ORIGINAL PAPER

Oxidative stress and hematocrit levels in eared doves (*Zenaida auriculata*) exposed to neonicotinoid-treated soybean cotyledons

August, 2025

Addy-Orduna, L.[1]; Cazenave, J.[2]

ABSTRACT

This study evaluates the effects of exposure to neonicotinoid-treated soybean cotyledons on oxidative stress markers and hematocrit in eared doves (*Zenaida auriculata*). Thirty-two doves (eight by treatment) were exposed to cotyledons from soybean treated with imidacloprid (IMI1: 0.4 mg a.i./g seed and IMI2: 1.2 mg a.i./g seed) and thiamethoxam (TMX: 0.7 mg a.i./g seed), and cotyledons from untreated soybean as a control, plus commercial seed mix (maintenance food). The concentrations of neonicotinoids used corresponded to those typically applied in soybeans as seed treatment in the field with commercial formulations. The exposure to the chemicals was intermittent, alternating weekly over a four-week period. This design simulates real-world scenarios where birds may encounter both treated and untreated fields, as well as a variety of seeds. Results indicate no significant changes in hematocrit, hepatic and cerebral glutathione S-transferase (GST), hepatic catalase (CAT), or hepatic lipid peroxidation (LPO) levels across treatments compared to the control. The neonicotinoid concentrations used in soybean seed treatments appear to be insufficient to induce oxidative damage in these birds under intermittent exposure conditions. These findings underscore the importance of studying various exposure scenarios to better understand the potential environmental risks posed by neonicotinoid-treated seeds.

Keywords: insecticides, avian toxicity, oxidative damage.

RESUMEN

Este estudio evalúa los efectos de la exposición a cotiledones de soja tratados con neonicotinoides sobre los marcadores de estrés oxidativo y los niveles de hematocrito en torcazas (Zenaida auriculata). Treinta y dos palomas (ocho por tratamiento) fueron expuestas a cotiledones de soja tratada con imidacloprid (IMI1: 0,4 mg i.a./g de semilla e IMI2: 1,2 mg i.a./g de semilla) y tiametoxam (TMX: 0,7 mg i.a./g de semilla), y cotiledones de soja no tratada como control, además de mezcla comercial de semillas (alimento de mantenimiento). Las concentraciones de neonicotinoides utilizadas correspondieron a las típicamente aplicadas en la soja como tratamiento de semillas en el campo con formulaciones comerciales. La exposición a los productos químicos fue intermitente, alternando semanalmente durante un período de cuatro semanas. Este diseño simula escenarios del mundo real en los que las aves pueden encontrar campos tratados y no tratados, así como una variedad de semillas. Los resultados no muestran cambios significativos en los niveles de hematocrito, glutatión S-transferasa (GST), catalasa hepática (CAT) o peroxidación lipídica hepática (LPO) entre los tratamientos en comparación con el control. Las concentraciones de neonicotinoides utilizadas en los tratamientos de semillas de soja parecen ser insuficientes para inducir daño oxidativo en estas aves en condiciones de exposición intermitente. Estos hallazgos subrayan la importancia de estudiar varios escenarios de exposición para comprender mejor los riesgos ambientales potenciales que plantean las semillas tratadas con neonicotinoides.

Palabras clave*: insecticidas, toxicidad aviar, daño oxidativo.*

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Paraná, Ruta 11 km 12.5, (3100) Paraná, Entre Ríos, Argentina. Correo electrónico: addyorduna.laura@inta.gob.ar
[2]Instituto Nacional de Limnología (INALI) del Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Universitaria, Colectora Ruta Nacional 168 km 0, (3000) Santa Fe, Argentina.

https://doi.org/10.58149/f9c9-wq48

INTRODUCTION

The widespread use of neonicotinoid insecticides in agriculture has raised significant concerns regarding their environmental impact, especially on non-target wildlife, including birds (Mineau and Kern, 2023; Mineau and Palmer, 2013). These systemic insecticides are frequently used as a preventive seed treatment in extensive crops, which can contribute to the development of resistance in pests, cause environmental contamination and possible damage to wildlife (Douglas and Tooker, 2015). Neonicotinoids bind to nicotinic acetylcholine receptors in the central nervous system of insects, causing constant stimulation that leads to paralysis and death of the insect. This action is highly specific in insects, but its persistence in the environment means that it can also affect non-target organisms (Simon-Delso *et al*., 2015). On the other hand, neonicotinoids are water-soluble and can accumulate in soils and water bodies, which increases their bioavailability and exposure to non-target species. In vertebrates, they have been shown to cause sublethal effects, including alterations in the immune and reproductive systems (Gibbons *et al*., 2015).

