Inhibition of Acetylcholinesterase in Wild Bird Populations Exposure to Pesticides

Lirio Elizabeth Zapata-Muñoz[1]
eli_250493@hotmail.com
https://orcid.org/0009-0004-9212-4621
Facultad de Ciencias Químico Biológicas
Universidad Autónoma de Guerrero
Chilpancingo, Guerrero, México

Roberto Carlos Almazán-Núñez
oikos79@yahoo.com.mx
https://orcid.org/0000-0002-9913-2737
Facultad de Ciencias Químico Biológicas
Universidad Autónoma de Guerrero
Chilpancingo, Guerrero, México

Isabel Hernández-Ochoa
mihernandez@cinvestav.mx
https://orcid.org/0000-0002-4427-6282
Departamento de Toxicología,
Centro de Investigación y de Estudios
Avanzados del Instituto Politécnico Nacional
(CINVESTAV)
Ciudad de México, México

Carlos Ortuño-Pineda
ortunoc@hotmail.com
https//orcid.org/0000-0002-1757-1964
Facultad de Ciencias Químico Biológicas
Universidad Autónoma de Guerrero
Chilpancingo, Guerrero, México.

Cecilia González- Calixto
cgonzalez@uagro.mx
https://orcid.org/0000-0002-0782-8551
Facultad de Enfermería
Universidad Autónoma de Guerrero
Acapulco, Guerrero, México

Mercedes Calixto-Galvéz
shany_mcg@hotmail.com
https://orcid.org/0009-0007-3258-6340
Facultad de Ciencias Naturales
Universidad Autónoma de Guerrero
Las Petaquillas, Guerrero, México

Salvador Muñoz-Barrios
17423@uagro.mx
https://orcid.org/0000-0002-4821-5356
Facultad de Ciencias Naturales
Universidad Autónoma de Guerrero
Las Petaquillas, Guerrero, México

Mayrut Urióstegui-Acosta[2]
uriosteguiacosta@uagro.mx
https://orcid.org/0000-0001-6213-183X
Facultad de Ciencias Naturales
Universidad Autónoma de Guerrero
Las Petaquillas, Guerrero, México

ABSTRACT

The widespread use of pesticides in agricultural fields has resulted in the exposure of wildlife species. Bird species have shown substantial population decline and range contraction in agroecosystems, which have been linked to the intensification of agriculture. In this study AChE evaluation in blood in different species of birds (Turdus rufopalliatus, Icterus pustulatus, Melanerpes chrysogenys, Crotophaga sulcirostris; both genders) in agricultural lands of the state of Guerrero, southern Mexico. The result show a tendency to susceptible the female birds at inhibition AChE. Our results also suggest that inhibition of AChE due to pesticides could have repercussions in the population of bird species.

Keywords: *Wild birds; Pesticides; Enzyme inhibition*

[1] Autor principal
[2] Correspondencia: uriosteguiacosta@uagro.mx

Inhibición de la Acetilcolinesterasa en la Exposición de Poblaciones de Aves Silvestres a los Pesticidas

RESUMEN

El uso generalizado de plaguicidas en los campos agrícolas ha resultado en la exposición de especies de vida silvestre. Las especies de aves han mostrado una disminución sustancial de su población y una contracción de su distribución en los agroecosistemas, lo que se ha relacionado con la intensificación de la agricultura. En este estudio se evalúa la AChE en sangre en diferentes especies de aves (Turdus rufopalliatus, Icterus pustulatus, Melanerpes chrysogenys, Crotophaga sulcirostris; ambos géneros) en tierras agrícolas del estado de Guerrero, sur de México. Los resultados muestran una tendencia a ser sensibles en las hembras a la inhibición de AChE. Nuestros resultados también sugieren que la inhibición de la AChE por plaguicidas podría tener repercusiones en la población de especies de aves.

