds of behavioral changes. All of these behavior disorders could bring about the stepwise behavioral response of organisms. The obtained results indicate that inhibition caused by thiram occurred at a concentration of 0,05 mg/L. Our results are in line with previously published data, namely those reported by Najimi *et al.* (1997) who described a significant cholinesterasic inhibition in gills of the seawater mussel species after acetaminophen acute exposure. In fact, some controversies exist regarding the degree of inhibition of AChE activity and its relation to the death of an organism. Some studies reported that AChE inhibition after exposure to lethal concentrations of anticholinesterasic compounds usually ranges from 70 % to 100 % (Barata *et al.*, 2001). A study established definitely the link between oxidations and cholinesterasic inhibition, by making clear that oxidant conditions were able to alter the conformational status of acetylcholinesterase of *Torpedo californica*, with obvious deleterious effects in its hydrolytic capabilities (Weiner *et al.*, 1994); (Oliveira *et al.*, 2015). Despite its undisputable ecological relevance, it is fundamental to increase the body of knowledge concerning the relation between neurotoxicity, and the pro-oxidative pathway, which seems to occur for environmental pollutants, which may have a potent but unsuspected neurotoxic effect, including thiram. The degrees of immobility of *D. magna* treated with different concentrations of thiram in standard 24h-48h acute toxicity tests see (Fig.1). There was a direct linkage of the biomarker to immobility of *D. magna* in our study. While immobility of *D. magna* was proportional to the concentration of the tested chemical in a dose-response manner, AChE activities changes were dependent on the tested concentration; this phenomenon is characterized as a dose-response with inhibition at low doses. Variations in the relationship between IC50-24h (related to AChE inhibition) and EC50-24h (related to immobility) in *D. magna* have been observed after parathion, dichlorvos and aldicarb exposure (Sturm and Hansen, 1999). Discrepancies could also be due to various factors such as genetic heterogeneity, diverse stocks compounds tested, and number of the brood or exposure medium, which could influence the EC50-48h and AChE specific activity variations as it was shown by Toumi *et al.* (2015) after Malathion exposure with the three strains of *D. magna*.

CONCLUSIONS

The aim of this study was to investigate on several toxic effects of thiram using an aquatic bio-monitoring method by toxicity assays on a bioindicator *Daphnia magna*. For achieving our object, acute and chronic tests have been performed for demonstrating harmful effects: immobilization, decrease of survive, longevity and number of molt. These experiments confirmed that the productivity was affected; thiram delays the maturity by affecting the day to first brood, the fertility by decreasing number of broods and total neonates produced. The findings enhance our understanding that these damages should be seriously considered, because

the use of AChE activity as biomarker proves neurotoxicity effects of thiram. Moreover, the results could classify thiram in the range of environmental stressor. This work has opened up several questions that need of further investigations in the future studies about the oxidative stress of thiram on *Daphnia magna* and more other freshwater species.

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AUTHORS CONTRIBUTIONS

Conceptualization: C. Belaid, I. Sbartai; Methodology: C. Belaid; Data collection: C. Belaid; Data validation: I. Sbartai; Data processing: C. Belaid; Writing—original draft preparation: C. Belaid I. Sbartai; Writing—review and editing: C. Belaid, I. Sbartai.

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CONFLICT OF INTEREST

The authors have no any competing financial, professional or personal interests from other parties.

REFERENCES

- Abbott W S, A method of computing the effectiveness of an insecticide. *J. econ. Entomol*, 18, 265-267, 1925.
- Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara J, Mejuto J C, García-Río L, The mobility and degradation of pesticides in soils and the pollution of groundwater resources, *Agriculture, Ecosystems & Environment*, 123, 247-260, 2008.
- Baird D J, Barber I, Bradley M, Soares, A M, Calow P, A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. *Ecotoxicology and Environmental Safety*, 21, 257-265, 1991.
- Barata C, Baird D J, Soares A M V M, Guilhermino L, Biochemical factors contributing to response variation among resistant and sensitive clones of *Daphnia magna* Straus exposed to ethyl parathion. *Ecotoxicology and Environmental Safety*, 49, 155-163, 2001.
- Casida J E, Esterase Inhibitors as Pesticides: Because of favorable biological properties they are displacing other types of established compounds. *Science*, 146, 1011-1017, 1964.
- Chen L, Fu X E, Zhang G, Zeng Y, Ren Z, Influences of Temperature, pH and Turbidity on the Behavioral Responses of *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) in the