Granivorous birds species are particularly vulnerable to exposure to neonicotinoids, as they may inadvertently consume treated seeds or germinating plant parts, such as cotyledons (Addy-Orduna *et al*., 2022). Previous studies have reported sublethal effects in birds exposed to neonicotinoids. In house sparrows, exposure to the neonicotinoid acetamiprid caused a significant decrease in sperm density and activity of the antioxidant enzyme superoxide dismutase (SOD) in sperm, indicating an increase in oxidative stress. This suggests an impact on the fertility of these birds at sublethal doses (Humann-Guilleminot *et al*., 2019). In red-legged partridges, exposure to wheat seeds treated with imidacloprid resulted in an increase in blood SOD activity, along with changes in biochemical oxidative stress parameters. In addition, there was a reduction in clutch size, delayed start of clutch, and a decrease in the immune response of their offspring, indicating impacts on reproduction and offspring health (Lopez-Antia *et al*., 2015).

In Argentina, soybeans are the main extensive crop, covering approximately 14,000,000 ha in the 2023/2024 season (SISA, 2024). Although maize seeds are more commonly pre-treated with neonicotinoids at the commercial level, soybean seeds are often treated by producers before sowing, particularly for prophylactic purposes or for weevil control (CASAFE, 2013; Cazado *et al*., 2014).

For a model granivorous species such as the eared dove (*Zenaida auriculata*), the treatment of soybeans with neonicotinoids represents a route of exposure to these chemicals not only through unburied seeds after sowing, but also through cotyledons (first leaves of the plant embryo) in the emergence phase of the crop (Addy-Orduna y Mateo, 2023). Given the wide territorial distribution of this crop, it is crucial to assess the potential exposure of native bird species to neonicotinoid residues, especially considering the lack of local studies on this topic.

As a measure of red blood cells, hematocrit can indicate an increase or decrease in the percentage of red blood cells, reflecting various physiological conditions, including toxin-induced anemia, hemolysis, dehydration, or oxidative stress in birds (Minias, 2015). On the other hand, oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and an organism's antioxidant systems (Halliwell and Gutteridge, 2015). ROS can damage lipids, proteins, and DNA, contributing to the development of various diseases (Sies, 2020). Oxidative stress is generated endogenously (cell metabolism. Biomarkers of oxidative stress, such as glutathione S-transferase (GST), catalase (CAT), and lipid peroxidation (LPO), are commonly used to assess the impact of pollutants on vertebrates, as they provide information on biochemical pathways activated in response to chemical stressors (Abbasi *et al*., 2017; Hellou *et al*., 2012). Elevated LPO levels, for example, indicate lipid membrane damage, while alterations in GST and CAT activities suggest disruptions in detoxification and antioxidant defense mechanisms (Abu Zeid *et al*., 2019; Isaksson, 2010). Through these established indicators, it is necessary to examine how field-relevant neonicotinoid concentrations, such as those applied in soybean seed treatments, affect oxidative stress markers in birds under exposure conditions that mimic natural agricultural scenarios. Accordingly, this study aimed to evaluate the effects of intermittent exposure to neonicotinoid-treated soy cotyledons on markers of oxidative stress and hematocrit in eared doves, a common bird species in South American agroecosystems. Hypothetically, repeated exposure to these cotyledons will induce a significant increase in oxidative stress markers and a variation in the hematocrit, reflecting a deterioration in the physiological state of the birds. By simulating real-world conditions in which birds alternate between treated and untreated food sources, this study provides insights into the ecological relevance of neonicotinoid exposure in agricultural landscapes. Our findings provide valuable data for the assessment of the risks of neonicotinoids in wildlife, addressing a critical knowledge gap in the context of South American agricultural practices.