Palabras clave: *Aves silvestres; Pesticidas; Inhibición enzimática*

Artículo recibido 28 agosto 2023
Aceptado para publicación: 20 setiembre 2023

INTRODUCTION

Bird species have a central role in ecosystems due they contribute to seed dispersal, pollination, pest control, recolonization, and restoration of disturbed environments (Sekercioglu et al., 2004). This wide ecological functionality of the birds has a positive impact on goods and services for the human populations. Additionally, bird species act as sentinels for detecting early environmental warnings to a particular hazard, especially those related to the use of pesticides for agricultural activities (Fry, 1995; USFWS, 2002). Some bird populations have substantially declined mostly due to intensification of pesticide use in different environments (Boatman et al., 2004; Birdlife, 2008; Mitra et al., 2011). At least 50 pesticides have been considered a threat~~en~~ for several bird species, as several of them use crops directly or indirectly to obtain food (Birdlife, 2008). For example, some bird species as granivores and frugivores depend on crops for their food (Rey, 2011). Other bird species, such as carnivores and insectivores consume food indirectly from prey found within agricultural landscapes (Alvarez-Alvarez et al. 2018) such as songbirds, game birds, raptors, and seabirds, as they use the crops to obtain food (Birdlife, 2008). In Mexico, pesticides are highly used to control pests on crops (AMIFAC, 2015; Cortés-Genchi et al., 2008; Ramos-Chavez et al., 2015; Ramirez-Vargas et al., 2017).

Pesticides have particular mechanisms of toxicity; for example, organophosphorus (OP) and carbamates (CB) are known to block the cholinesterase (AChE) enzyme, which is responsible for controlling the nerve impulse, and various developmental functions, hence, they may cause adverse effects on different animal species, such as birds (Busby et al., 1991; Abass, 2014). In general, On the other hand, herbicides are may be toxic to birds likely depending on the period and frequency of exposure. For example, (MacKinnon and Freedman., 1993); however, some herbicides, such as trifluralin, have been considered as nontoxic to this animal group from studies performing acute toxicity but toxic from studies performing multiple exposures to birds (EPA. 1996). Also, glyphosate (OP) has been shown to cause dramatic decreases in bird populations of mixed wood clear-cuts in Nova Scotia, Canada (MacKinnon and Freedman, 1993). The inhibition of AChE leads to excessive amounts of Acetylcholine (Ach) at synapses (Fukuto, 1990; Pohanka, 2011), causing overstimulation and disruption of neurotransmission in both central and peripheral nervous systems (Namba et al., 1971). Exaggerated manifestations of nicotinic and muscarinic receptors appear as a result of these actions (Mortensen, 1986). This scenario occurs when birds receive only sublethal exposure to pesticides, primarily by ingesting contaminated food items (Matsumura, 1975). Previous studies have examined dietary pesticide exposure in birds (Balcomb et al., 1984). However, the aim of this study was to assess the AChE activity in some bird species exposed to pesticides in agricultural lands of the state of Guerrero, southern Mexico. In addition, we obtained information on the pesticides that farmers use in their agricultural activities.

MATERIALS AND METHODS

Study area and characteristics of the agricultural lands

Tepechicotlan ejido is located in the state of Guerrero, southern Mexico (17° 30' 00.38" N, 99° 25' 52.90" W, and 17° 28' 38.24" N, 99° 23' 37.31" W). The study site has an altitude of 1028 meters above sea level. The agricultural area 212 ha and each farmer possess an average of one ha, where 81.25% of the farmers rent the land. The main crops that are managed in the region are corn (93.5%), followed by beans (37.5%), jicama (31.25%), and with minor proportion papaya and chili (6.25%).

Survey application and species selected

In order to obtain information on the pesticides used and the frequency of which are applied in the study area, we performed randomly a total of 20 interviews with farmers. On the other hand, we selected four bird species (Crotophaga sulcirostris, Melanerpes chrysogenys, Turdus rufopalliatus, and Icterus pustulatus) to assess AChE inhibition due to pesticides. The main criterion for the selection of these species was that they were associated with agroecosystems. Besides, they were considered species with high abundance in the study area to facilitate their capture (Alvarez-Alvarez et. al., 2018). To compare the AChE activity between males and females, five individuals of each sex were captured. We used poultry (Coturnix coturnix) as a control group to evaluate AChE levels. Birds were captured using 5 mist nets placed inside and in the borders of the agricultural areas. Mist nets were opened daily from 7:00 to 15:00 h for 60 days. Once birds were captured, sex was determined (male or female) and for the species without sexual dimorphism, we followed the guidelines of Pyle (1997). In addition, plastic rings were placed on their legs (tarsus) to avoid duplicate the individuals previously captured.

