Effect of the insecticide chlorpyrifos on behavioral and metabolic aspects of the spider Polybetes pythagoricus

EFFECT OF THE INSECTICIDE CHLORPYRIFOS ON BEHAVIORAL AND METABOLIC ASPECTS OF THE SPIDER POLYBETES PYTHAGORICUS

Molina Gabriel[a], Laino Aldana[a], Arreghetti Florencia[b], Lacava Mariangeles[c], Romero Sofía[a], Mijailovsky Sergio[a], Garcia Carlos Fernando[a]*.

[a] Instituto de Investigaciones Bioquímicas de La Plata "Prof. Dr. Rodolfo R. Brenner", La Plata, Argentina

[b] CONICET- Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Ciudad Autónoma de Buenos Aires, Argentina

[c] Centro Universitario de Rivera, Universidad de La República, Rivera, Uruguay

[*] Corresponding author: cfgarcia1123@yahoo.com.ar

ACKNOWLEDGEMENTS

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT-2017-0684 and PICT 2019-02166), and UNLP Argentina M232. The authors are grateful to Rosana del Cid for her help with the text, Mario Ramos for the figure design, Jorge Barneche for help in capturing spiders and Juan Girotti for his comments regarding chromatographic techniques. Dr. Donald F. Haggerty, a retired academic career investigator and native English speaker, edited the final version of the manuscript.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.5607.

AUTHOR CONTRIBUTIONS

- Author 1, author 2 conceived research.
- Author 1, 4, 5 and author 6 conducted experiments.
- Author 1, 2 and author 7 contributed material.
- Author 3 analysed data and conducted histologycal analyses.
- Author 1 and author 4 conducted statistical analyses
- Author 2, 3 and author 7 wrote the manuscript.
- Author 2, and author 7 secured funding.
- All authors read and approved the manuscript.

DATA AVAILABILITY STATEMEN

All authors agree with the SETAC policy. All data is available and accessible

All the data of the work are available in the manuscript. if there is any doubt please contact the authors

ACKNOWLEDGEMENTS

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT-2017-0684 and PICT 2019-02166), and UNLP Argentina M232. The authors are grateful to Rosana del Cid for her help with the text, Mario Ramos for the figure design, Jorge Barneche for help in capturing spiders and Dr. Juan Girotti for his comments regarding chromatographic techniques. Dr. Donald F. Haggerty, a retired academic career investigator and native English speaker, edited the final version of the manuscript.

ABSTRACT

The toxicity of pesticides to organisms depends on the total amount of chemical exposure. Toxicity can be minimized if the organism recognizes the pesticide and alters its behavior. Furthermore, the physical barrier of cuticular hydrocarbons can prevent the entrance of the pesticide into the organism. Finally,

if the pesticide enters the body, the organism experiences physiological changes favoring detoxification and the maintenance of homeostasis. We analyzed the behavioral and metabolic response of the spider *Polybetes pythagoricus* at different times of exposure to the organophosphates pesticide chlorpyrifos.

First, we observed that the individuals are capable of recognizing and avoiding surfaces treated with pesticides based on a behavioral analysis. Subsequently, we characterized cuticular hydrocarbons as a possible barrier against pesticides. Afterwards, we observed that the pesticide provoked histological damage, mainly at the level of the midgut diverticula. Finally, we analyzed the activity of several of the spider's enzymes linked to oxidative stress after exposure to chlorpyrifos for different lengths of time (6, 24, and 48 h). We observed that catalase manifested a high activity at the start, whereas superoxide dismutase and glutathione S-transferase evidenced a significant change in activity at 48 h. The lipid peroxidation became high at 6 h, but decreased at 48 h. In conclusion, although *Polybetes pythagoricus* can avoid contact with chlorpyrifos, this pesticide causes the activation of the antioxidant system when it enters the body. This work turns out to be a significant contribution to the ecotoxicology of spiders.

Key words

spider, chlorpyrifos, oxidative stress, hydrocarbons, reactive oxygen species

Abbreviations

AChE: acetylcholinesterase; **CAT:** catalase; **GPx:** Glutathione peroxidase; **GR:** Glutathione reductase; **GST:** Glutathione S transferase; **HC:** hydrocarbons; **MD:** midgut diverticula; **MDA:** malondialdehide; **ROS:** reactive oxygen species; **SOD:** superoxide dismutase.

INTRODUCTION

The manipulation of agroecosystems by humans may eventually cause an alteration in the natural balance between populations of herbivore species and their predators (Maloney et al., 2003), causing an uncontrolled population growth of

animals that could affect crops. The use of pesticides, however, implies a threat to wildlife since they produce a serious contamination of the soil, air, and water (Aktar et al., 2009; Degrendele, 2016; Sumon et al., 2018; Yadav, 2015) as well as a significant problem for human health (Aktar et al., 2009; Carvalho, 2017; Gomes et al., 2020; Pimentel, 1992).

The European Food Safety Authority has recently confirmed a concern about possible human genotoxicity and developmental neurotoxicity caused by chlorpyrifos, an organophosphate pesticide (FSN, 2019) (IUPAC name: O,O-diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate). Although chlorpyrifos is banned in certain European countries (Foong et al., 2020), in some US states (Huang, 2020) and in China (Mara, 2013), the use of the agent is still prevalent elsewhere. The application of this pesticide has spread worldwide (Aktar et al., 2009; Degrendele, 2016; Sumon et al., 2018; Yadav, 2015), being one of the most currently used (Ding et al., 2019; Kralj et al., 2007; Shaffo et al., 2018). Chlorpyrifos is stable under different conditions and UV-resistant (Dar et al., 2019). The compound's half-life in soils is variable, ranging from a few days under air-dry soils (Awasthi and Prakash, 1999) to 4 years with high concentrations and a low rate of biotic and abiotic degradation (John and Shaike, 2015). Chlorpyrifos acts as an inhibitor of the enzyme AChE in target organisms (Díaz-Barriga Arceo et al. 2015), but also affects nontarget organisms such as spiders (Erban et al., 2019).

Spiders are mainly hunters that feed almost exclusively on insects (Nyffeler et al., 1994; Nyffeler, 1999) and represent one of the most ubiquitous groups of predatory organisms in the animal kingdom (over 50,000 species) (World Spider Catalog 2022), being present in the different agroecosystems (Nyffeler and Suderland, 2003) as well as in their environs (Bayrama and Luff, 1993). Therefore, spiders constitute a valuable group as biological control agents (Maloney et al., 2003; Michalko et al., 2018; Riechert and Lockley, 1984), but the ability of spiders to function in that capacity is limited by the use of pesticides,

which affect them adversely (Pekár, 2012, 2013; Riechert and Lockley, 1984) by decreasing their capability of capturing prey (Lacava, 2021; Michalko and Košulič, 2015; Rezac et al., 2010; Tahir et al 2015). As with other arthropods, spiders are subject to three possible routes of pesticide absorption: by surface contact, topically or orally of either pesticide-contaminated water or prey that have accumulated the pesticide in different tissues (Adedara et al 2016; Van den Bossche, P., 1996), with superficial contact being the most harmful (Everts et al., 1991; Mullie et al., 1991).

Nevertheless, information related to the behavior of spiders on surfaces exposed to pesticides is scarce. The exposure of the spider *Pardosa palustris* to either Nurelle™ (organophosphate + pyrethroid) or Decis™ (pyrethroid) was reported to affect their behavior (Pekár and Benes, 2008), as also the fresh residues phosalone and permethrin had repellent effect on *Clubiona spp., Dictyna spp*., *Pardosa spp*., (Pekár and Haddad, 2005).

When organisms do not avoid surfaces with pesticides, a mechanical barrier still exists that pesticides must penetrate: namely, the cuticle (Juárez et al., 2010; Zhu et al., 2013; Bass et al., 2014; Balabanidou et al., 2016). The spider's cuticle consists of four superimposed layers with an external epicuticle composed of lipids (Trabalon and Garcia, 2021), with the cuticular hydrocarbons (HCs) being prominent among those lipids (Trabalon et al., 1997). This lipid layer could be significant in the arthropod's response to pesticide exposure since removal of that cuticular lipid has been demonstrated to accelerate pesticide penetration in insects (Motoyama et al., 1992). Moreover, when pesticides finally become internalized in the body, they most likely cause histologic damage in addition to activating different detoxification mechanisms. The analysis of detoxification responses after pesticide exposure would provide us with an understanding of how the resistance of target organisms is generated and how nontarget organisms can combat an exposure (Hemingway, 2000; Kostaropoulos et al., 2001). These detoxification mechanisms act on exogenous toxic molecules and work in conjunction with

homeostatic mechanisms to avoid the oxidative damage generated by ROSs that may affect the oxidation of molecules such as lipids (Abdelfattah and El-Bassiony, 2022; Di Nica et al., 2019; Laino and García, 2020; Wilczek et al., 2008) and tissue damage (Mahmood et al., 2018; Plata-Rueda et al., 2020). The antioxidant system is represented by enzymes such as SOD, CAT, and GPx (Rikans and Hornbrook, 1997), GR, and GSTs (Ketterer et al., 1983).

Different aspects of the detoxification system of biological pest control insects have been previously described (Booth, 2007; Rodrigues et al., 2014; Janssens and Stoks, 2017; Khan et al., 2021). The scarcity of information on the detoxification capability of spiders and their behavior upon exposure to pesticides through surface contact underscores the long-standing underestimation and lack of knowledge of these topics. Moreover, the few studies related to the subject have been carried out with respect to a single exposure time (generally 24 h), and thus far no work has been published involving an analysis of the enzymatic antioxidant system against pesticides after different exposure times. This field needs to be addressed because the exposure time is a relevant variable when the effects of a toxicant on enzymatic activity are studied. In order to investigate these topics, in the work reported here, we used the species *Polybetes pythagoricus* as a model for the family Sparassidae those being generalist hunter spiders present in different crops such as banana, sugar-apple, and sorghum (Patil et al., 2020), castor corn, cotton, paddy, and pigeon pea (Rain et al., 2016; Solanki and Kumar, 2013), and sugar cane (Prajapati and Surani, 2018). Our objectives were: 1) to analyze the behavior of *P. pythagoricus* on surfaces treated with chlorpyrifos, 2) to determine the HC profile present in the epicuticle, 3) to examine the histological effects of the insecticide on the tissues, 4) to evaluate the pesticide impact on the AChE activity and lipid peroxidation, and 5) to determine the response of the enzymes of the antioxidant system at different times of exposure to chlorpyrifos.

MATERIALS AND METHODS

Specimen collection and maintenance

P. pythagoricus is neither protected nor in danger of extinction. Adult females of *P. pythagoricus* were collected from *Eucalyptus* sp. trees in an area where no agricultural activities are performed, to ensure that where not previously exposed to organophosphates, around the city of La Plata (Buenos Aires Province, Argentina; 34° 53' 55" S, 57° 55' 48" W). The spiders were housed individually in circular terrariums (10 cm diameter x 5 cm high) without any substrate and were kept at 20 ± 1 °C, under a 14:10 h light-dark photoperiod.

Insecticide

The insecticide chlorpyrifos (CAS#**:** 2921-88-2) used in the present study was donated by Gleba laboratories, Argentina, (purity 98%). Chlorpyrifos was solubilized in acetone and all assays were performed at the concentration corresponding to the maximum recommended field dose 1,120.85 g of active ingredient/hectare Environmental protection Agency (EPA). www.epa.org).

Behavioral assays

Behavioral assays were carried out to evaluate the spiders' selectivity for surfaces treated with chlorpyrifos.

We evaluated of a possible avoidance behavior of the acetone residues (the solvent used to solubilize the insecticide) by analyzing the behavior of twelve spiders. Each one was placed one at a time on a filter paper of surface area 288 cm^2 divided into half sectors, with one being treated with 800 µl acetone and dried by a stream of air for 10 min and the other remaining untreated. Before the experiment, the filter papers were put in a glass terrarium 24.1 cm long, 12 cm wide, and 10.3 cm high, with this terrarium containing the filter paper being placed in an opaque box 58.3 cm long, 36 cm wide, and 36 cm high, with a red light. The aim of this terrarium system was to isolate each individual from external visual stimuli. Fig. S1 of the supplementary material is a pair of photographs illustrating the experimental set-up.

The displacements and movements of the spiders were recorded for 4 h, with a 4k Noblex Acn4k1 camera and Debut Professional v 6.67 (NCH) video-

capture software and subsequent processing with EthoVision v 3.1 (Noldus information Technology, Wageningen, The Netherlands) video-tracking software. This software analyzed the displacement every 5 min during the 4 h experiment for each spider. These times were used because they successfully captured the mobility of our spider model in preliminary tests. Finally, the results obtained for each spider constituted the average time spent (or remaining) in each of the two sectors of the terrarium.

The evaluation of the avoidance behavior of surfaces treated with chlorpyrifos was performed by analyzing twelve spiders' behavior. Each spider was placed one at a time on a filter paper of the same size, but this time with half of the area treated with 800 µl of acetone and the other half with 800 µl of the insecticide solution (1.6 mg/144 cm^2, equal to the maximum field dose) (similar to Pekar and Haddad, 2005) in that same solvent and both dried by a stream of air for 10 min.