MATERIALS AND METHODS

Capture and management of birds

The doves were captured in the field at the Paraná Agricultural Experimental Station (EEA), Argentina, with authorization from the Government of Entre Ríos (Resolution 1721). The birds were housed in group cages (2 m wide × 9 m long × 2.5 m high) at the EEA Paraná facilities, following the guidelines for the use of wild birds in research (Fair *et al*., 2010). Subsequently, they were randomly assigned to individual cages (50 × 50 × 50 cm) for acclimatization and experimental management. They were provided with water, grit and a commercial mixture of pigeon seeds (wheat, maize, sorghum, millet, and rapeseed) *ad libitum*, and ventilation was controlled to maintain photoperiod, temperature and humidity conditions according to the outside environment.

Treatments and experimental design

The study was conducted in January and February to ensure adequate environmental conditions for the germination of soybean seedlings. Concentrations of neonicotinoids typically applied in the field were used in soybean crops, using the commercial formulations IMIDA NOVA 60 FS® (imidacloprid) and CRUISER 35 FS® (thiamethoxam). Soybean seeds of the variety 5909 from Nidera Seeds® were used. Treatments were prepared by coating untreated seeds (600 g per treatment) with 3 ml of a neonicotinoid solution. For IMI1 (70 ml of formulated product per 100 kg of seeds), the solution was made with 1.96 ml of IMIDA NOVA 60 FS® and 12.04 mL of water. For IMI2 and TMX (200 ml of formulated product per 100 kg of seeds), the solution was prepared using 5.6 mL of the corresponding commercial product (IMIDA NOVA 60 FS® for IMI2, CRUISER 35 FS® for TMX) and 8.4 mL of water. All seeds were shaken vigorously for 5 minutes to ensure uniform coating.

Treated seeds were then sown in trays at a density of 20 seeds per tray. Seedlings were offered to the birds between 5 and 7 days post-sowing, once they had reached the cotyledon stage. During each exposure period, birds received two trays per day, each with up to 20 cotyledonary seedlings.

A total of 32 doves were used, randomly assigned to one of four treatments: 1) CON: cotyledons from untreated seeds; 2) IMI1: cotyledons from seeds treated with imidacloprid at 0.4 mg a.i./g seed (insecticidal use); 3) IMI2: cotyledons from seeds treated with imidacloprid at 1.2 mg a.i./g seed (repellent use); 4) TMX: cotyledons from seeds treated with thiamethoxam at 0.7 mg a.i./g seed.

Exposure of birds to treated cotyledons was intermittent to simulate consumption in a field setting. The experiment included the following stages: 1) Acclimatization (7 days): the birds were kept in individual cages with *ad libitum* maintenance feeding. 2) Pre-exposure (10 days): birds were gradually adapted to cotyledons by offering trays with seedlings and progressively reducing the amount of maintenance food from 20 g to 5 g per day. 3) Exposure 1 (5 days): doves consumed cotyledons treated according to the assigned treatment, with limited access to maintenance feed. Daily, at 8:00 a.m., two trays with a maximum of 20 seedlings each were replenished and from 1 p.m. onwards, 5 g of maintenance food was offered; 4) Post-exposure 1 (7 days): only *ad libitum* maintenance food was offered. 5) Exposure 2 (5 days): repeat exposure regimen to treated cotyledons. 6. Post-exposure 2 (5 days): identical conditions to the previous stage (Exposure) but with access to untreated cotyledons instead of treated ones (figure 1).

Neonicotinoid analysis in cotyledons

Neonicotinoid residues in treated and untreated cotyledons were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS), using an Agilent 1100® chromatograph and an Agilent 6110® mass detector, and following the procedure described in López-Antia *et al*. (2013). Neonicotinoid concentrations in the cotyledons were reported as mean ± SE in mg of active ingredient per gram of cotyledon.

Hematocrit and oxidative stress measurements

At the end of the post-exposure period 2, the birds were euthanized in a CO_2 chamber and weighed. Blood was drawn from the heart to determine hematocrit by centrifugation of 2-3 capillary tubes at 1400 rcf for 5 minutes. Hematocrit was measured as the packed cell volume percentage. The sex was confirmed by observing the reproductive system. The liver was weighed and its relative weight was calculated as the hepato-somatic index (table 1). Liver and brain samples (n=8 per treatment) were frozen in liquid nitrogen and stored at -80°C until oxidative stress marker analysis.