Bird capture and handling were conducted in accordance with the guidelines to the use of wild birds in research (Fair et. al., 2010). The protocol was reviewed and approved by Subsecretaria de Gestión para la Protección Ambiental through the Dirección General de Vida Silvestre with official number (No. SGPA/DGVS/11451/19 to the Universidad Autónoma de Guerrero, Mexico). The species included in our study are not listed as threatened species birds in the Mexican Official Standard or the NOM-059-SEMARNT-2010 for native wild protection in Mexico (SEMARNAT 2010).

Animal sampling

Heparin blood collection tubes and other safety materials were used. Blood was drawn from the brachial/ulnar vein, the alar vein, or the middle metatarsal vein (leg vein). The sample obtained was preserved to 4°C until its analysis.

Analytical procedures

Erythrocyte AChE activity was evaluated as a biomarker of exposure to pesticides based on the technique proposed by Ellman et al. (1961). This technique evaluates the hydrolysis index of acetylthiocholine (substrate) generating thiocholine iodide, which by reaction with dithiobisnitrobenzoic acid (DTNB) at pH 7.6 will generate the 5-thio-2-nitrobenzoate anion, which was evaluated at 412 nm at 25°C/6 min.–The activity was expressed as µmol thiocholine/min/mL of blood.

Statistical analysis

Differences in AChE activity for the males and females of six bird species was evaluated using a Mann-Whitney U test. Differences were considered significant when $P < 0.05$. This analysis was performed using the statistical program SPSS 22.

RESULTS

Pesticides used in the study area

The main pesticides used by farmers in agricultural lands of the study area are Disparo (43.75%), followed by Paraquat (31.25%), and Finale (25%; Table 1). showing the percentage of application, these belong to the organophosphorous and bipyridil chemical group.

Evaluation of the AChE activity in birds

AChE activity showed significant differences between the females of Turdus rufopalliatus and the control (U = -1.964, p = 0.05), and with the female of Icterus pustulatus (U = -0.00466, p ≤ 0.05; Figure 1). No significant difference in the AChE activity was found between males, and only significant differences were observed when comparing individuals of both sexes of Icterus pustulatus (U = -0.00390, p= 0.0025).

Table 1
Frequency and classification of the pesticides most used in the study area

Commercial name Or tradename	✦Frequency %	Active ingredient	Chemistry group	Toxicity	Who
Disparo	7(43.75 %)	Ethyl chlorpyrifos Permethrin	Organophosphate Pyrethroids	Moderately Hazardous	II
Paraquat	7 (43.25 %)	1-1´-Dimethyl-4-4- Bipyridylo	Bipyridylium	Moderately Hazardous	II
Finale	4 (25 %)	4-[Hidroxi(Metil)Fosfinoil]-Dl-Homoalaninato de Amonio	Organophosphate	Slightly Hazardous	IV
Tackle 2as	4 (25 %)	4- [Hydroxymethyl) Phosphinyl] -Dl-Ammonium Homoalanine	Nitrobenzoate	Slightly Hazardous	III
Harness	4 (25 %)	2-Chloro-N-(2-Ethyl-6-Methylphenyl)Acetamide	Chloroacetamide	Moderately Hazardous	II
Tamaron Monitor 600	3 (18.75 %)	(Rs)-O,S-Dimetil Fosforamidotioato	Organophosphate	Highly Dangerous	IB
Hierbamina	4(25 %)	2,4-Dichlorophenoxyacetic Acid (2,4-D)	Chorofenoxi	Moderately	II