Extraction, purification, and determination of cuticular HCs

With the aim of analyzing the cuticular HCs of *P. pythagoricus*, the whole body of adult female spiders (n = 3) was washed with redistilled water for 3 min to remove any water-soluble contaminants. After being anesthetized with cold, each spider was transferred to a glass tube and submerged in 5 ml of redistilled hexane (Carlo Erba Reagents, Milano, Italy) for 5 min to extract the total cuticular lipids (Sessa et al. 2021). The hexane volume was reduced under a nitrogen stream and the HCs separated from the total lipids by adsorption chromatography on a minicolumn (10 mm x 5 mm internal diameter) of activated Biosil A (Bio-Rad Laboratories, Richmond, CA) and eluted with redistilled hexane (4 mL). Each sample was taken up in 2 µl of hexane and analyzed by capillary gas chromatography in a Hewlett-Packard gas chromatograph 6890 (Wilmington, DE) operating in the splitless mode at 320 °C and fitted with nonpolar fused silica (0.25 mm) DB-5 capillary column (30 m x 0.32 mm inner diameter) with helium as carrier gas and the flame-ionization detector being held at 320 °C. The

temperature was programmed from 50 °C (holding time 2 min) to 180 °C at 20 °C/min, and then 180 ºC (holding time 10 min) to 310 °C at 3 °C/min. The internal standard used was a docosane (C22) at 10µg/ml (Sigma-Aldrich, San Luis, USA).

Toxicity assays

Toxicity assays were carried out by treating adult females of *P. pythagoricus* (12 spiders) with 10 µl of the chlorpyrifos solution applied topically with a Hamilton syringe on the dorsal area of the opisthosoma (52.6 µg/individual). The control group contained adult female spiders with acetone applied topically (12 spiders). The spiders were divided into independent groups (n= 4 individuals per group) and a control group and a treated group were sacrificed at 6 hours, others at 24 hours, and others at 48 hours after treatment. All treated spiders survived exposure to the pesticide. A dorsal incision was made in the opisthosoma integument in order to remove the midgut diverticula (MD), which is a metabolically active organ in chemical-compound detoxification (Laino and Garcia, 2020). The MD were dissected as described by Laino et al. (2009), and weighed on an analytical balance (Mettler-Toledo New Classic MS-204). The MD used for enzymatic assays were homogenized in 50 mM potassium phosphate buffer pH 7.4 containing a protease-inhibitor cocktail at 8 µl/1000 µg (Sigma-Aldrich Chemicals, St. Louis, MO, USA) and then centrifuged for 20 min at 10,000 *g*. The protein concentration of each sample was determined by the method of Lowry et al. (1951).

Histological analyses

Adult females treated with chlorpyrifos for 24 and 48 h and control females (8 spiders for each group) were used for histological analysis. We only worked with these times because they were the times that affected more the enzyme activity. These MD samples were dehydrated by a series of ascending aqueous-ethanol concentrations and then immersed in an infiltration solution (glycol-methacrylate resin, Leica Historesin™) and ethanol 96º (1/1 v/v) for 2 h. The MD were placed in infiltration solution for 24 h at 4 ºC and then transferred to molds

with the infiltration solution and a hardener. Blocks were cut in 5 μm sections with an electronic microtome (Leica RM 2155™), and the sections stained with hematoxylin-eosin. The samples were examined under a light microscope (AXIOPLAN 2 Zeiss™) and photographic records made with an image-analysis system (Axiovision Rel 4.4).

Enzymatic-activity assays

The effect of the insecticide chlorpyrifos on the activity of the antioxidant enzyme system, AChE, and lipid peroxidation of the spider *P. pythagoricus* was evaluated. The activity of each enzyme was expressed as a percent of the respective control value, the latter being 100%.

AChE activity was measured by a modification of the technique of Ellman and Callaway (1961). The reaction contains S-butyrylthiocholine iodide and 5,5-dithio-bis-(2-nitrobenzoic acid). One unit of AChE activity was defined as the hydrolysis of 1 μmol of S-butyrylthiocholine/min.

The lipid peroxidation levels were assayed according to Ohkawa et al. (1979) by measuring the concentrations of thiobarbituric-acid–reactive substances, which method quantifies the levels of MDA generated as one of the main products of fatty acid peroxidation. CAT activity was evaluated by the method of Aebi (1984), quantitating the decrease in absorbance of H_2O_2 at 240 nm in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7) and 10 mM H_2O_2. One CAT unit was defined as the enzyme activity catalyzing 1 μmol of H_2O_2 reduction/min. SOD activity was quantified according to Misra and Fridovich (1972) by measuring the effect of endogenous SOD on the autoxidation of epinephrine in a 50 mM glycine buffer (pH 10.2). One SOD unit was defined as the enzyme activity inhibiting by 50% the rate of autocatalytic adrenochrome formation/min (absorbance recorded at 480 nm).

GPx activity was determined by the method of Flohé and Gunzler (1984) by quantitating at 340 nm the consumption of NADPH coupled to the depletion of

reduced glutathione (GSH) upon addition of GR to the reaction mixture. One GPx unit was defined as the enzyme activity consuming 1 μmol of NADPH/min.

GR activity was assessed via the technique of Calberg and Mannervik (1985), linking the reduction of oxidized glutathione to NADPH oxidation. A unit of GR activity was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH/ min at 340 nm.

GST activity was assayed following the method described by Habig et al. (1974) with the 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The reaction mixture contained 1 mM CDNB and 1 mM GSH. One GST unit was defined as the enzyme activity conjugating GSH with 1 μmol of CDNB/min at 340 nm.

Statistical analyses

The average times obtained from the behavioral experiment were compared through the use of a one-sample hypothesis test (Student t test, $\alpha = 0.05$). The null hypothesis was considered as randomness in each spider's movements with the assumption that spiders spent half of the total time (to contrast mean = 2.5 min) in each area of the terrarium. The alternative hypothesis considered that the spiders spent a time of less than 2.5 min in the sector with the treated surface -acetone in control assays, or insecticide in the treatment assays-, which shortness of duration meant that those surfaces produced repulsion. A logarithmic transformation was applied to the data when required.

The statistical comparison of the different treatment times was done by means of a one-way analysis of variance (ANOVA), after a previous control of data normality and homogeneity of variances. The Kruskal-Wallis test was applied if those tests were not fulfilled. The results of the metabolic biomarkers of oxidative stress are expressed as the mean ± the standard deviation (SD). Significant differences ($p < 0.05$) were compared through the Tukey or Bonferroni *post-hoc* tests. The data were analyzed through the use of the Statsgraphics Centurion XVI v. 16.2.04 statistical software.

RESULTS

Fig. 1 summarizes the average time spent (or remaining) in the behavioral assay. The results illustrated in the top right inset of the figure rule out a possible avoidance behavior of the solvent ($t = 0.240$, $p = 0.8113$), whereas those depicted in the central panel confirm a significant repulsion ($t = -4.332$, $p = 0.0006$) by chlorpyrifos (Fig. 1).

The MD of control spiders consisted of a simple epithelium composed of digestive and secretory cells surrounded by interstitial connective tissue (Fig. 2, Panel A). The digestive cells were larger and more numerous than the secretory cells and also contained vacuoles in the cytoplasm. The secretory cells contained cytoplasm filled with deeply stained droplets (Fig. 2, Panels A and B). Spiders after a 24 h exposure to chlorpyrifos exhibited an increase in the size of the interstitial connective tissue, with some necrotic midgut tubules (Fig. 2, Panel C), whereas the 48 h treatment revealed most of the tubules in a state of necrosis (Fig. 2, Panel D).

Within the profile of the cuticular HCs of *P. pythagoricus* (Fig. 3), 99 HC could be identified, mainly multi-isomeric methyl-, dimethyl- and trimethyl-branched components. The saturated branched-chain components of very-long-chain dimethyl (44.8%) and monomethyl (13.0%) saturated species, mostly C_{35}–C_{45}, were the predominant constituents. An isomer multiplicity of 11x, 13x, and 15x was found in most of the peaks, with x varying mainly from 15 to 29. Furthermore, 26% of the linear HCs were found between C_{22} and C_{37}. Table 1 lists the identification of each HC performed by CGC–mass spectrometry.

When chlorpyrifos entered the spider's body, changes in the AChE activity (Fig. 4) occurred after 24 and 48 h.

Lipid peroxidation (Fig. 5), was considerably higher at 6 and 24 h than after 48 h of exposure to chlorpyrifos ($F = 6.700$, $p = 0.0296$), where the values decreased down to a final value of 75 ± 53%.

The CAT and SOD activity (Fig. 6, Panels A and B respectively) were assayed at the different exposure times. CAT exhibited a higher percent activity at

6 h (145 ± 21%) with a clear decrease in activity occurring after 48 h of chlorpyrifos treatment ($F = 60.71$, $p = 0.0001$). SOD increased in activity to 168 ± 61% at 24 h ($F = 14.64$, $p = 0.0049$) over the corresponding values at 6 h and 48 h. The activity of GPx, GR, and GST (Fig. 7, Panels A–C respectively) enzymes related to glutathione metabolism—evidenced an increase after 24 h in all instances over the values at 6 h and 48 h. The variation in GST activity was significant at an average percent increase of 211 ± 70% ($F = 6.71$, $p = 0.027$).

DISCUSSION

The use of insecticides in agriculture can affect the physiological processes and behavioral aspects of nontarget inhabitants as well as cause alterations in different trophic levels influencing the relationships among organisms (Beauvais et al., 1999; Bellas et al., 2005; Galloway and Handy, 2003). In the present study, we observed that *P. pythagoricus* was capable of avoiding the surfaces with the organophosphate chlorpyrifos probably due to the exploration performed by contact chemoreceptors on the legs (pretarsus and tarsus) and on the pedipalps (Foelix and Chu-Wang, 1973). Although the sublethal impact of pesticides clearly varies in relation to the method used in a study and the model species (Dinter and Poehling, 1995; Evans et al., 2010), the present results coincide with the observations on *Clubiona* spp., *Dictyna* spp., *Pardosa* spp., and *Xysticus* spp. When confronted with surfaces with the organophosphate phosalone (Pekár and Haddad, 2005) and on *Pardosa palus* when exposed to Nurelle™ (chlorpyrifos and cypermethrin) or Decis™ (deltamethrin) (Pekár and Benes, 2008).

During the 4 h analyzed in our experiments, the first reaction of the spiders was to move out of the contaminated zone, as was also observed in *Oedothorax* sp. Spiders and other biological controllers (Everts et al., 1991; Michalková and Pékar, 2009). This response may have been caused by an avoidance of exploratory movements by the individuals because of the hostile environment (Wrinn et al., 2012). We need to emphasize that these effects are clearly produced by the active component of the insecticide chlorpyrifos and not by the additives, co-adjuvant, or

other ingredients of those formulations; which components in many instances can contribute significantly to toxicity (Mesnage and Antoniou, 2018). As previously mentioned, in spiders one possible portal of entry of insecticides is across the cuticle. This barrier has great significance since the cuticle can block xenobiotic absorption (Balabanidou et al., 2016; Bass et al., 2014; Juárez et al., 2010; Zhu et al., 2013). The removal of cuticular lipids has been demonstrated to accelerate the entry of insecticides in insects (Motoyama et al., 1992) and, moreover, insect resistance was correlated with an increase in lipids (Juárez, 1994; Juárez et al., 2010; Pedrini et al., 2009). In spiders, the cuticular HCs are present in great abundance, constituting the majority of the lipids observed in, for example, *Tegenaria atrica* at some 86% of the lipid extract (Trabalon et al., 1996). As mentioned above, HCs are the major component of the epicuticle of spiders and a possible synthesis of lipids by the epicuticle and transport to that tissue have been reported in *P. pythagoricus* (Trabalon and Garcia, 2021). In the present work, a considerable proportion of long-chain HCs (C_{33} to C_{45}) were registered, which species are probably the main HCs responsible for inhibiting the entry of the different insecticides. Longer-chain HCs have been demonstrated to prevent more effectively the entry and exit of molecules, thus constituting better waterproofing components (Balabanidou et al., 2016; Gibbs and Pomonis, 1995). In addition, it was observed that the increase in branched-chain HCs generates a thickening of the cuticle, thus impeding or slowing the entry of insecticides (Balabanidou et al., 2018). In that regard, in *P. pythagoricus* a large amount of HCs was observed, as had been found in *Anopheles gambiae* phenotypes that were pyrethroid- and organochlorine-insecticide resistant such as C_{28}, C_{29}, C_{30}, dimethyl C_{39}, and dimethyl C_{41} (Balabanidou et al., 2019). Nevertheless, new studies are certainly necessary to gain an understanding of how the cuticular-HC barrier functions in spiders.

Histological damage has been proposed as a biomarker of pesticide effects (Arrighetti et al., 2018; El-Saad et al., 2017). Numerous studies on arthropods

have evaluated histological changes and injuries caused by insecticides (dos Santos Jr. et al., 2020; Lavarías et al., 2017; Stentiford et al., 2005; Kalita et al., 2016; Plata-Rueda et al., 2020; Serra et al., 2021), but these sorts of studies on spiders are extremely scarce (El-Khouly et al., 2016). The necrosis generated in the MD after exposure of animals to chlorpyrifos coincides with what has been observed in the crustaceans *Penaeus monodon* and *Litopenaeus vannamei* (Nguyen Hong Son et al., 2015), other invertebrates (Ahmed et al., 2021; Sharaf et al., 2013), and vertebrates (Kammon et al., 2010; Verma et al., 2020).