Treatment	Female-Male	Hepato-somatic index
	(%)	(mean ± SE, mg/mg)
CONTROL	50-50	22.50 ± 1.38
IMI1	50-50	27.66 ± 1.91
IMI2	60-40	23.23 ± 2.30
TMX	30-70	28.55 ± 2.33

Table 1. Sex proportion and relative liver mass parameters in birds exposed to the different treatments.

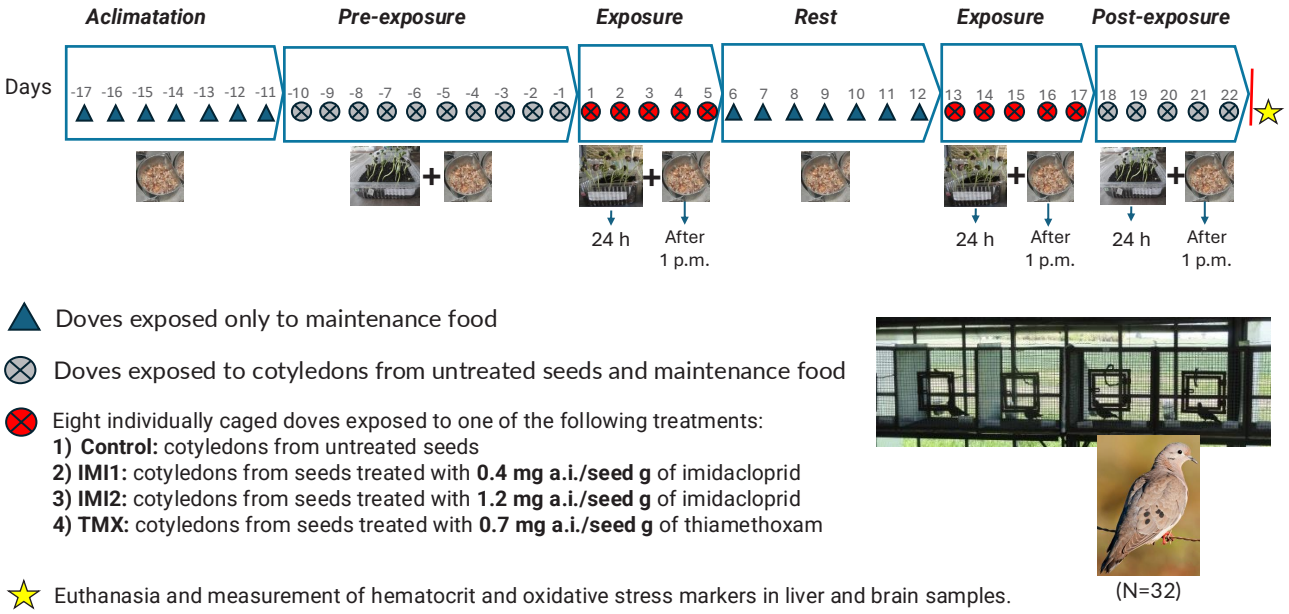

Figure 1. Stages and treatments of the experimental design used.

Oxidative stress markers included glutathione S-transferase (GST) and catalase (CAT) activities, and lipid peroxidation (LPO) levels, in liver and brain. The tissues were homogenized in a phosphate buffer with EDTA and KCl. The homogenates were centrifuged at 10,000 × g for 10 minutes at 4°C, and the supernatants were used to measure the activity of antioxidant enzymes and lipid peroxidation (Reglero *et al*., 2009). GST was determined according to Habig *et al*. (1974), with results expressed in mU/mg of protein. CAT was measured according to Beutler (1982), with activity expressed in U/mg of protein. LPO was determined by the measurement of thiobarbituric acid reactive substances (TBARS) according to Yagi (1976) and expressed as nmol MDA/mg protein. The protein concentration was determined by the Bradford method (Bradford, 1976), using bovine albumin as a standard.

Statistical analysis

Data were analyzed using linear models or generalized linear models, with treatment, sex, body weight (BW), and the hepato-somatic index as factors. The R program (R Core Team, 2021; version 4.4.0) and the tidyverse (Wickham *et al*., 2019), car (Fox and Weisberg, 2019), dplyr (Wickham *et al*., 2023), DHARMa (Hartig, 2022), emmeans (Lenth, 2024), and ggplot2 (Wickham, 2016) packages were used. Dunnett's post-hoc tests were used to compare each treatment group against control. All statistical analyses were conducted with a significance threshold of $p < 0.05$.