				Hazardous	
Fastac	1 (6.25 %)	(S)-A-Cyano-3-Phenoxybencyl (1r,3r)-3- (2,2-Dichlorovinyl)-2,2-Dimethylcyclopropanecarboxylate	Pyrethroids	Moderately Hazardous	II
Gesarol	1 (6.25 %)	1,1,1-Trichloro-2,2-Bis (4-Chlorofenil) Etano	Organochlorado	Moderately Hazardous	II
Decis	1 (6.25 %)	(S) -α-Cyano-3-Phenoxybenzyl (1r, 3r) -3- (2,2-Dibromovinyl) -2,2-Dimethylcyclopropanecarboxylate	Pyrethroids	Moderately Hazardous	II
Faena	1 (6.25 %)	N- (Phosphonomethyl) Glycine-Isopropylamine	Phosphonomethyl-Glycine	Slightly Hazardous	I
Benomilo	1 (6.25 %)	Methyl 1- (Butylcarbamoyl) Benzimidazol-2-Ylcarbamate	Benzimidazole	Slightly Hazardous	IV
Cipermetrina	1 (6.25 %)	(S) -α-Cyano-3-Phenoxybenzyl (1rs, 3rs, 1rs, 3sr) -3- (2,2-Dichlorovinyl) -2,2-Dimethylcyclopropanecarboxylate	Pyrethroids	Slightly Hazardous	III
Folidol	1 (6.25 %)	O, O-Dimethyl O-4-Nitrophenyl Phosphorothioate	Organophosphate	Extremely Dangerous	IA

✦ Data frequency expressed %
WHO= World Health Organization

Figure 1

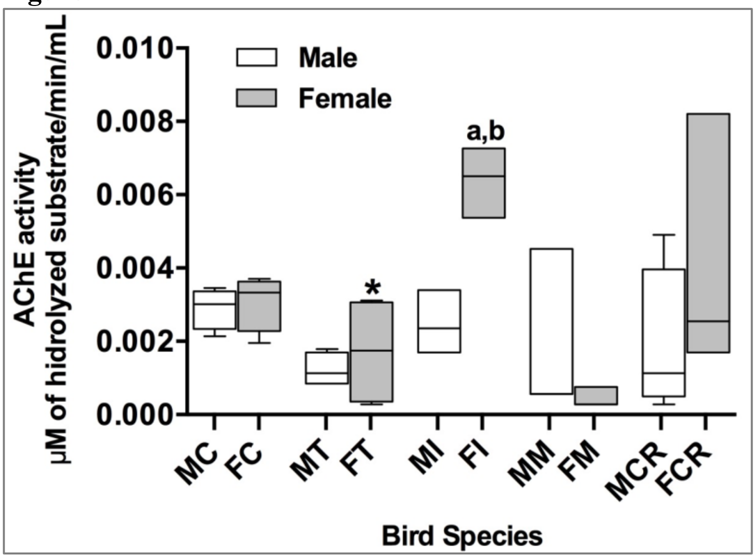

Figure 1. AChE activity in bird species associated with agricultural lands in the state of Guerrero, southern Mexico.

MC= Male Control; FC= Female Control; MT= Male Turdus rufopalliatus; FT= Female T. rufopalliatus; MI= Male Icterus pustulatus; FI= Female I. pustulatus; MM= Male Melanerpes chrysogenys; FM= Female M. chrysogenys; MCR= Male Crotophaga sulcirostris; FCR= Female C. sulcirostris.

*Significant compared to Control Female.

[a]Significant compared to Female Turdus.

[b]Significant against males of the same species.

DISCUSSION

In this study acetylthiocholine was the substrate used for the determination of AChE activity in the five-bird species. Our results showed that the activity of the enzyme was different between females of Icterus pustulatus and Turdus rufopalliatus. Also, the males of Icterus pustulatus showed significant differences in the AChE activity respect to the females of the same species. The effects that have been observed in birds due to pesticide poisoning are varied. The main route of intoxication in birds is through food, including water intake, but this can also occur by inhalation. The type of pesticide determines the degree of toxicity. Thus, it has been in other studies that observed that parathion is more toxic than diazinon and malathion in birds (Hooper et al., 1989). However, different bird species under the same conditions of consumption of contaminated food by pesticides may present different percentages of inhibition AChE, apparently due to their physiological capacity to eliminate the pesticides (Neithammer and Baskett, 1983). OP–carbamate toxicosis has been reported in many bird species, including parrots, pigeons, raptors, and waterfowl (Franson and Little, 1996). Common clinical signs in birds exposed to anticholinesterase compounds include lethargy, dyspnea, diarrhea, ocular discharge, paresis, and nonspecific neurologic signs (Franson and Little, 1996; Franson and Smith, 1999).