- Biomonitor. Procedia Environmental Sciences, 13, 80-86, 2012.
- Chevalier J, Grote M, Keller M, Pandard P, Cachot J, A new multi-cell exposure system for continuous tracking of *Daphnia* behavior for toxicity assessments. *J Environ Anal Toxicol*, 5, 2161-0525, 2014.
- Crossland N O, Hillaby J M, Fate and effects of 3, 4-dichloroaniline in the laboratory and in outdoor ponds: II. chronic toxicity to *Daphnia* SPP. and other invertebrates. *Environmental toxicology and chemistry*, 4, 489-499, 1985.
- Day K, Kaushik N K, An assessment of the chronic toxicity of the synthetic pyrethroid, fenvalerate, to *Daphnia galeata mendotae*, using life tables. *Environmental Pollution*, 44, 13-26, 1987.
- Ebert D, A genome for the environment. *Science*, 331, 539-540, 2011.
- Ellman G L, Courtney K D, Andres Jr V, Featherstone R M, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, 7, 88-95, 1961.
- Fernandez-Casalderrey A, Ferrando M D, Andreu-Moliner E, Chronic toxicity of methylparathion to *Daphnia magna*: Effects on survival, reproduction, and growth. *Bulletin of environmental contamination and toxicology*, 54, 43-49, 1995.
- Ferrando M D, Sancho E, Andreu-Moliner E, Effects of lindane on *Daphnia magna* during chronic exposure. *Journal of Environmental Science & Health Part B*, 30, 815-825, 1995.
- Ferrando M D, Sancho E, Andreu-Moliner E, Effects of lindane on *Daphnia magna* during chronic exposure. *Journal of Environmental Science & Health Part B*, 30, 815-825, 1995.
- Friberg-Jensen U, Nachman G, Christoffersen K S, Early signs of lethal effects in *Daphnia magna* (Branchiopoda, Cladocera) exposed to the insecticide cypermethrin and the fungicide azoxystrobin. *Environmental toxicology and chemistry*, 29, 2371-2378, 2010.
- International Standard Organisation ISO 10706, Water quality—Determination of Long Term Toxicity of Substances to *Daphnia magna* Straus (Cladocera, Crustacea). International Organization for Standardization, Geneva, Switzerland, 2000.
- International Standard Organisation ISO 6341, Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test. Switzerland: International Organization for Standardization, Geneva, Switzerland, 1996.
- Kyriakopoulou K, Anastasiadou P, Machera K, Comparative toxicities of fungicide and herbicide formulations on freshwater and marine species. *Bulletin of environmental contamination and toxicology*, 82, 290, 2009.
- Le Q A V, Sekhon S S, Lee L, Ko J H, Min J, *Daphnia* in water quality biomonitoring-“omic” approaches. *Toxicology and Environmental Health Sciences*, 8, 1-6, 2016.
- Leblanc G A, Mclachlan J B, Molt-independent growth inhibition of *Daphnia magna* by a vertebrate antiandrogen. *Environmental toxicology and chemistry*, 18, 1450-1455, 1999.
- Lee C C, Peters P J, Neurotoxicity and behavioral effects of thiram in rats. *Environmental health perspectives*, 17, 35-43, 1976.
- Lee J, Ji K, Kim J, Park C, Lim K H, Yoon T H, Choi K, Acute toxicity of two CdSe/ZnSe quantum dots with different surface coating in *Daphnia magna* under various light conditions. *Environmental toxicology*, 25, 593-600, 2010.
- Li L, Zheng B, Liu L, Biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends. *Procedia environmental sciences*, 2, 1510-1524, 2010.
- Manar R, Bessi H, Vasseur P, Reproductive effects and bioaccumulation of chlordane in *Daphnia magna*. *Environmental toxicology and chemistry*, 28, 2150-2159, 2009.
- Metcalfe J L, Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. *Environmental pollution*, 60, 101-139, 1989.
- Najimi S, Bouhaimi A, Daubeze M, Zekhnini A, Pellerin J, Narbonne J F, Moukrim A, Use of acetylcholinesterase in *Perna perna* and *Mytilus galloprovincialis* as a biomarker of pollution in Agadir Marine Bay (South of Morocco). *Bulletin of Environmental Contamination and Toxicology*, 58, 901-908, 1997.
- Neves M, Castro B B, Vidal T, Vieira R, Marques J C, Coutinho J A P, Gonçalves A M M, Biochemical and populational responses of an aquatic bioindicator species, *Daphnia longispina*, to a commercial formulation of a herbicide (Primextra® Gold TZ) and its active ingredient (S-metolachlor). *Ecological indicators*, 53, 220-230, 2015.
- Offem B O, Ayotunde E O, Toxicity of lead to freshwater invertebrates (Water fleas; *Daphnia magna* and *Cyclop* sp) in fish ponds in a tropical floodplain. *Water, air, and soil pollution*, 192, 39-46, 2008.
- Okamoto A, Yamamuro M, Tatarazako N, Acute toxicity of 50 metals to *Daphnia magna*. *Journal of Applied Toxicology*, 35, 824-830, 2015.
- Oliveira L L, Antunes S C, Gonçalves F, Rocha O, Nunes B, Evaluation of ecotoxicological effects of drugs on *Daphnia magna* using different enzymatic biomarkers. *Ecotoxicology and environmental safety*, 119, 123-131, 2015.
- Olson D L, Christensen G M, Effects of water pollutants and other chemicals on fish