RESULTS

Neonicotinoid concentrations in cotyledons

Average concentrations of neonicotinoids in each treatment were (mean ± SE) 0.0359 ± 0.0049 mg a.i./g cotyledon in IMI1 cotyledons, 0.1570 ± 0.0170 mg a.i./g cotyledon in IMI2 cotyledons, 0.0114 ± 0.0011 mg a.i./g cotyledon in TMX cotyledons, and no neonicotinoids were detected in CON cotyledons. Considering an average body mass of 127 g for the eared dove (Addy-Orduna *et al*., 2019), the amount of neonicotinoid ingested by the consumption of cotyledons corresponded to approximately 0.28, 1.24 and 0.09 mg a.i./kg BW for IMI1, IMI2 and TMX treatments, respectively.

Hematocrit

No significant differences in hematocrit were observed among treatments ($p = 0.5942$). The mean hematocrit values obtained were (mean ± SE) 49.72 ± 2.70% in CON doves, 46.97 ± 2.66% in IMI1 doves, 45.91 ± 2.24% in IMI2 doves, and 50.39 ± 3.79% in TMX doves.

Oxidative stress markers

CAT activity was only detected in the liver. No significant effects on GST and CAT activities, or LPO levels were found in treated birds compared to controls ($p > 0.05$ in all cases). Although hepatic LPO levels tended to be higher in treated groups (0.064 ± 0.011, 0.050 ± 0.005, and 0.068 ± 0.018 nmol TBARs/mg protein in IMI1, IMI2, and TMX, respectively) than in the control (0.043 ± 0.004 nmol TBARs/mg protein), these differences were not statistically significant ($p>0.05$ in all cases, figure 2).

DISCUSSION

The concentrations of neonicotinoids detected in the cotyledons indicate successful systemic absorption of these insecticides. This finding aligns with previous studies demonstrating the systemic nature of these chemicals within plants (Alford and Krupke, 2017), confirming that cotyledons serve as an additional pathway of neonicotinoid exposure for wildlife associated with agroecosystems. However, despite the doves' ingestion of

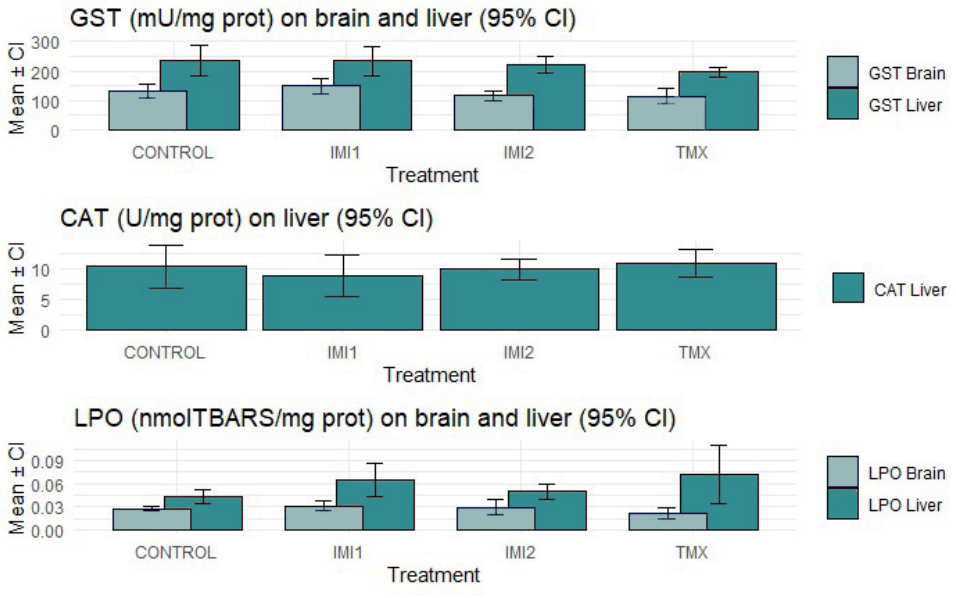

Figure 2. Activities of GST and CAT, and LPO in the brain and liver in each treatment. Error bars indicate the 95% confidence interval (CI).

treated cotyledons, no significant changes were observed in hematocrit or oxidative stress biomarkers, including hepatic and brain GST activity, hepatic CAT activity, or LPO levels, between exposed doves and control groups. Although hepatic LPO levels tended to be higher in treated groups, this increase was not statistically significant, suggesting that, under these experimental conditions, the doves effectively neutralized or excreted the neonicotinoids, limiting their physiological impact.