AChE inhibition has been used for a long time as a biomarker of organophosphate toxicity in vertebrates (Boccioni et al., 2021; Fairbrother et al., 1991; Sandahl et al., 2005; Yen et al., 2011), aquatic invertebrates (Day and Scott, 1990; Edwards and Fisher, 1991; Hyne and Maher, 2003) and, to a lesser extent, in terrestrial invertebrates (Booth and O'Halloran, 2001; Engenheiro et al., 2005; Goven et al., 1993; Scott-Fordsmand and Weeks, 2000; Ribeiro et al.,1999). The activity of this enzyme in spiders has been described only for *Lycosa hilaris* (Van Erp, 2002), *Pardosa lugubris* (Babczyńska and Migula, 2002), and *Agelena labyrinthica* (Babczyńska et al., 2006). When *P. pythagoricus* was treated with chlorpyrifos, no inhibition of AChE was observed after 6 h. A 60% of inhibition was recorded 24 h after treatment, however; which difference coincides with the report of *A. labyrinthica* after being treated with dimethoate (Babczyńska et al., 2006). When *P. pythagoricus* was treated with chlorpyrifos, no inhibition of AChE was observed after 6 h of treatment. This result could be explained by the binding of the insecticide to the hemolymphatic lipoproteins that could cause structural changes that modify the functionality of those components, as was previously observed in *P. pythagoricus* (Cunningham et al., 2002), making a transport of insecticides to the organs difficult. Furthermore, differences in the response as a result of variation between individuals—*i. e.*, phenotypic plasticity (Nikinmaa and Antilan, 2019)—has prevented the observation of a clear inhibition over short time intervals. A 60% of inhibition was recorded 24 h after treatment, however; which

difference coincides with the report of *A. labyrinthica* after being treated with dimethoate (Babczyńska et al., 2006). This irreversible inhibition is progressive until at least 48 h (at an 80% decrease), as was reported by Van Erp et al. (2002) for *Lycosa hilaris*, a wolf spider (a 55% decrease). We need to emphasize that no mortality was observed in the individuals treated under the assay conditions, even with the high percentages of AChE inhibition at 48 h, in contrast to the inhibition of 55% that had been observed in *Lycosa hilaris*, where mortality had been recorded at even 30% (Van Erp et al., 2002) produced by the acetylcholine accumulation in the nerve endings.

Lipid peroxidation has been found to be one of the main consequences of oxidative stress, potentially leading to the breakdown of cells, tissues, and organs (Kidd, 1991; Ramesthagam and Ramasamy, 2006). Toxicological studies in arthropods in which acute exposures are performed have usually analyzed lipid peroxidation after 24 and 48 h of treatment (Alzahrani, 2019; Sarikaya et al., 2011; Yuan et al., 2019) since free radicals and ROS are expected to be eliminated during that time period by the antioxidant-enzyme system (*cf*. further on). In the present experiments with *P. pythagoricus*, chlorpyrifos treatments at 6 and 24 h caused high levels of lipid peroxidation in the MD possibly due to the high percentage of unsaturated fatty acids present in this organ (75%), which would be the main target of the free radicals (Laino et al., 2009). Moreover, the antioxidant enzymes either were at a low activity at that time or became activated later since at 48 h the levels of lipid peroxidation were considerably decreased. The first line of defense against ROS is the SOD and CAT, enzymes that sometimes act complementarily as has been observed in the spider *Xerolycosa nemoralis* (Lycosidae) (Wilczek et al., 2013) and the insects *Lymantria dispar* (Lepidoptera) (Aslanturk et al., 2011) and *Oxyachinensis* (Orthoptera: Acrididae) (Wu et al., 2011) after exposure to different organophosphate insecticides. In our example, a simultaneous induction of SOD and CAT was not observed since CAT activity increased after at 6 h and SOD after 24 h of exposure. This non simultaneous

induction of the two enzymes had also been observed in the insect *Sogatella furcifera* (Delphacidae) upon treatment with abamectin. The authors reported that CAT was induced at a maximum activity after 6 h of exposure and then decreased after 48 h; whereas SOD exhibited a low percent induction at 6 h, a maximum at 12 h, and then a decrease after 48 h of treatment (Zhou et al., 2019).

Glutathione is a tripeptide playing a key role in detoxification reactions and frequently being found in cells in the reduced form (GSH). The tripeptide acts as an antioxidant, providing reduction equivalents in the GPx-catalyzed reaction with GSH being oxidized to GSSG and the reduced form being later reconstituted by the enzyme GR. Furthermore, GSH acts as a substrate of GST catalyzing a conjugation of the tripeptide with different organic compounds (Hermes-Lima, 2004; Thair, 2016). The activity of GPx and GR in *P. pythagoricus* was usually higher in the individuals treated with chlorpyrifos after 24 h of exposure than after either 6 or 48 h, similar to the pattern of GPx activity in the spider *A. labyrinthica* treated with an organophosphate for 24 h (Babczyńska et al., 2006).

GST, for its part, intervenes in the metabolism of xenobiotics, participating in their detoxification. The transferase catalyzes the nucleophilic attack of a great number of compounds (Mannervik and Danielson, 1988) and the conjugation of lipophilic compounds with GSH, thus facilitating the elimination of that conjugate from the cell (Halliwell and Gutteridge, 2007; Hayes and Pulford, 1995). For instance, although a GST induction was observed in the larvae of *Trogoderma granarium* (Dermestidae) at short intervals (5 h) after fumigation with methylbromide, acrylonitrile, ethylenedichloride, or other agents (Shivanandappa and Rajendran, 1987), in the spiders *A. labyrinthica* and *P. lugubris* that induction was not observed after a 24 h exposure to an organophosphate (Babczyńska et al., 2006). In our experiments with *P. pythagoricus*, the increase in the GST activity after a 24 h application of the organophosphate was probably caused by an elevation in the xenobiotic's concentration in the MD after circulation, while the subsequent decrease after 48 h was most likely attributable to the detoxification

activity of GST. Similar results after a 48 h treatment were observed in the activities of GPx, GR, and GST in juveniles of the wolf spider *Pardosa saltans* after exposure to comparable concentrations of chlorpyrifos (Laino et al. 2021).

CONCLUSIONS

Finally, we can conclude from the enzymatic analysis that chlorpyrifos is capable of penetrating the organism (producing the inhibition of AChE) and producing an increase in lipid peroxidation after between 6 and 24 h of exposure. At 48 h we observed a decrease in lipid peroxidation after an increase in the activities of SOD, CAT, and GST at 6 and 24 h. Unfortunately, no literature was found providing an analysis of the antioxidant enzyme activity at different times after acute exposure to organophosphate, which absence makes correlations with other arthropods and a more conclusive interpretation impossible.

Our study has demonstrated that the spider *P. pythagoricus* 1) recognizes and is repelled by the organophosphate insecticide chlorpyrifos, undergoing an avoidance behavior; 2) evidences histological alterations in the MD caused by exposure to chlorpyrifos; 3) exhibits an inhibition of AChE activity and lipid peroxidation after being treated with chlorpyrifos at different times; and 4) responds to exposure to the insecticide by activating antioxidant enzymes (CAT, SOD, and GST) at different times for detoxification and a reduction of oxidative stress.

The results of this work are important for agriculture because it demonstrates how spiders respond when faced with insecticides. These new findings open up an intriguing and unexplored field of research on these biological pest controllers.

LEGENDS TO THE FIGURES

Table 1. Hydrocarbons of cuticle of *Polybetes. pythagoricus*

Peak	RT (min)	Area %	K I	Hydrocarbon	Diagnosticions
1	10.9	0.01	21	C22:1	308

	0		95		
2	10.99	0.01	2200	nC22	310
3	12.55	0.04	2292	C23:1	322
4	12.68	0.59	2300	nC23	324
5	13.75	0.01	2361	4-methyl C23	70/71,294/295;323
6	13.94	0.00	2371	3-methyl C23	56/57,308/309;323
7	14.34	0.01	2394	C24:1	336
8	14.44	0.09	2400	nC24	338
9	15.66	0.04	2456	4-methyl C24	70/71,308/309;337
10	16.28	0.02	2485	C25:1	350
11	16.60	3.84	2500	nC25	352
12	17.66	0.09	2555	4-methyl C25	70/71,322/323;351
13	17.86	0.07	2566	3-methyl C25	56/57,336/337;351
14	18.51	1.32	2600	nC26	366
15	19.74	0.33	2649	4-methyl C26	70/71,336/337;365
16	21.00	12.18	2700	nC27	380
17	21.30	0.08	2717	11-; 13-methyl C27	168/169,252/253;196/197,224/225;379
18	21.35	0.01	2720	9-methyl C27	140/141,280/281;379
19	21.45	0.01	2726	7-methyl C27	112/113,308/309;379
20	21.63	0.16	2737	5-methyl C27	84/85,336/337;379
21	21.87	0.11	2751	4-methyl C27	70/71,350/351;379
22	22.11	0.41	2764	3-methyl C27	56/57,364/365;379
23	22.71	1.22	2800	nC28	394
24	23.26	0.04	2825	12-;13-;14-methyl C28	182/183,252/253;196/197,
					238/239;210/211,224/225;393
25	23.93	0.34	2854	4-methyl C28	70/71,364/365;393
26	24.95	4.84	2900	nC29	408
27	25.39	0.24	2924	11-; 13-; 15-methyl C29	168/169,280/281;196/197,252/253;224/225;407

28	25.45	0.05	2927	9-methyl C29	140/141,308/309;407
29	25.58	0.27	2935	7-methyl C29	112/113,336/337;407
30	25.76	0.18	2945	5-methyl C29	84/85,364/365;407
31	25.99	0.11	2957	4-methyl C29	70/71,378/379;407
32	26.26	0.59	2972	3-methyl C29	56/57,392/393;407
33	26.37	0.07	2978	5,15-; 5,17-dimethyl C29	84/85,379;196/197,267;224/225,239;421
34	26.76	0.33	3000	nC30	422
35	26.93	0.08	3008	3,7-; 3,11-; 3,15-dimethyl C29	56/57,407;336/337,127;280/281,183;224/225,239;421
36	27.37	0.14	3029	x-methyl C30 (x=10,11,12,13,14,15)	154/155,308/309;168/169,294/295;182/183,280/281;196/197, 266/267;210/211,252/253;224/225,238/239;421
37	27.48	0.04	3034	x-methyl C30 (x=7,8,9)	112/113,350/351;126/127,336/337;140/141,322/323;421
38	27.65	0.07	3042	6-methyl C30	98/99,364/365;421
39	28.06	0.60	3061	4-methyl C30	70/71,392/393;421
40	28.26	0.09	3071	6,16-; 6,18-dimethyl C30	98/99,379;224/225,253;196/197,281;435
				3-methyl C30	56/57,406/407;421
41	28.54	0.06	3084	4,16-dimethyl C30	70/71,407;224/225,253;435
42	28.88	1.47	3100	nC31	436
43	29.64	5.56	3139	9-; 11-; 13-; 15-methyl C31	140/141,336/337;168/169,308/309;196/197,280/281;224/225, 252/253;435
44	29.78	0.59	3146	7-methyl C31	112/113,364/365,435
45	29.90	0.11	3152	5-methyl C31	84/85,392/393,435
46	30.03	0.94	3159	11,15-; 13,17-dimethyl C31	168/169,323;252/253,239;196/197,295; 224/225,267;449
47	30.39	0.98	3176	7,19-dimethyl C31; 3-methyl C31	112/113,379,196/197,295,449; 56/57,420/421,435
48	30.58	2.63	3186	5,17-; 5,19-dimethyl C31	84/85,407,224/225,267;196/197,295;449

Table 1. Hydrocarbon of cuticle of *P. pythagoricus* (continued)

49	30.94	0.72	3205	3,7-; 3,9-; 3,11-; 3,13-; 3,15-dimethyl C31	56/57,435;364/365,127;336/337,155;308/309,183;280/281,211; 252/253,239;449
50	31.54	1.75	3237	x-methyl C32 (x=10,11,12,13,14,15,16)	154/155,336/337;168/169,322/323;182/183,308/309;196/197, 294/295;210/211,280/281;224/225,266/