- acetylcholinesterase (in vitro). Environmental Research, 21, 327-335, 1980.
- Pereir J L, Mendes C D, Gonçalves F, Short-and long-term responses of *Daphnia* spp. to propanil exposures in distinct food supply scenarios. Ecotoxicology and environmental safety, 68, 386-396, 2007.
- Pereira J L, Mendes C D, Gonçalves F, Short-and long-term responses of *Daphnia* spp. to propanil exposures in distinct food supply scenarios. Ecotoxicology and environmental safety, 68, 386-396, 2007.
- Sturm A, Hansen P D, Altered Cholinesterase and Monooxygenase Levels in *Daphnia magna* and *Chironomus riparius* Exposed to Environmental Pollutants. Ecotoxicology and Environmental Safety, 42, 9-15, 1999.
- Sturm A, Hansen P D, Altered Cholinesterase and Monooxygenase Levels in *Daphnia ma*; 127 *Chironomus riparius* Exposed to Environmental Pollutants. Ecotoxicology and Environmental Safety, 42, 9-15, 1999.
- Toumi H, Boumaiza M, Millet M, Radetski C M, Felten V, Féraud J F, Is acetylcholinesterase a biomarker of susceptibility in *Daphnia magna* (Crustacea, Cladocera) after deltamethrin exposure?. Chemosphere, 120, 351-356, 2015.
- Toumi H, Boumaiza M, Millet M, Radetski C M, Felten V, Fouque C, Féraud J F, Effects of deltamethrin (pyrethroid insecticide) on growth, reproduction, embryonic development and sex differentiation in two strains of *Daphnia magna* (Crustacea, Cladocera). Science of the total Environment, 458, 47-53, 2013.
- Van Leeuwen C J, Maas-Diepeveen J L, Niebeek G, Vergouw W H A, Griffioen P S, Luijken M W, Aquatic toxicological aspects of dithiocarbamates and related compounds. I. Short-term toxicity tests. Aquatic toxicology, 7, 145-164, 1985.
- Villarroel M J, Sancho E, Ferrando M D, Andreu E, Acute, chronic and sublethal effects of the herbicide propanil on *Daphnia magna*. Chemosphere, 53, 857-864, 2003.
- Weiner L, Kreimer D, Roth E, Silman I, Oxidative stress transforms acetylcholinesterase to a molten globule-like state. Biochemical and biophysical research communications, 198, 915-922, 1994.
- Winner R W, A comparison of body length, brood size and longevity as indices of chronic copper and zinc stresses in *Daphnia magna*. Environmental Pollution Series A, Ecological and Biological, 26, 33-37, 1981.