Overall, these findings are consistent with previous literature on the effects of neonicotinoids on hematological parameters, such as hematocrit. Balani *et al*. (2011) also reported no significant changes in hematocrit after daily oral exposure to sublethal doses of thiamethoxam (1.25, 1.67, and 2.5 mg a.i./kg bw) in chickens over a 28-day period. Conversely, Gul *et al*. (2017) observed a reduction in hematocrit levels in chickens exposed to higher daily oral doses of thiamethoxam (250, 500, 750, and 1000 mg a.i./kg bw) at both 15 and 30 days. In a similar study by these authors using slightly lower doses (50, 100, 200, and 400 mg a.i./kg bw), a decrease in hematocrit was also noted *(Gul et al*., 2018). In red-legged partridges (*Alectoris rufa*) fed wheat treated with low doses of imidacloprid (0.7 and 1.4 mg a.i./kg bw—doses closer to those used in this study) for ten days, Lopez-Antia *et al*. (2013) observed a reduction in hematocrit levels. The difference between these results and those for eared doves may be due to greater sensitivity of partridges to imidacloprid compared to doves and/or the continuous exposure in partridges versus the intermittent exposure in doves. Additionally, in the South American songbird species, the grayish baywing (*Agelaioides badius*), hematocrit temporarily decreased and returned to normal within 48 hours after a single imidacloprid dose of 35 mg a.i./kg bw (Poliserpi *et al*., 2021). In the same species, with lower imidacloprid doses (0.053 and 0.514 mg a.i./kg bw) administered via treated millet, Poliserpi and Brodeur (2023) found no effects on hematocrit, which is consistent with the present study.

Glutathione-S-transferase (GST) utilizes the tripeptide glutathione (GSH) as a substrate to conjugate it with xenobiotic compounds and reactive species, enabling efficient detoxification (Narayanankutty *et al*., 2019). Consequently, reductions in GSH or GST indicate alterations in redox balance and toxicity (Reed, 1990). Unlike the lack of effect observed in eared doves, other studies have reported decreases in GST and GSH levels in certain tissues following exposure to higher doses of imidacloprid than those tested here (Abu Zeid *et al*., 2019), as well as with lower doses of the chemical (Poliserpi *et al*., 2023, 2021; Poliserpi and Brodeur, 2023; Sasidhar *et al*., 2014). However, low doses of imidacloprid in red-legged partridges produced no significant effect on GSH levels (Lopez-Antia *et al*., 2015).

Different doses of neonicotinoids appear not to affect catalase (CAT) levels in birds, as CAT levels did not significantly differ from controls in this study or in reviewed studies that measured this enzyme (Poliserpi *et al*., 2023, 2021; Poliserpi and Brodeur, 2023). Exceptions include doses of 3 and 6 mg a.i./kg bw, which (Abu Zeid *et al*., 2019) found to decrease CAT activity in the liver and brain following gavage administration of imidacloprid to domestic pigeons (*Columba livia*).

The impact of imidacloprid on lipid peroxidation (LPO) has also yielded variable results. Consistent with our findings, Lopez-Antia *et al*. (2015) found no differences in MDA levels between control and low-dose imidacloprid-treated red-legged partridges. In contrast, Sasidhar *et al*. (2014) observed an increase in TBARS in the liver of chickens exposed to low concentrations of imidacloprid.

The differences found among studies could be explained by variations in species sensitivity, the active ingredient tested, the dose magnitude, and/or its administration route, as well as the exposure duration. These factors likely contribute to the differing responses to neonicotinoids, highlighting the need for further research into species-specific sensitivities and detoxification mechanisms in wildlife exposed to these pesticides in agricultural settings. This underscores the importance of considering species-specific physiology and ecology when interpreting the toxicological effects of neonicotinoid exposure, as different bird species may vary considerably in their susceptibility and response mechanisms.