					267;238/239,252/253;449
51	31.68	0.19	3244	x-methyl C32 (x=6,7,8)	98/99,392/393;112/113,378/379;126/127,364/365;449
52	31.96	0.59	3258	4-methyl C32	70/71,420/421;449
				x,y-dimethyl C32 (x=10,12,14; y=16,18,20)	154/155,351;182/183,323;210/211,295;463
				8,12-dimethyl C32	126/127,379;308/309,197;463
53	32.23	0.13	3273	6,16-; 6,18-; 6,20-dimethyl C32	98/99,407;224/225,253;196/197,281;168/169,309;463
54	32.52	0.08	3288	4,16-; 4,18-, 4,20-dimethyl C32	70/71,435;224/225,253;196/197,281;168/169,309;463
55	32.74	0.31	3300	nC33	464
56	33.58	6.32	3344	9-; 11-; 13-; 15-; 17-methy C33	140/141,364/365;168/169,336/337;196/197,308/309;224/225,280/281;252/253;463
57	34.20	10.25	3375	9,x-dimethyl C33 (x=19,21,23,25)	140/141,379;477
				11,x-dimethyl C33 (x=15,17,19,21,23)	168/169,351;477
				13,x-dimethyl C33 (x=15,17,19,21)	196/197,323;477
				15,x-dimethyl C33 (x=17,19)	224/225,295;477
58	34.54	2.64	3393	5,15-; 5,17-; 5,19-dimethyl C33	84/85,435;224/225,295;252/253,267;280/281,239;477
59	34.67	0.27	3400	7,11,17-; 7,13,17-trimethyl C33	112/113,421;252/253,281;183,351;211,323;491
60	34.90	1.20	3412	3,11-; 3,13-; 3,15-dimethyl C33	56/57,463;336/337,183;308/309,211;280/281,239;477
61	35.30	0.98	3433	x-methyl C34 (x=10,11,12,13,14,15,16,17)	154/155,364/365;182/183,336/337;210/211,308/309;477
62	35.53	0.22	3445	3,7,9,13-tetramethyl C33	56/57,491;127,421,169,379,308/309,239;505
63	35.74	1.54	3456	x,y-dimethyl C34 (x=10,12,14; y=16,18,20,22)	154/155,379;182/183,351;210/211,323;491
64	36.19	0.61	3479	x,y,z-trimethyl C34 (x=10,12; y=14,16; z=18,20)	154/155,393;182/183,365;225,323,253,295;505
65	36.30	0.20	3485	4,16-; 4,18-; 4,20-dimethyl C34	70/71,463;280/281,253;252/253,281;224/225,309;491
66	36.53	0.08	3500	nC35	492
67	36.76	0.04	3508	4,12,18-; 4,12,20-trimethyl C34	70/71,477;197,351;252/253,295;224/225,323;505
68	37.17	2.09	3530	11-; 13-; 15-; 17-methyl C35	168/169,364/365;196/197,336/337;224/225,308/309;252/253,280/281;491
69	37.85	5.97	3568	9,x-dimethyl C35 (x=17,19,21,23,25,27)	140/141,407;505
				11,x-dimethyl C35 (x=17,19,21,23,25)	168/169,379;505
				13,x-dimethyl C35 (x=17,19,21,23)	196/197,351;505
				15,x-dimethyl C35	224/225,323;505

				(x=17,19,21)	
				17,19-dimethyl C35	252/253,295;505
70	38.15	2.89	3585	5,17-; 5,19-; 5,21-dimethyl C35	84/85,463;280/281,267;252/253,295;224/225,323;505
				11,15,19-trimethyl C35	168/169,393;239,323;252/253,309;519
71	38.28	0.27	3593	7,11,17-trimethyl C35	112/113,449;183,379;280/281,281;519
72	38.55	1.70	3608	3,11-; 3,15-; 3,17-dimethyl C35	56/57,491;308/309,239;280/281,267;364/365,183;505
73	38.99	0.94	3633	x-methyl C36 (x=14,16,18)	210/211,336/337;238/239,308/309;266/267,294/295;505
				3,9,15-; 3,11,15-trimethyl C35	56/57,505;155,407;183,379;308/309,253;519
74	39.39	0.69	3655	12,18-;12,20-;14,18-;14,20-;16,20-dimethyl C36	182/183,379;210/211,351;238/239,323;280/281,281; 252/253,309;519
				10,18-dimethyl C36	154/155,407;280/281,281;519
				8,18-dimethyl C36	126/127,435;280/281,281;519
75	39.71	0.28	3673	10,14,18-; 12,16,20-trimethyl C36	154/155,421,225,351,280/281,295;182/183,393,253,323, 252/253,323;533
76	39.86	0.24	3682	4,14-; 4,16-; 4,18-dimethyl C36	70/71,491;336/337,225;308/309,253;280/281,281;519
77	40.10	0.02	3700	nC37	520

Table 1. Hydrocarbon of cuticle of *P. pythagoricus* (continued)

78	40.31	0.06	3707	4,10,16-; 4,12,16-trimethyl C36	70/71,505;169,407;197,379;308/309,267;533
79	40.68	1.44	3728	9-; 11-; 13-; 15-; 17-; 19-methyl C37	140/141,420/421;168/169,392/393;196/197,364/365;224/225, 336/337;252/253,308/309;280/281;519
80	41.29	4.65	3765	9,x-dimethyl C37 (x=19,21,23,25,27,29)	140/141,435;533
				11,x-dimethyl C37 (x=19,21,23,25,27)	168/169,407;533
				13,x-dimethyl C37 (x=19,21,23,25)	196/197,379;533
				15,x-dimethyl C37 (x=19,21,23)	224/225,351;533
				17,19-dimethyl C37	252/253,323;533
81	41.65	2.90	3787	5,15-; 5,17-dimethyl C37	84/85,491;336/337,239;308/309,267;533
				11,15,19-; 13,15,19-trimethyl C37	168/169,421;196/197,393;239,351,280/281,309;547
82	41.75	0.17	3793	7,11,15-trimethyl C37	112/113,477;183,407;336/337,253;547
83	41.97	0.83	3807	3,15-; 3,17-; 3,19-dimethyl C37	56/57,519;336/337,239;308/309,267;280/281,295;533
				5,11,15-; 5,13,15-trimethyl C37	84/85,505;183,407;211,379;336/337,253;547
84	42.32	0.27	3828	x-methyl C38 (x=12,...,19)	182/183,392/393;210/211,364/365;238/239,336/337;533

				3,9,17-; 3,11,15-trimethyl C37	56/57,533;155,407;183,379;308/309,281;336/337,253;547
85	42.76	0.38	3856	12,18-;12,22-;12,24-;14,18-;14,22-dimethyl C38	182/183,407;210/211,379;308/309,281;252/253,337;224/225,365;547
				10,18-; 10,22-dimethyl C38	154/155,435;308/309,281;252/253,337;547
				8,18-; 8,22-dimethyl C38	126/127,463;308/309,281;252/253,337;547
86	43.07	0.25	3875	10,14,18-; 12,16,20-; 14,18,22-trimethyl C38	154/155,449;225,379;308/309,295;182/183,421;253,351;280/281,323;210/211,393;281,323;252/253,351;561
87	43.22	0.09	3884	4,16-; 4,18-; 4,22-dimethyl C38	70/71,519;336/337,253;308/309,281;252/253,337;547
88	43.95	0.63	3929	11-; 13-; 15-; 17-; 19-methyl C39	168/169,420/421;196/197,392/393;224/225,364/365;252/253,336/337;280/281,308/309;547
89	44.49	1.79	3958	9,x-dimethyl C39 (x=15,17,19,21,23,25,27,29,31)	140/141,463;561
				11,x-dimethyl C39 (x=15,17,19,21,23,25,27,29)	168/169,435;561
				13,x-dimethyl C39 (x=15,17,19,21,23,25,27)	196/197,407;561
				15,x-dimethyl C39 (x=17,19,21,23,25)	224/225,379;561
				17,x-dimethyl C39 (x=19,21,23)	252/253,351;561
				19,21-dimethyl C39	280/281,323;561
90	44.84	0.88	3977	5,15-; 5,17-dimethyl C39	84/85,519;364/365,239;336/337,267;561
				11,x,y-; 13,x,y-trimethyl C39	168/169,449;196/197,421;575
91	45.21	0.24	3997	3,15-; 3,17-; 3,19-dimethyl C39	56/57,547;364/365,239;336/337,267;308/309,295;561
				5,x,y-trimethyl C39 (x=11,13,15; y=17,19,21,23)	84/85,533;575
92	45.63	0.05	4020	x-methyl C40 (x=12,...,20)	182/183,420/421;238/239,364/365;266/267,308/309;561
93	46.11	0.04	4047	12,x-dimethyl C40	182/183,435;575
94	46.51	0.02	4068	4,x-dimethyl C40	70/71,547;575
95	47.58	0.05	4127	13-; 15-; 17-methyl C41	196/197,420/421;224/225,392/393;252/253,364/365;575
96	48.25	0.43	4155	11,15-; 11,17-dimethyl C41	168/169,463;364/365,267;392/393,239;589
				13,15-; 13,17-dimethyl C41	196/197,435;364/365,267;392/393,239;589
97	48.68	0.09	4175	5,15-; 5,17-dimethyl C41	84/85,547;392/393,239;364/365,267;589
				11,x,y-trimethyl C41	168/169,477;603
98	53.45	0.36	4350	11,21-; 11,23-dimethyl C43	168/169,491;308/309,351;336/337,323;617

				13,21-; 13,23-dimethyl C43	196/197,463;308/309,351;336/337,323;617
99	60.50	0.10	4549	11,23-dimethyl C45	168/169,519;336/337,351;645
				13,23-dimethyl C45	196/197,491;336/337,351;645

Table 1: Cuticular hydrocarbons of *Polybetes pythagoricus*. The methyl, dimethyl, trimethyl, and tetramethyl hydrocarbons are highlighted in green, the saturated ones in red, and the major species in blue.

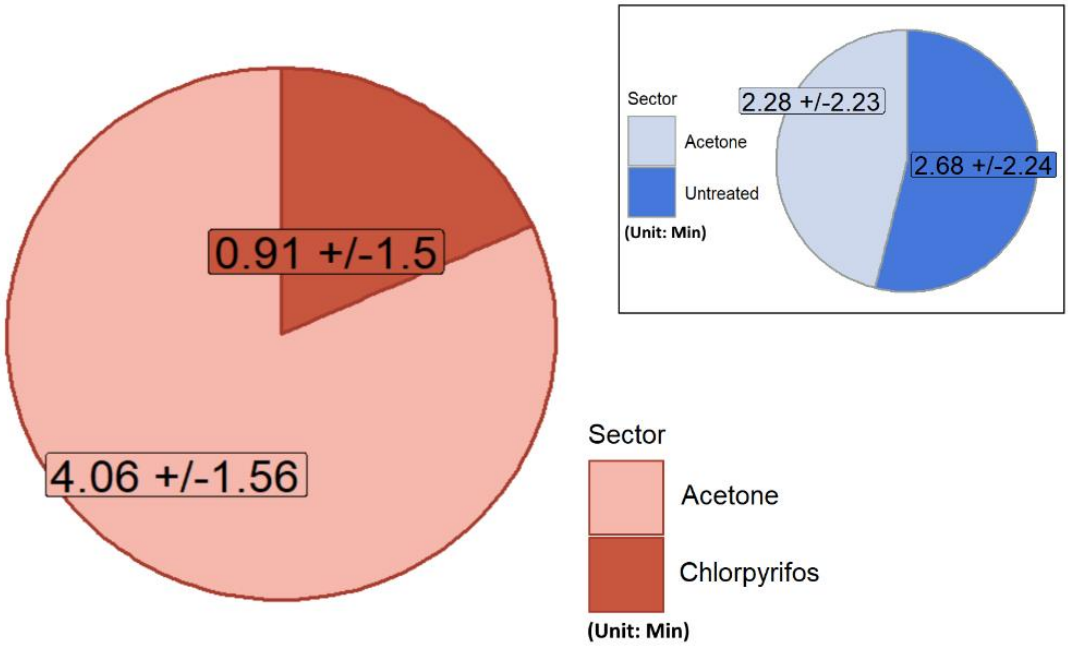

Fig. 1. Duration time of *Polybetes pythagoricus* in sectors with and without acetone and with and without chlorpyrifos. In the two graphics, the average duration times in min spent in a given sector of the experimental box (*ordinate*) for the two sectors of the terrarium (*abscissa)* are plotted. Inset: left, acetone treated filter paper; right, untreated filter paper. Centre graphic: left, chlorpyrifos treated filter paper; right, acetone treated filter paper.

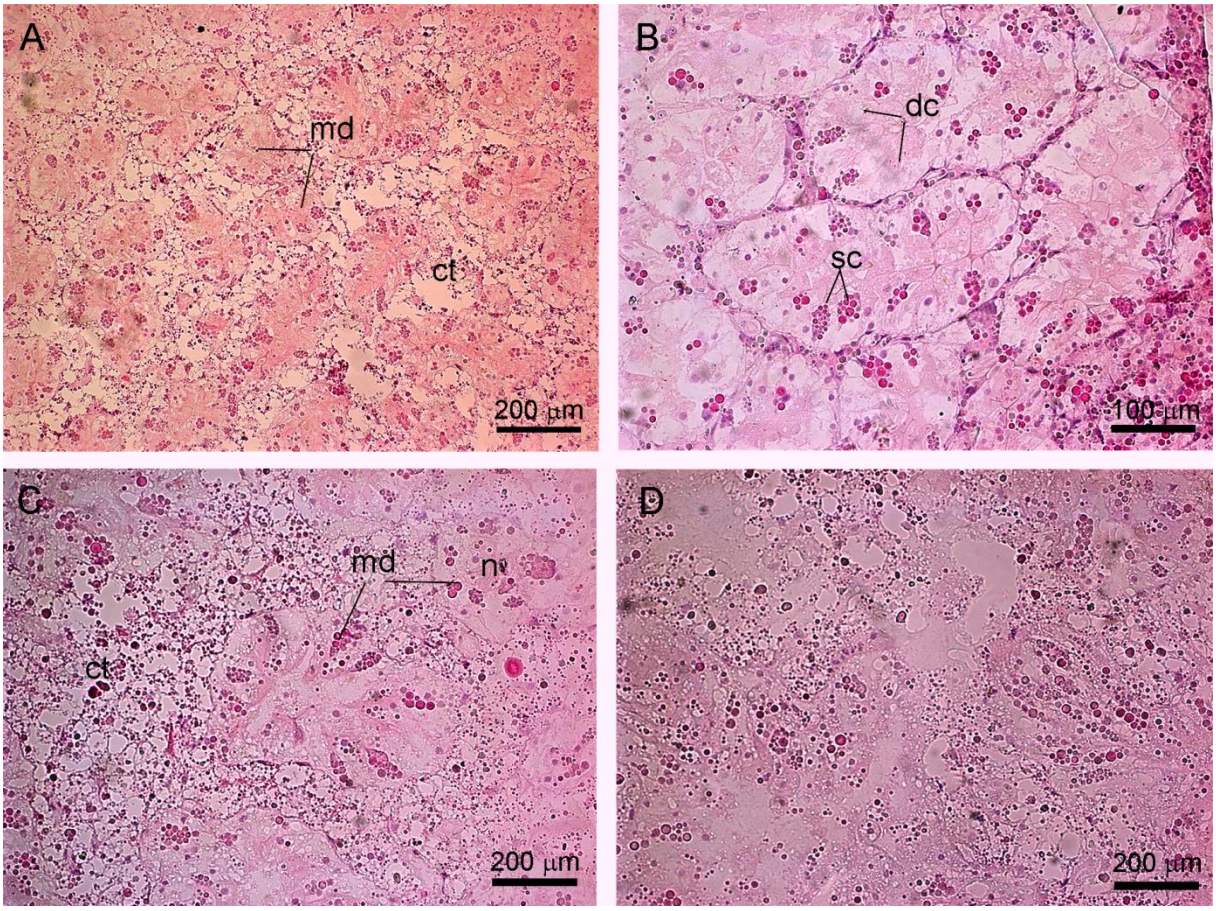

Fig. 2. *Polybetes pythagoricus* histological analysis. Panel A. General view of the midgut diverticula (MD) of a control spider illustrating the tubular structure surrounded by interstitial connective tissue (ct). Panel B. Detail of the midgut epithelium formed by digestive cells (dc) and secretory cells (sc). Panel C. section from a spider exposed to a field dose of chlorpyrifos for 24 h with part of the MD manifesting necrosis (n) and an increment in the connective tissue (ct). Panel D. Detail of the MD section after exposure to a field dose of chlorpyrifos for 48 h, revealing necrosis in most of the tissue.