In this study, the birds were captured in different periods of the year, and we speculate that AChE activity was different according to the time in which were collected. Corson et al., (1998) suggested that the amounts of pesticide residues in fields are similar so diet preferences and daily intake become more important. For most birds, foraging location influences exposure more than a diet. Birds that forage more frequently in rangeland experience lower AChE inhibitions, because no insecticide applications occur there. Exposure and inhibition decrease as crop growth and birds spend less time in crop fields (Corson et al., 1998).

The results found in this study show a tendency to susceptible the female birds at inhibition AChE. Another possible explanation of this result is the difference in the activity of the females and males of the bird species studied in the food search. For example, in some species, the females are more active in the pre-incubation and incubation period (Lou et al., 2017), which could leave more exposed to the females to chemical products applied in the agricultural lands. Our results also suggest that inhibition of AChE due to pesticides could have repercussions in the population of bird species of the tropics, which is a region highly threatened in the world by different anthropogenic factors (Mitra et al., 2011). Some species analyzed in this study have biological importance because are endemic to Mexico (i.e., Melanerpes chrysogenys), or quasiendemic (Turdus rufopalliatus), and play a central role in the ecological processes like seed dispersal, and pest control in the semi-arid environments of southern Mexico, so the effects by pesticides must be reverted.

ACKNOWLEDGEMENTS

The authors thank Jorge Luis Benitez Marino and the personnel of Integrated Wildlife Laboratory their help during simple collection; as well as Dr. Alfredo Mendez Bahena for his contributions. This work was partially supported by Red Temática de Toxicología de Plaguicidas (CONACYT-2280045).

REFERENCES BIBLIOGRAPHICAL

Abass, K. S. 2014. A method for fast assessment of OP/CB exposure in the Japanese quail (Coturnix coturnix japonica) using combined esterases enzyme activity as biomarkers. Enzyme research. 2014: 1-15.

Álvarez-Álvarez, E. A., Corcuera, P., and Almazán-Núñez, R. C. 2018. Spatiotemporal variation in the structure and diet types of bird assemblages in tropical dry forest in southwestern Mexico. The Wilson Journal of Ornithology, 130(2):457-469.

AMIFAC. Asociación Mexicana de la Industria Fitosanitaria, A.C. 2015. México. http://amifac.org.mx/nosotros.html. Accessed Aug 10 2015

Balcomb, R., Bowen, C.A., Wright, D., Law, M., 1984. Effects on wildlife of at-planting corn applications of granular carbofuran. J. Wildl. Manag. 48: 1353–1359

Birdlife. 2008. State ofthe World's Birds: lndicators far Our Changing World. BirdLife lnternational, Cambridge, UK.;

Boatman, N. D., Brickle, N. W., Hart, J. D. Milsom, T.D., Morris, A. J., Murray, A. W. A. 2004. Evidence for the indirect effects of pesticides on farmland birds. Ibis. 146 (s2): 131-43.

Busby, D. G., White, L. M., Pearce, P. A. 1991. Brain acetylcholinesterase activity in forest songbirds exposed to a new method of UULV fenitrothion spraying. Arch. Environ. Contam. Toxicol. 20: 25–31.

Corson, M. S., Mora, M. A., Grant, W. E. 1998. Simulating cholinesterase inhibition in birds caused by dietary insecticide exposure. Ecological modelling. 105(2-3): 299-323.

Cortés-Genchi, P., Villegas-Arrizón, A., AguilarMadrid, G., María del Pilar Paz-Román, M.P., Maruris-Reducindo, M., Juárez-Pérez, C.A. 2008. Síntomas ocasionados por plaguicidas en trabajadores agrícolas. Revista Médica del Instituto Mexicano de Seguro Social. 46(2): 145-152.

Ellman, G. L , Courtney, K. D., Andres, V., Feather-Stone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol.7:88-95

EPA. Office of Prevention, Pesticides, and Toxic Substances. 1996. Registration eligibility decision (RED): trifluralin. Washington, D.C., April

Fair, J., E. Paul, and J. Jones, Eds. 2010. Guidelines to the Use of Wild Birds in Research. Washington, D.C.: Ornithological Council.