In practical terms, our findings support the idea that when neonicotinoid-treated soybean seeds are properly sown and covered, and the use of treated areas by granivorous birds is intermittent and limited to the early emergence stage of the crop, the risk of acute oxidative damage is low. However, this does not rule out potential effects of prolonged or repeated exposure, or exposure to other crops with higher treatment rates. Therefore, recommendations include improving sowing practices to reduce seed exposure on the soil surface and encouraging additional research in local species to assess sublethal and long-term impacts under field conditions.

CONCLUSIONS

The lack of significant changes in oxidative stress markers and hematocrit suggests that the concentrations of neonicotinoids typically used in soybean treatments may not pose a direct risk of oxidative stress to birds under conditions of intermittent exposure. The observed non-significant trend toward higher LPO in treated birds could indicate that while acute oxidative damage is unlikely at these doses, cumulative exposure could still be of concern under certain conditions. This study highlights the importance of conducting research under different exposure scenarios that may reflect different aspects of the complexity of field conditions in different crops in which neonicotinoids are used.

ACKNOWLEDGMENTS

We thank the institutions that financed this study through their projects, especially INALI and INTA.

REFERENCES

ABBASI, N.A.; ARUKWE, A.; JASPERS, V.L.B.; EULAERS, I.; MENNILO, E.; IBOR, O.R.; FRANTZ, A.; COVACI, A.; MALIK, R.N. 2017. Oxidative stress responses in relationship to persistent organic pollutant levels in feathers and blood of two predatory bird species from Pakistan. Science of The Total Environment 580, 26-33. https://doi.org/10.1016/j.scitotenv.2016.11.197

ABU ZEID, E.H.; ALAM, R.T.M.; ALI, S.A.; HENDAWI, M.Y. 2019. Dose-related impacts of imidacloprid oral intoxication on brain and liver of rock pigeon (Columba livia domestica), residues analysis in different organs. Ecotoxicology and Environmental Safety 167, 60-68. https://doi.org/10.1016/j.ecoenv.2018.09.121

ADDY-ORDUNA, L.; CAZENAVE, J.; MATEO, R. 2022. Avoidance of neonicotinoid-treated seeds and cotyledons by captive eared doves (Zenaida auriculata, Columbidae). Environmental Pollution 304, 119237. https://doi.org/10.1016/j.envpol.2022.119237

ADDY-ORDUNA, L.M.; BRODEUR, J.C.; MATEO, R. 2019. Oral acute toxicity of imidacloprid, thiamethoxam and clothianidin in eared doves: A contribution for the risk assessment of neonicotinoids in birds. Science of The Total Environment 650, 1216-1223. https://doi.org/10.1016/j.scitotenv.2018.09.112

ALFORD, A.; KRUPKE, C. 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS ONE 12, e0173836. https://doi.org/10.1371/journal.pone.0173836

BALANI, T.; AGRAWAL, S.; THAKER, A.M. 2011. Hematological and biochemical changes due to short-term oral administration of imidacloprid. Toxicology International 18, 2-4.

BEUTLER, E. 1982. Catalase. In *Red Cell Metabolism: A Manual of Biochemical Methods* (3rd ed.). New York: Grune & Stratton.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

CASAFE. 2013. Guía de productos fitosanitarios, décimosexta edición 2013-2015. ed. LARA Producciones Editoriales, Buenos Aires, Argentina.

CAZADO, L.E.; CASMUZ, A.S.; SCALORA, F.; MURÚA, M.G.; SOCÍAS, M.G.; GASTAMINZA, G.A.; WILLINK, E. 2014. El picudo negro de la soja, Rhyssomatus subtilis Fiedler (Coleoptera: Curculionidae). EEAOC Avance Agroindustrial 35, 34-42.

DOUGLAS, M.R., TOOKER, J.F. 2015. Large-Scale Deployment of Seed Treatments Has Driven Rapid Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S. Field Crops. Environmental Science & Technology 49, 5088-5097. https://doi.org/10.1021/es506141g

FOX, J., WEISBERG, S. 2019. An R Companion to Applied Regression, Third. ed. Sage, Thousand Oaks CA.