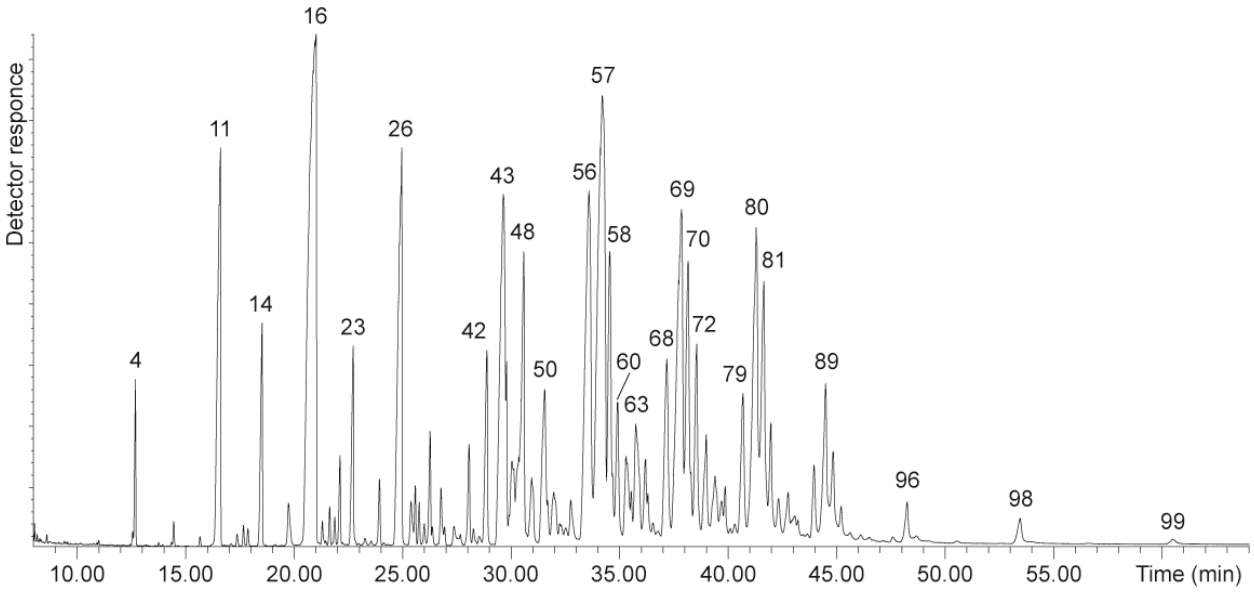

Fig. 3. Chromatograms of capillary gas chromatography of hydrocarbon profiles of *Polybetes pythagoricus*. The figure illustrates a representative chromatic record of detector response with respect to time in min with the retention times of the various peaks being marked above.

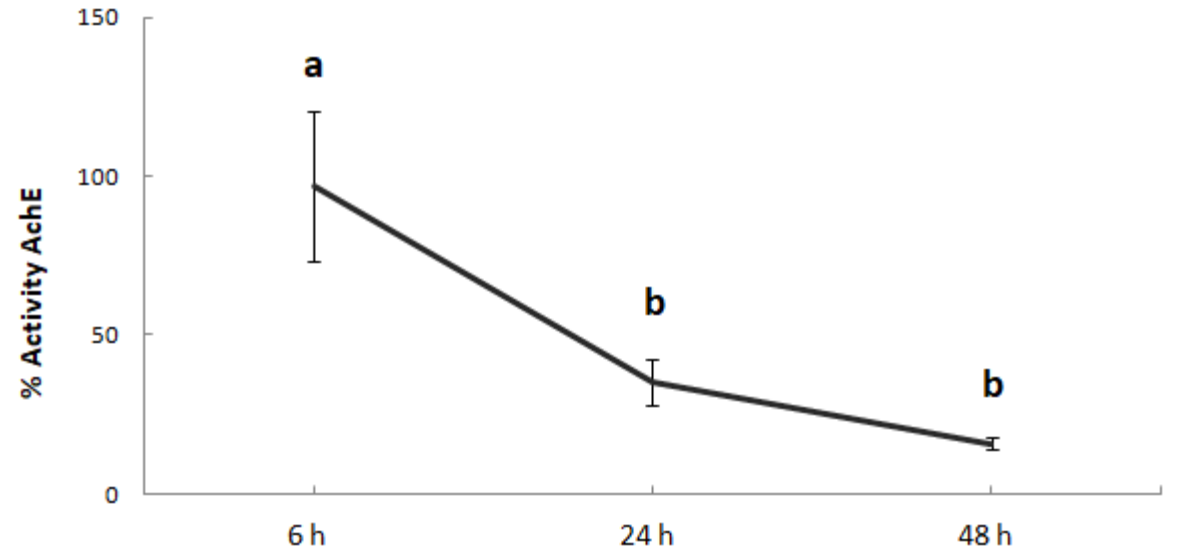

Fig. 4. Variation of the activity of acetylcholinesterase (AChE) of *Polybetes phytagoricus* in relation to the control at different times. In the figure, the percent AChE activity is plotted on the *ordinate* as a function of time of exposure to chlorpyrifos in h on the *abscissa*. The data were compared by the ANOVA. Different letters indicate significant differences among the data.

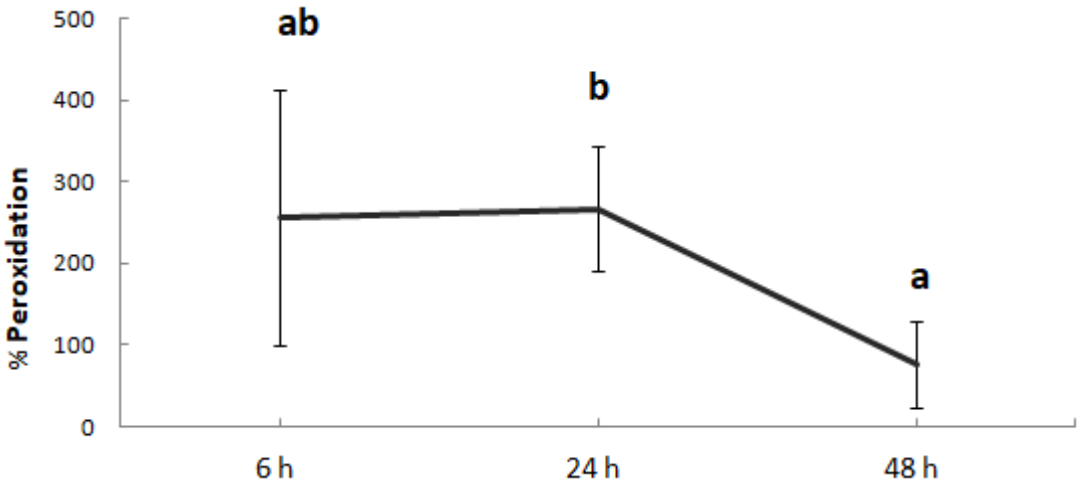

Fig. 5. Variation in the activity of lipid peroxidation measured after exposure of *Polybetes pythagoricus* to chlorpyrifos. In the figure, the percent variation in peroxidation, is plotted on the *ordinate* as a function of time of exposure to chlorpyrifos in h on the *abscissa*. The data were compared by the ANOVA. Different letters indicate significant differences among the data.

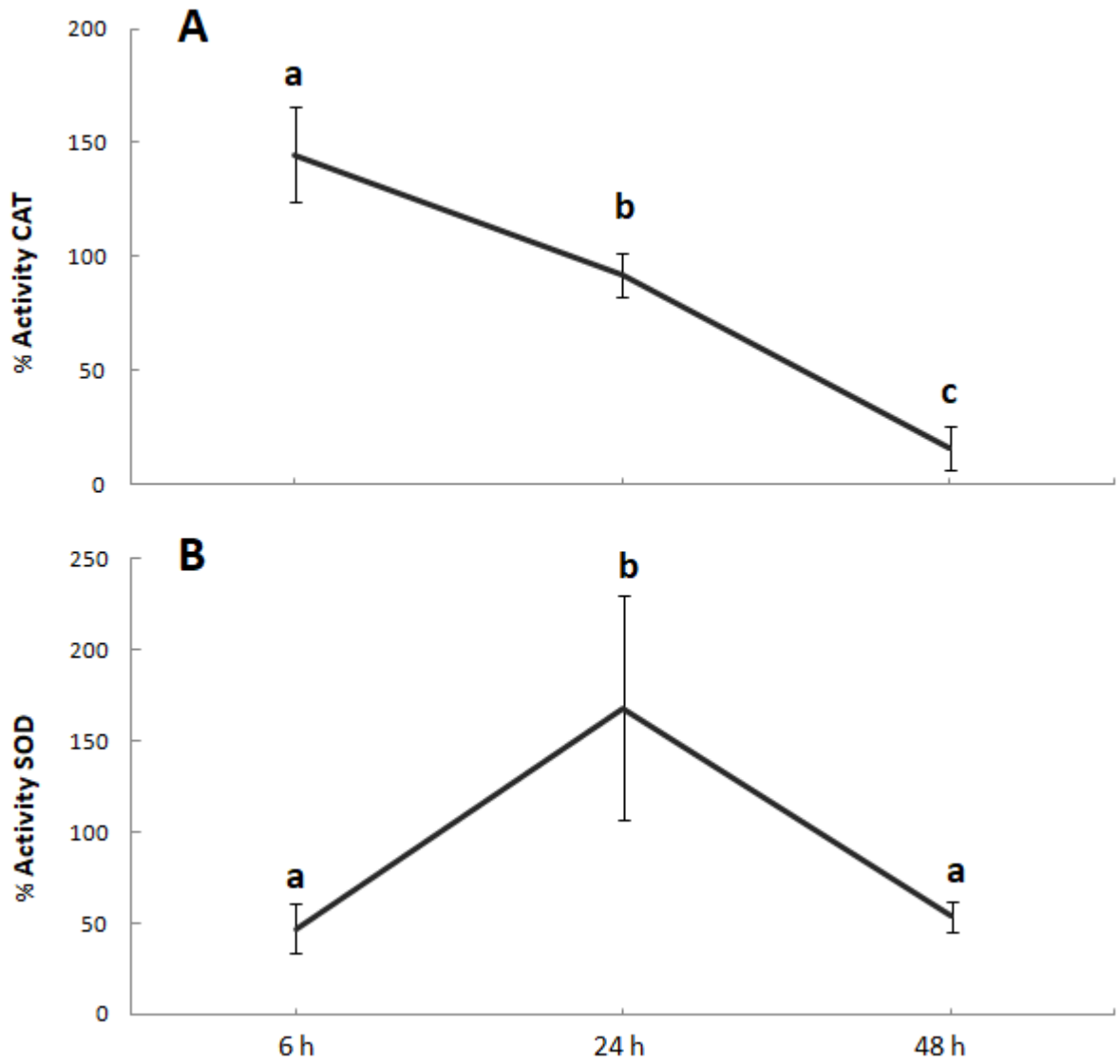

Fig. 6. Variation of the activity of catalase (CAT), (Panel A) and superoxide dismutase (SOD), (Panel B) after exposure of *Polybetes phytagoricus* to chlorpyrifos. In each panel, the percent variation in enzyme activity is plotted on the *ordinate* as a function of time in h on the *abscissa*. The data were compared by the ANOVA. Different letters indicate significant differences among the data.