Franson, J. C., & Smith, M. R. 1999. Poisoning of wild birds from exposure to anticholinesterase compounds and lead: diagnostic methods and selected cases. In Seminars in Avian and Exotic Pet Medicine. 8(1): 3-11.

Franson, J. C., and S. E. Little. 1996. Diagnostic findings in 132 great horned owls. J. Raptor. Res. 30: 1–6.

Fry, D. M. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environmental Health Pespectives. Suppl 7:165-71.

Fukuto, T. R. 1990.Mechanism of action of organophosphorus and carbamate insecticides. Environ. Health Perspect. 87: 245–254.

Hooper, M. J., Detrich, P. J., Weisskopf, C. P., Wilson, B. W. 1989. Organophosphorous insecticide exposure in hawks inhabiting orchards during winter dormant- spraying. Bull Environ Contam Toxicol. 42:651-659

Lou, Y., Shi, M., Fang, Y., Swenson, J. E., Lyu, N., Sun, Y. 2017. Male vigilance and presence are important for foraging by female Chinese grouse in the pre-incubation period. Wildlife Biology doi: 10.2981/wlb.00257.

MacKinnon, D.S., and Freedman, B. 1993. Effects of silvicultural use of the herbicide glyphosate on breeding birds of regenerating clearcuts in Nova Scotia, Canada. J Appl Ecol. 30(3): 395–406.

Matsumura, F., 1975. Toxicology of Insecticides. Plenum, New York, 503 pp

Mitra, A., Chatterjee, C., and Mandal, F. B. 2011. Synthetic chemical pesticides and their effects on birds. Res J Environ Toxicol. 5: 81-96.

Mortensen, M. L. 1986. Management of acute childhood poisoning caused by selected insecticides and herbicides. Pediatr Clin North Am. 33:421–445

Namba, T., Nolte, C. T., Jackrel, J., Grob, D. 1971. Poisoning due to organophosphate insecticides. Acute and chronic manifestations. Am J Med. 50:475–492.

Neithammer, K. R., Baskett, T. S. 1983. Cholinesterase inhibition of birds inhabiting wheat field treated with Methyl Parathion and Toxaphene. Arch Environ Contam Toxicol. 12: 471-475.

Pohanka, M. 2011. Cholinesterases, a target of pharmacology and toxicology. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 155: 219–229.

Pyle, P. 1997. Identification guide to North American Birds. Part I. Columbidae to Ploceidae. Slate Creek Press, Bolinas, California, USA.

Ramírez-Vargas, M. A., Huerta-Beristain, G., Gúzman-Gúzman, I. P., Alarcón-Romero, L. D., Flores-Alfaro, E., Rojas-García, A. E., Moreno-Godínez, M. E. 2017. Methamidophos induces cytotoxicity and oxidative stress in human peripheral blood mononuclear cells. Environ Toxicol. 32(1):147-155.

Ramos-Chavéz, L. A., Sordo, M., Calderón-Aranda, E., Castañeda-Saucedo, E., Ostrosky-Wegman, P., Moreno-Godínez, M.E. 2015. A permethrin/allethrin mixture induces genotoxicity and cytotoxicity in human peripheral blood lymphocytes. J Toxicol Environ Health A. 78(1):7-14.

Sekercioglu, C. H., Daily G. C. and Ehrlich P. R. 2004. Ecosystem consequences of bird declines.Proc. Natl. Acad. Sci. USA., 101: 18042-1804.

SEMARNAT. 2010. SEGOB (Ed.), Mexican Official Standard NOM-059-SEMARNAT-2010. Environmental protection of Mexico – species nativesnative's wildlife wild-risk categories and specifications for their inclusion, exclusion or change – list of species at risk, Diario Oficial de la Federación: Executive Branch of the United States of México.

U.S. Fish and Wildlife Service. 2002. Birds of conservation concern 2002. Division of Migratory Bird Management, Arlington, Virginia. 99 pp. <http://migratorybirds.fws.gov/reports/bcc2002.pdf>.

Rey, P. J. 2011. Preserving frugivorous birds in agro-ecosystems: lessons from Spanish olive orchards. Journal of Applied Ecology 48, 228–237