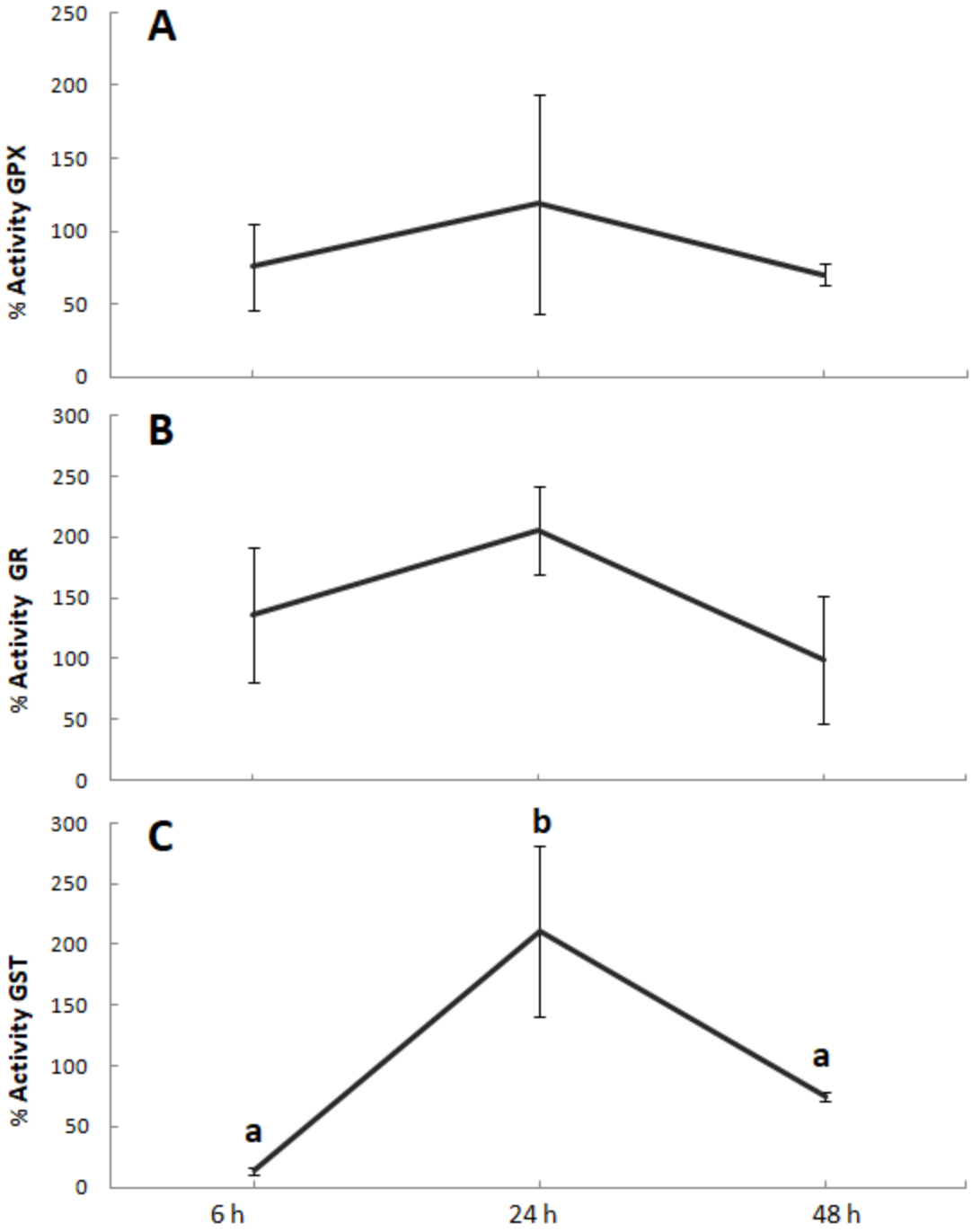

Fig. 7. Variation of the activity of glutathione peroxidase (GPx), (Panel A), glutathione reductase (GR), (Panel B), and glutathione S-transferase (GST), (Panel C) after exposure of *Polybetes phytagoricus* to chlorpyrifos. In each panel, the percent variation in enzyme activity is plotted on the *ordinate* as a

function of time in h on the *abscissa*. The data were compared by the ANOVA. Different letters indicate significant differences among the data.

Supplementary data

Fig. S1. Photographs of the boxes where the experiments were carried out, involving the two impregnated sectors of filter paper, A and B, in each box. Upper panel: A, acetone; B untreated. Lower panel: A chlorpyrifos; B, acetone.

REFERENCES

Abdelfattah, E. A. & El-Bassiony, G. M., 2022. Impact of malathion toxicity on the oxidative stress parameters of the black soldier fy *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). *Scientifc Reports* 12, 4583. https://doi.org/10.1038/s41598-022-08564-8

Aebi, H., 1984. Catalasein vitro. *Methods in Enzymology* 105, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3

Ahmed, F. A. G. & El-Sobki, A. E. A. M., 2021. Biochemical and Histological Responses of Red Palm Weevil, *Rhynchophorus ferrugineus* Exposed to Sub-lethal Levels of Different Insecticide Classes. *Egyptian Academic Journal of Biological Sciences* 13(1), 293-308. https://doi.org/10.21608/eajbsf.2021.211778

Aktar, M. W., Sengupta, D. S. & Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology* 2(1), 1-12. https://doi.org/10.2478/v10102-009-0001-7

Alzahrani, A. M., 2019. Ultrastructural damage and biochemical alterations in the testes of red palm weevils (*Rhynchophorus ferrugineus*) exposed to imidacloprid. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-019-04968-8

Arrighetti, F., Ambrosio, E., Astiz, M., Rodrigues Capítulo, A. & Lavarías, S., 2018. Differential response between histological and biochemical biomarkers in the apple snail *Pomacea canaliculata* (Gasteropoda:

Amullariidae) exposed to cypermethrin. *Aquatic Toxicology* 194, 140-151. https://doi.org/10.1016/j.aquatox.2017.11.014

Aslanturk, A., Kalender, S., Uzunhisarcikli, M. & Kalender, Y., 2011. Effects of Methidathion on Antioxidant Enzyme Activities and Malondialdehyde Level in Midgut Tissues of *Lymantria dispar* (Lepidoptera) larvae. *Journal of the Entomological Research Society* 13(3), 27-38. https://doi.org/10.81043/aperta.21151

Awasthi, M. D. & Prakash, N. B., 1999. Persistence of Chlorpyrifos in Soils under Different Moisture Regimes. *Pesticide Science* 50(1), 1–4. https://doi.org/10.1002/(sici)1096-9063(199705)50:13.0.co;2-x

Babczyńska, A. & Migula, P., 2002. Cadmium-Fenitrothion Interaction in the Spider Pardosa lugubris and the Fruit Fly *Drosophila melanogaster*. *Bulletin of Environmental Contamination and Toxicology* 69, 586–592. https://doi.org/10.1007/s00128-002-0101-y

Babczyńska, A., Wilczek, G. & Migula, P., 2006. Effects of dimethoate on spiders from metal pollution gradient. *Science of the Total Environment* 370, 352-359. https://doi.org/10.1016/j.scitotenv.2006.06.024

Balabanidou, V., Grigoraki, L. & Vontas, V., 2018. Insect cuticle: a critical determinant of insecticide resistance. *Current Opinion in Insect Science* 27, 68-74. https://doi.org/10.1016/j.cois.2018.03.001

Balabanidou, V., Kampouraki, A., MacLean, M., Blomquist, G., Tittiger, C., Juárez, M., Mijailovsky, S., Chalepakis, G., Anthousi, A., Lynd, A., Antoine, S., Hemingway, J., Ranson, H., Lycett, G. & Vontas, J., 2016. Cytochrome P450 associated with insecticide resistance catalyzes cuticular hydrocarbon production in *Anopheles gambiae*. *Proceedings of the National Academy of Sciences* 113, 9268–9273. https://doi.org/10.1073/pnas.1821201116

Balabanidou, V., Mary Kefi, M., Aivaliotis, M., Koidou, V., Girotti, J.R., Mijailovsky, S., Juárez, M., Papadogiorgaki, E., Chalepakis, G.,

Kampouraki, A., Nikolaou, C., Ranson, H. & Vontas, J., 2019. Mosquitoes cloak their legs to resist insecticides. *Proceedings of the Royal Society B: Biological Sciences* 286(1907). https://doi.org/10.1098/rspb.2019.1091

Bass, C., Puinean, A., Zimmer, C., Denholm, I., Field, L., Foster, S., Gutbrod, O., Nauen, R., Slater, R. & Williamson, M., 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology* 51, 41–51. https://doi.org/10.1016/j.ibmb.2014.05.003

Bayram, A. & Luff, M.L., 1993. Winter abundance and diversity of lycosids (Lycosidae, Araneae) and other spiders in grass tussocks in a field margin. *Pedobiologia* 37(6), 357-364.

Beauvais, S.L., Atchison, G.J., Stenback, J.Z. & Crumpton, W.G., 1999. Use of cholinesterase activity to monitor exposure of *Chironomus riparius* (Diptera: Chironomidae) to a pesticide mixture in hypoxic wetland mesocosms. *Hydrobiologia* 416, 163–170. https://doi.org/10.1023/a:1003819621659

Bellas, J., Beiras, R., Marino-Balsa, J. & Fernandez, N., 2005. Toxicity of organic compounds to marine invertebrate embryos and larvae: a comparison between the sea urchin embryogenesis bioassay and alternative test species. *Ecotoxicology* 14(3), 337–353. https://doi.org/10.1007/s10646-004-6370-y

Boccioni, A. C. P., Lajmanovich, R.C., Peltzer, P.M., Attademo, A.M. & Martinuzzi, C.S., 2021. Toxicity assessment at different experimental scenarios with glyphosate, chlorpyrifos and antibiotics in *Rhinella arenarum* (Anura: Bufonidae) tadpoles. *Chemosphere* 128475 https://doi.org/10.1016/j.chemosphere.2020.128475

Booth, L. H. & O'Halloran, K., 2001. A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus

insecticides diazinon and chlorpyrifos. *Environmental Toxicology and Chemistry* 20(11), 2494–2502. https://doi.org/10.1002/etc.5620201115

Booth L H, Wratten S. D. & Kehrli P., 2007. Effects of Reduced Rates of Two Insecticides on Enzyme Activity and Mortality of an Aphid and Its Lacewing Predator. *Journal of Economic Entomology* 100, 11-19. https://doi.org/10.1603/0022-0493(2007)100[11:eorrot]2.0.co;2

Calberg, E. & Mannervik, A., 1985. Glutathione reductase. *Methods Enzimol* 113(484-495.

Carvalho, F. P., 2017. Pesticides, environment, and food safety. *Food and Energy Security* 6(2), 48–60. https://doi.org/10.1002/fes3.108

Cunningham, M. L., Garcia, C. F., Gonzales-Baró, M.R., Garda, H. & Pollero, R., 2002. Organophosphorus insecticide fenitrothion alters the lipid dynamics in the spider *Polybetes pythagoricus* high density lipoproteins. *Pesticide Biochemistry and Physiology* 73, 37-47. https://doi.org/10.1016/S0048-3575(02)00016-0

Dar, M. A., Kaushika, G. & Villarreal-Chiub, J. F., 2019. Pollution status and bioremediation of chlorpyrifos in environmental matrices by the application of bacterial communities: A review. *Journal of Environmental Management* 239, 124-136. https://doi.org/10.1016/j.jenvman.2019.03.048

Day, K. E. & Scott, I. M., 1990. Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. *Aquatic Toxicology* 18(2), 101–113. https://doi.org/10.1016/0166-445x(90)90021-g

Degrendele, C., Okonski, K., Melymuk, L., Landlová, L., Kukucka, P., Audy, O., Kohoutek, J., Cupr, P. & Klánová, J., 2016. Pesticides in the atmosphere: a comparison of gas-particle partitioning and particle size distribution of legacy and current-use pesticides. *Atmospheric Chemistry*

and Physics (Print) 16, 1531-1544. https://doi.org/10.5194/acp-16-1531-2016

Di Nica, V., González, A. B. M., Lencioni, V. & Villa, S., 2019. Behavioural and biochemical alterations by chlorpyrifos in aquatic insects: an emerging environmental concern for pristine Alpine habitats. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-019-06467-2

Díaz-Barriga Arceo, S., Martínez-Tabche, L., Álvarez-González, I., López López, E. & Madrigal-Bujaidar, E., 2015. Toxicity induced by dieldrin and chlorpyrifos in the freshwater crayfish *Cambarellus montezumae* (Cambaridae). *Revista de Biología Tropical* 63(1), 83-96. https://doi.org/10.15517/rbt.v63i1.13665

Ding, T., Zhang, Y., Zhu, Y., Du, S., Zhang, J., Cao, Y., Wang, Y., Wang, G. & He, L., 2019. Deriving water quality criteria for China for the organophosphorus pesticides dichlorvos and malathion. *Environmental Science and Pollution Research* 26(33), 34622-34632. https://doi.org/10.1007/s11356-019-06546-4

Dinter, A. & Poehling, H.-M., 1995. Side-effects of insecticides on two erigonid spider species. *Entomologia Experimentalis et Applicata* 74(2), 151-163. https://doi.org/10.1111/j.1570-7458.1995.tb01887.x

dos Santos Junior, V.C., Martínez, L.C., Plata-Rueda, A., Fernandes, F.L., de Souza Tavares, W., Zanuncio, J.C. & Serr, J.E., 2020. Histopathological and cytotoxic changes induced by spinosad on midgut cells of the non-target predator *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae). *Chemosphere* 238, 124585. https://doi.org/10.1016/j.chemosphere.2019.124585

Edwards, C. A. & Fisher, S.W. (1991). The use of cholinesterase measurements in assessing the impact of pesticides on terrestrial and aquatic

invertebrates. *In* "Cholinesterase inhibiting insecticides – impacts on wildlife and environment" (P. Mineau, ed.). Elsevier.

El-Khouly, N. M., Rahil, A. A. & Dwidar, E. F., 2016. Effect of three inseticides on some biological and histological aspect of the spider *Anelosimus aulicus* (Koch). *Fayoum Journal of Agricultural Research and Development* 30(2), 37-52.

El-Saad, A. M. A., Kheirallah, D. A. & El-Samad, L. M., 2017. Biochemical and histological biomarkers in the midgut of *Apis mellifera* from polluted environment at Beheira Governorate, Egypt. *Environmental Science and Pollution Research* 24, 3181–3193. https://doi.org/10.1007/s11356-016-8059-1

Ellman, G. L. & Callaway, E., 1961. Erythrocyte cholinesterase-levels in mental patients. *Nature* 192, 1216.

Engenheiro, E. L., Hankard, P.K., Sousa, J. P., Lemos, M. F., Weeks, J. M. & Soares, A.M.V.M., 2005. Influence of dimethoate on acetylcholinesterase activity and locomotor function in terrestrial isopods. *Environmental Toxicology and Chemistry* 24, 603-609. https://doi.org/10.1897/04-131r.1

Erban, T., Sopko, B., Vaclavikova, M., Tomesova, D., Halesova, T. & Rezac, M., 2019. Pesticide comparison of *Phylloneta impressa* (Araneae: Theridiidae) females, cocoons and webs with prey remnants collected from a rape field before the harvest. *Pest Management Science* 76, 1128-1133.

Evans, S.C., Shaw, E. M. & Rypstra, A. L., 2010. Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. *Ecotoxicology* 19, 1249–1257. https://doi.org/10.1007/s10646-010-0509-9

Everts, J.W., Aukema, B., Mulliré, W.C., van Gemerden, A., Rottier, A., van Katz, R. & van Gestel, C.A.M., 1991. Exposure of the Ground Dwelling Spider *Oedothorax apicatus* (Blackwall) (Erigonidae) to Spray and

Residues of Deltamethrin *Arch. Environ. Contam. Toxicol.* 20, 13-19. https://doi.org/10.1007/BF01065322

Fairbrother, K., 1991. Methods used in determination of cholinesterase activity. *Chemicals in Agriculture*, 35-72.

Flohé, L. & Gunzler, W. A., 1984. Assays of glutathione peroxidase. *Methods Enzimology* 105(114-121. https://doi.org/10.1016/s0076-6879(84)05015-1

Foelix, R. F. & Chu-Wang, I. W., 1973. The morphology of spider sensilla II. chemoreceptors. *Tissue and Cell* 5(3), 461-478. https://doi.org/10.1016/S0040-8166(73)80038-2

Foong, S.Y., Ma, N. L., Lam, S. S., Peng, W., Low, F., Lee, B. H. K., Alstrup, A.K.O. & Sonne, C., 2020. A recent global review of hazardous chlorpyrifos pesticide in fruit and vegetables: Prevalence, remediation and actions needed. *Journal of Hazardous Materials* 400(123006) https://doi.org/10.1016/j.jhazmat.2020.123006

FSN, 2019. Food Safety News (FSN). Eu votes against renew. Chlorpyrifos approval. https://www.foodsafetynews.com/2019/12/eu-votes-against-renewingchlorpyrifos-approval/

Galloway, T. & Handy, R., 2003. Immunotoxicity of organophosphorous pesticides. *Ecotoxicology and Environmental Safety* 12, 345–363. https://doi.org/10.1023/a:1022579416322

Gibbs, A. & Pomonis, J. G., 1995. Physical properties of insect cuticular hydrocarbons: The effects of chain length, methyl-branching and unsaturation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 112(2), 243-249. https://doi.org/10.1016/0305-0491(95)00081-X

Gomes, H. D. O., Menezes, J. M. C., da Costa, J. G. M., Coutinho, H. D. M., Teixeira, R.N.P. & do Nascimento, R.F., 2020. A socio-environmental perspective on pesticide use and food production. *Ecotoxicology and*

Environmental Safety 197, 11062. https://doi.org/10.1016/j.ecoenv.2020.110627

Goven, A. J., Fitzpatrick, L. C., Eyambe, G. S., Venables, B. J. & Cooper, E. L., 1993. Cellular biomarkers for measuring toxicity of xenobiotics: Effects of polychlorinated biphenyls on earthworm *Lumbricus terrestris* coelomocytes. *Environmental Toxicology and Chemistry* 12(5), 863-870. https://doi.org/10.1002/etc.5620120510

Habig, W., Pabst, M. J. & Jakoby, W. B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 22(7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Halliwell, B.J. & Gutteridge, J. 2007. "Free radicals in biology and medicine," 4th/Ed. Oxford University Press, Oxford, United Kingdom.

Hayes, J. D. & Pulford, D. J., 1995. The Glutathione S-Transferase Supergene Family: Regulation of GST and the Contribution of the lsoenzymes to Cancer Chemoprotection and Drug Resistance. *Critical Reviews in Biochernisty and Molecular Biology* 30(6), 445-600. https://doi.org/10.3109/10409239509083491

Hemingway, J., 2000. The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochemistry and Molecular Biology* 30(11), 1009–1015. https://doi.org/10.1016/s0965-1748(00)00079-5

Hermes-Lima, M., Ramos-VAsconcelos, G.R., Cardoso, L. A., Rivera, P. M. & Drew, K.L. (2004). Animal Adaptability to Oxidative Stress: Gastropod Estivation and Mammalian Hibernation. *In* "Life in the Cold: Evolution, Mechanisms, Adaptation, and Application. Twelfth International Hibernation Symposium". Biological Papers of the University of Alaska.

Huang, X., Cui, H. & Duan, W., 2020. Ecotoxicity of chlorpyrifos to aquatic organisms: A review. *Ecotoxicology and Environmental Safety* 200, 110731. https://doi.org/10.1016/j.ecoenv.2020.110731

Hyne, R. V. & Maher, W. A., 2003. Invertebrate biomarkers: links to toxicosis that predict population decline. *Ecotoxicology and Environmental Safety* 54(3), 366–374. https://doi.org/10.1016/S0147-6513(02)00119-7

Janssens, R. & Stoks, L. 2017. Chlorpyrifos-induced oxidative damage is reduced under warming and predation risk: Explaining antagonistic interactions with a pesticide. *Environmental Pollution*. 226, 79-88. https://doi.org/10.1016/j.envpol.2017.04.012

John, E.M. & Shaike, J.M., 2015. Chlorpyrifos: pollution and remediation. *Environmental Chemistry Letters* 13(3), 269–291. https://doi.org/10.1007/s10311-015-0513-7

Juárez, M. P., Pedrini, N., Girotti, J. R. & Mijailovsky, S.J. (2010). Pyrethroid resistance in Chagas disease vectors: The case of Triatoma infestans cuticle. Review. *In* "Resistant Pest Management Newsletter", Vol. 19, pp. 59-61. Center for Integrated Plant Systems (CIPS) Insecticide Resistance Action Committee (IRAC) Western Regional Coordinating Committee (WRCC-60).

Juárez, P., 1994. Inhibition of cuticular lipid synthesis and its effect on insect survival. *Archives of Insect Biochemistry and Physiology* 25(3), 177-191. https://doi.org/10.1002/arch.940250302

Kalita, M. K., Haloi, K. & Devi, D., 2016. Larval Exposure to Chlorpyrifos Affects Nutritional Physiology and Induces Genotoxicity in Silkworm Philosamia ricini (Lepidoptera: Saturniidae). *Frontiers in Physiology* 7, 535. https://doi.org/10.3389/fphys.2016.00535

Kammon, A. M., Brar, R. S., Banga, H. S. & Sodhi, S., 2010. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. *Veterinarski Arhiv* 80(5), 663-672. https://hrcak.srce.hr/62160

Ketterer, B., Coles, B. & Meyer, D.J., 1983. The Role of Glutathione in Detoxication. *Environmental Health Perspectives* 49(59-69.

Khan, M. M., Hafeez, M., Elgizawy, K., Wang, H., Zhao, J., Cai, W., Ma, W. & Hua, H. 2021. Sublethal effects of chlorantraniliprole on *Paederus fuscipes* (Staphylinidae: Coleoptera), a general predator in paddle field. *Environmental Pollution* 291, 118171. https://doi.org/10.1016/j.envpol.2021.118171

Kidd, P.M., 1997. Glutathione: Systemic Protectant Against Oxidative and Free Radical Damage. *Alternative Medicine Review* 2(3), 155-176.

Kostaropoulos, I., Papadopoulos, A. I., Metaxakis, A., Boukouvala, E. & Papadopoulou-Mourkidou, E., 2001. Glutathione S–transferase in the defence against pyrethroids in insects. *Insect Biochemistry and Molecular Biology* 31(4-5), 313–319. https://doi.org/10.1016/s0965-1748(00)00123-5

Kralj, M. B., Černigoj, U., Franko, M. & Trebše, P., 2007. Comparison of photocatalysis and photolysis of malathion, isomalathion, malaoxon, and commercial malathion--products and toxicity studies. *Water Research* 41(19), 4504–4514. https://doi.org/10.1016/j.watres.2007.06.016

Lacava, M., García, L. F., Viera, C. & Michalko, R., 2021. The pest-specific effects of glyphosate on functional response of a wolf spider. *Chemosphere* 262, 127785. https://doi.org/10.1016/j.chemosphere.2020.127785

Laino, A., Cunningham, M. L., Garcia, F. & Heras, H., 2009. First insight into the lipid uptake, storage and mobilization in arachnids: role of midgut diverticula and lipoproteins. *Journal of Insect Physiology* 55(12), 1118-1124. https://doi.org/10.1016/j.jinsphys.2009.08.005

Laino, A. & Garcia, C.F., 2020. Study of the effect of cypermethrin on the spider *Polybetes phytagoricus* in different energy states. *Pesticide Biochemistry and Physiology*. https://doi.org/doi.org/10.1016/j.pestbp.2020.104559

Laino, A., Romero, S., Cunningham, M., Molina, G., Gabellone, C., Trabalon, M. & Garcia, C.F., 2021. Can Wolf Spider Mothers Detect Insecticides in the Environment? Does the Silk of the Egg-Sac Protect Juveniles from Insecticides? *Environmental Toxicology and Chemistry* 40(10), 2861-2873. https://doi.org/10.1002/etc.5157

Lavarías, S., Arrighetti, F. & Siri, A., 2017. Histopathological effects of cypermethrin and *Bacillus thuringiensis* var. *israelensis* on midgut of *Chironomus calligraphus* larvae (Diptera: Chironomidae). *Pesticide Biochemistry and Physiology* 139, 9-16. https://doi.org/10.1016/j.pestbp.2017.04.002

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biology and Chemistry* 193, 265-275.

Mahmood, S., Khan, N., Iqbal, K.J., Ashraf, M. & Khalique, A., 2018. Evaluation of water hyacinth (*Eichhornia crassipes*) supplemented diets on the growth, digestibility and histology of grass carp (*Ctenopharyngodon idella*) fingerlings. *Journal of Applied Animal Research* 46(1), 24-28. https://doi.org/10.1080/09712119.2016.1256291

Maloney, D., Drummond, F. A. & Alford, R., 2003. Spider Predation in Agroecosystems: Can Spiders Effectively Control Pest Populations? *Maine Agricultural and Forest Experiment Station Technical Bulletin* 190.

Mannervik, B. & Danielson, U. H., 1988. Glutathione Transferases—Structure and Catalytic Activit. *Critical Reviews in Biochemistry* 23(3), 283-337 https://doi.org/10.3109/10409238809088226

MARA, 2013. Ministry of Agriculture and Rural Affairs (MARA) of the People's Republic of China. Announcement No. 2032 (In Chinese). http://www.moa.gov.cn/govpublic/ZZYGLS/201312/t20131219_3718683.htm

Mesnage, R. & Antoniou, M. N., 2018. Ignoring Adjuvant Toxicity Falsifies the Safety Profile of Commercial Pesticides. *Frontiers in Public Health* 5. https://doi.org/10.3389/fpubh.2017.00361

Michalko, R. & Košulič, O., 2015. Temperature-dependent effect of two neurotoxic insecticides on predatory potential of Philodromus spiders. *Journal of Pest Science* 89(2), 517–527. https://doi.org/10.1007/s10340-015-0696-5

Michalko, R., Pekár, S. & Entling, M. H., 2018. An updated perspective on spiders as generalist predators in biological control. *Oecologia* 189, 21–36. https://doi.org/10.1007/s00442-018-4313-1

Michalková, V. & Pekár, S., 2009. How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae). *Biological Control* 51, 444–449.

Misra, H. P. & Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247, 3170-3175.

Motoyama, N., Suganuma, T. & Maekoshi, Y., 1992. Biochemical and Physiologial Characteristics of Insecticide Resistance in Diamondback Moth. *Proceedings of the Second International Workshop, Tainan, Taiwan 10-14 December 1990*, 411-418.

Mullié, W. C. & Everts, J. W., 1991. Uptake and elimination of [14C]deltamethrin by Oedothorax apicatus (Arachnida; Erigonidae) with respect to bioavailability. *Pesticide Biochemistry and Physiology* 39(1), 27-34. https://doi.org/10.1016/0048-3575(91)90210-D

Nguyen Hong, S., Viet, T. Q., Dang Hoang, O., Do Phuong, C., Lan Huong, B., Dinh Tien, D. & Nguyen Huy, M., 2015. Acute toxic and hepatopancreas syndrome caused by Chlopyrifos ethyl to black tiger shrimp (*Penaeus monodon*) and white shrimp (*Litopenaeus vannamei*) in Mekong River

Delta of Vietnam. *International Journal of Agricultural Technology* 11(5), 1097-1108.

Nikinmaa, M. & Anttila, K., 2019. Individual variation in aquatic toxicology: Not only unwanted noise. *Aquatic Toxicology* 207, 29-33. https://doi.org/10.1016/j.aquatox.2018.11.021

Nyffeler, M. (1999). Prey selection of spiders in the field. *In* "Spiders in Agroecosystems: Ecological Processes and Biological Control" (M. H. Greenstone and K. D. Sunderland, eds.), Vol. 27, pp. 6.

Nyffeler, M. & Sterling, W. L., 1994. Comparison of the feeding niche of polyphagous insectivores (Araneae) in a Texas cotton plantation: Estimates of niche breadth and overlap. *Environmental Entomology* 23(5), 1294-1303.

Nyffeler, M. & Sunderland, K. D., 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. *Agriculture, Ecosystems & Environment,* 95(2-3), 579–612. https://doi.org/10.1016/s0167-8809(02)00181-0

Ohkawa, H., Ohishi, N. & Yagi, K., 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annales of Biochemistry* 95(351-358.

Patil, R., Patil, Y., Salunkhe, P. & Patil, P., 2020. Diversity and distribution of agrobiont spiders (Arachnida: Araneae) from different agro-ecosistems of Anjani village, M.S. (India) *International Journal of Research and Analytical Reviews* 455-460. https://doi.org/10.1729/Journal.20966

Pedrini, N., Mijailovsky, S. J., Girotti, J. R., Stariolo, R., Cardozo, R. M., Gentile, A. & Juárez, M.P., 2009. Control of Pyrethroid-Resistant Chagas Disease Vectors with Entomopathogenic Fungi. *PLoS Neglected Tropical Diseases* 3(5), e434. https://doi.org/10.1371/journal.pntd.0000434

Pekár, S., 2012. Spiders (Araneae) in the pesticide world: an ecotoxicological review. *Pest. Manag. Sci.* https://doi.org/10.1002/ps.3397

Pekár, S. (2013). Side effect of synthetic pesticides on spider. *In* "Spider Ecophysiology" (W. Nentwing, ed.). Springer, Berling.

Pekár, S. & Benes, J., 2008. Aged pesticide residues are detrimental to agrobiont spiders (Araneae). *Journal of Applied Entomology* https://doi.org/10.1111/j.1439-0418.2008.01294.x(

Pekár, S. & Haddad, C. R., 2005. Can agrobiont spiders (Araneae) avoid a surface with pesticide residues? *Pest Management Science* 61(12), 1179-1185. https://doi.org/10.1002/ps.1110

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. & D'Amore, M., 1992. Environmental and Economic Costs of Pesticide Use. *Bioscience* 42(10), 750–760. https://doi.org/10.2307/1311994

Plata-Rueda, A., Martins de Menezesa, C. H., dos Santos Cunha, W., Alvarenga, T.M., Barbosa, B.F., Zanuncio, J.C., Martínez, L.C. & Serrão, J.E., 2020. Side-effects caused by chlorpyrifos in the velvetbean caterpillar Anticarsia gemmatalis (Lepidoptera: Noctuidae). *Chemosphere*, 127530. https://doi.org/10.1016/j.chemosphere.2020.12

Prajapati, J. N., Patel, S. R., Surani, P. M. & Radadia, G. G., 2018. Agrobiont spiders (Araneae) from Five Ecosystems of Navsari Agricultural University, Navsari, Gujarat, India. *International Journal of Chenical Studies* 6(3), 2547-2550.

Rain, F. F., Howlader, A. J. & Bashar, K., 2016. Diversity and abundance of spider fauna at different habitats of Jahangirnagar University Campus, Bangladesh. *Journal of Entomology and Zoology Studies* 4(5), 87-93.

Rameshthangam, P. & Ramasamy, P., 2006. Antioxidant and membrane bound enzymes activity in WSSV-infected Penaeus monodon Fabricius. *Aquaculture* 254, 32–39. https://doi.org/10.1016/j.aquaculture.2005.10.011

Rezac, M., Pekar, S. & Jitka, S., 2010. The negative effect of some selective insecticides on the functional response of a potential biological control agent, the spider *Philodromus cespitum*. *Bio Control* 55, 503–510.

Ribeiro, S., Guilhermino, L., Sousa, J.P. & Soares, A. M. V. M., 1999. Novel Bioassay Based on Acetylcholinesterase and Lactate Dehydrogenase Activities to Evaluate the Toxicity of Chemicals to Soil Isopods. *Ecotoxicology and Environmental Safety* 44(3), 287-293. https://doi.org/10.1006/eesa.1999.1837

Riechert, S. E. & Lockley, T., 1984. Spiders as biological control agents. *Annual Review of Entomology* 29, 229-320.

Rikans, L. E. & Hornbrook, K. R., 1997. Lipid peroxidation, antioxidant protection and aging. *Biochimica et Biophysica Acta* 1362(2-3), 116-127. https://doi.org/10.1016/s0925-4439(97)00067-7

Rodrigues A. R. S., Siqueira, H. A. A. & Torres, J. B. 2014. Enzymes mediating resistance to lambda-cyhalothrin in *Eriopis connexa* (Coleoptera: Coccinellidae). *Environmental Pollution* 110, 36-43. https://doi.org/10.1016/j.pestbp.2014.02.005

Sandahl, J. F., Baldwin, D. H., Jenkins, J.J. & Scholz, N. L., 2005. Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry* 24(1), 136-145. https://doi.org/10.1897/04-195r.1

Sarikaya, S. B. O., Topal, F., Şentürk, M., Gülçin, İ. & Supuran, C. T., 2011. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. *Bioorganic and Medicinal Chemistry Letters* 21(14), 4259–4262. https://doi.org/10.1016/j.bmcl.2011.05.071

Scott-Fordsmand, J. J. & Weeks, J. M., 2000. Biomarkers in earthworms. *Reviews of Environmental Contamination and Toxicology* 165(117–159. https://doi.org/10.1002/etc.5620120510

Serra, R. S., Cossolin, J. F. S., Santos de Resende, M. T. C., de Castro, M. A., Oliveira, A.H., Martínez, L.C. & Serrão, J.E., 2021. Spiromesifen induces histopathological and cytotoxic changes in the midgut of the honeybee *Apis mellifera* (Hymenoptera: Apidae). *Chemosphere*, 129439. https://doi.org/10.1016/j.chemosphere.2020.129439

Sessa, L., Calderón-Fernández, G. M., Abreo, E., Altier, N., Mijailovsky, S. J., Girotti, J.R. & Pedrini, N., 2021. Epicuticular hydrocarbons of the redbanded stink bug *Piezodorus guildinii* (Heteroptera: Pentatomidae): sexual dimorphism and alterations in insects collected in insecticide-treated soybean crops. *Pest Management Science*. https://doi.org/10.1002/ps.6528

Shaffo, F. C., Grodzki, A. C., Schelegle, E. S. & Lein, P. J., 2018. The organophosphorus pesticide chlorpyrifos induces sex-specific airway hyperreactivity in adult rats. *Toxicological Sciences* 165(1), 244–253. https://doi.org/10.1093/toxsci/kfy158

Sharaf, H. M., Salama, M. A. & Abd El-Atti, M. S., 2013. Biochemical and Histological Alterations in the Digestive Gland of the Land Snail *Helicella vestalis* (Locard, 1882) Exposed to Methiocarb and Chlorpyrifos in the Laboratory. *International Journal of Science and Research* 4(7), 2319-7064. https://doi.org/10.4172/2157-7099.1000327

Shivanandappa, T. & Rajendran, S., 1987. Induction of glutathione S-transterase by fumigants in larvae of the Khapra beetle, *Trogoderma granarium* (E.). *Pesticide Biochemistry and Physiology* 28(1), 121-126. https://doi.org/10.1016/0048-3575(87)90120-9

Solanki, R. & Kumar, D., 2014. Effect of Pesticides on Spider Population in Cotton Agro-System of Vadodara (Gujarat). *Journal of Science & Technology* 3(1), 48-52.

Stenersen, J. 2007. "Chemical pesticides. Mode of action and toxicology," CRC Press, New York.

Stentiford, G.D. & Feist, S. W., 2005. A histopathological survey of shore crab (*Carcinus maenas*) and brown shrimp *(Crangon crangon*) from six estuaries in the United Kingdom. *Journal of Invertebrate Pathology* 88, 136–146. https://doi.org/10.1016/j.jip.2005.01.006

Sumon, K. A., Rashid, H., Peeters, E. T., Bosma, R. H. & Van den Brink, P. J., 2018. Environmental monitoring and risk assessment of organophosphate pesticides in aquatic ecosystems of north-west Bangladesh. *Chemosphere* 206(92–100). https://doi.org/10.1016/j.chemosphere.2018.04.167

Tahir, H. M., Khizar, F., Naseem, S., Yaqoob, R. & Samiullah, K., 2016. Insecticide resistance in the ground spider Pardosa sumatra (Thorell, 1890; Araneae: Lycosidae). *Insect Biochemistry and Physiology* 93(1), 55–64. https://doi.org/10.1002/arch.21341

Tahir, H. M., Yaqoob, R., Naseem, S., Sherawat, S.M. & Zahra, K., 2015. Effects of Insecticides on Predatory performance of Spiders. *Biologia (Pakistan)* 61(1), 127-131.

Trabalon, M., Bagnéres, A.G., Hartman, N. & Vallet, A. M., 1996. Change in cuticular compounds composition during th gregarious period and after dispersal of the young in *Tegenaria atrica*, (Araneae, Agelinidae). *Insect Biochemistry and Molecular Biology* 26, 77-84. https://doi.org/10.1016/0965-1748(95)00065-8

Trabalon, M., Bagnères, A.G. & Roland, C., 1997. Contact Sex Signals in Two Sympatric Spider Species, Tegenaria domestica and Tegenaria pagana. *Journal of Chemical Ecology* 23, 747–758. https://doi.org/10.1023/B:JOEC.0000006408.60663.db

Trabalon, M. & Garcia, C. F., 2021. Transport pathways of hydrocarbon and free fatty acids to the cuticle in arthropods and hypothetical models in spiders. *Comparative Biochemistry and Physiology, Part B* 252, 110541. https://doi.org/10.1016/j.cbpb.2020.110541

Van Erp, S., Booth, L., Gooneratne, R. & K., O. H., 2002. Sublethal responses of wolf spiders (Lycosidae) to organophosphorous insecticides. *Environmental Toxicology* 17, 449-456.

Verma, D.K., Tripathi, R., Das, V. K. & Pandey, R. K., 2020. Histopathological Changes in Liver and Kidney of Heteropneustes fossilis (Bloch) on Chlorpyrifos Exposure. *The Scientific Temper* 11(1-2), 141-147.

Wilczek, G., Babczyńska, A. & Wilczek, P., 2013. Antioxidative responses in females and males of the spider *Xerolycosa nemoralis* (Lycosidae) exposed to natural and anthropogenic stressors. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 157, 119-131.

Wilczek, G., Babczynska, A., Wilczek, P., Dolezych, B., Migula, P. & Mlynska, H., 2008. Cellular stress reactions assessed by gender and species in spiders from areas variously polluted with heavy metals. *Ecotoxicology and Environmental Safety* 70(1), 127-37. https://doi.org/10.1016/j.ecoenv.2007.03.005

World Spider Catalog (2022). World Spider Catalog. Version 23.5. (N. H. M. Bern, ed.).

Wrinn, K. M., Evans, S.C. & Rypstra, A.L., 2012. Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider. *Chemosphere* 87(4), 390-396. https://doi.org/10.1016/j.chemosphere.2011.12.030

Wu, H., Zhang, R., Liu, J., Guo, Y. & Ma, E., 2011. Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). *Chemosphere* 83(4), 599–604. https://doi.org/10.1016/j.chemosphere.2010.12.004

Yadav, I. C., Devi, N. L., Syed, J. H., Cheng, Z., Li, J., Zhang, G. & Jones, K.C., 2015. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: A

comprehensive review of India. *Science of The Total Environment* 511, 123-137. https://doi.org/10.1016/j.scitotenv.2014.12.041

Yen, J., Donerly, S., Levin, E. D. & Linney, E. A., 2011. Differential acetylcholinesterase inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish. *Neurotoxicology and Teratology* 33(6), 735–741. https://doi.org/10.1016/j.ntt.2011.10.004

Yuan, J., Guo, J., Wang, W., Guo, G., Lian, Q. & Gu, Z., 2019. Acute toxicity of cypermethrin on the juvenile of red claw crayfish *Cherax quadricarinatus*. *Chemosphere* 124468. https://doi.org/10.1016/j.chemosphere.2019.124468

Zhou, C., Zhu, C. X., Fu, H., Li, X., Chen, L., Lin, Y., Lai, Z. & Guo, Y., 2019. Genome-wide investigation of superoxide dismutase (SOD) gene family and their regulatory miRNAs reveal the involvement in abiotic stress and hormone response in tea plant (*Camellia sinensis*). *PloS One* 14(10), e0223609. https://doi.org/10.1371/journal.pone.0223609

Zhu, F., Gujar, H., Gordon, J., Haynes, K., Potter, M. & Palli, S., 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. *Scientific Reports* 3(1456). https://doi.org/10.1038/srep01456