GIBBONS, D.; MORRISSEY, C.; MINEAU, P. 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental Science and Pollution Research 22, 103-118. https://doi.org/10.1007/s11356-014-3180-5

GUL, S.T.; KHAN, A.; AHMAD, M.; ANWAR, M.F.; KHATOON, A.; SALEEMI, M.K.; AKRAM, M.N.; 2018. Effect of sub-lethal doses of thiamethoxam (a neonicotinoid) on hemato-biochemical parameters in broiler chicks. Toxin Reviews 37, 144-148. https://doi.org/10.1080/15569543.2017.1336731

GUL, S.T.; KHAN, A.; FAROOQ, M.; NIAZ, S.; AHMAD, M.; KHATOON, A.; HUSSAIN, R.; SALEEMI, M.K.; HASSAN, M.F. 2017. Effect of sub lethal doses of Thiamethoxam (A Pesticide) on hemato-biochemical values in cockerels. Pakistan Veterinary Journal 37, 135-138.

HABIG, W.H.; PABST, M.J.; JAKOBY, W.B. 1974. Glutathione S-Transferases: The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249, 7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8

HALLIWELL, B.; GUTTERIDGE, J.M. 2015. Free radicals in biology and medicine. Oxford university press, USA.

HARTIG, F. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.

HELLOU, J.; ROSS, N.W.; MOON, T.W. 2012. Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environmental Science and Pollution Research 19, 2007-2023. https://doi.org/10.1007/s11356-012-0909-x

HUMANN-GUILLEMINOT, S.; TASSIN DE MONTAIGU, C.; SIRE, J.; GRÜNIG, S.; GNING, O.; GLAUSER, G.; VALLAT, A.; HELFENSTEIN, F. 2019. A sublethal dose of the neonicotinoid insecticide acetamiprid reduces sperm density in a songbird. Environmental Research 177, 108589. https://doi.org/10.1016/j.envres.2019.108589

ISAKSSON, C. 2010. Pollution and Its Impact on Wild Animals: A Meta-Analysis on Oxidative Stress. EcoHealth 7, 342-350. https://doi.org/10.1007/s10393-010-0345-7

LENTH, R.V. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means.

LOPEZ-ANTIA, A.; ORTIZ-SANTALIESTRA, M.E.; MOUGEOT, F.; MATEO, R. 2015. Imidacloprid-treated seed ingestion has lethal effect on adult partridges and reduces both breeding investment and offspring immunity. Environ Res 136. https://doi.org/10.1016/j.envres.2014.10.023

LOPEZ-ANTIA, A.; ORTIZ-SANTALIESTRA, M.E.; MOUGEOT, F.; MATEO, R. 2013. Experimental exposure of red-legged partridges (Alectoris rufa) to seeds coated with imidacloprid, thiram and difenoconazole. Ecotoxicology 22, 125-138.

MINEAU, P.; KERN, H. 2023. Neonicotinoid insecticides: failing to come to grips with a predictable environmental disaster. ABC.

MINEAU, P.; PALMER, C. 2013. The impact of the nation's most widely used insecticides on birds. American Bird Conservancy, The Plains.

MINIAS, P. 2015. The use of haemoglobin concentrations to assess physiological condition in birds: a review. Conserv Physiol 3, cov007. https://doi.org/10.1093/conphys/cov007

NARAYANANKUTTY, A.; JOB, J.T.; NARAYANANKUTTY, V. 2019. Glutathione, an Antioxidant Tripeptide: Dual Roles in Carcinogenesis and Chemoprevention. Current Protein and Peptide Science. https://doi.org/10.2174/1389203720666190206130003

POLISERPI, M.B.; BRODEUR, J.C. 2023. Behavioral and physiological changes in the passerine Agelaioides badius following the ingestion of coated seeds with imidacloprid in a 30-day experiment. Science of The Total Environment 905, 167078. https://doi.org/10.1016/j.scitotenv.2023.167078

POLISERPI, M.B.; CRISTOS, D.; PÉREZ-IGLESIAS, J.M.; BRODEUR, J.C. 2021. Tissue distribution and sublethal effects of imidacloprid in the South American grayish baywing (Agelaioides badius). Chemosphere 284, 131327. https://doi.org/10.1016/j.chemosphere.2021.131327

POLISERPI, M.B.; NOYA ABAD, T.; DE GERÓNIMO, E.; APARICIO, V.; BRODEUR, J.C. 2023. Behavioral and physiological response of the passerine bird Agelaioides badius to seeds coated with imidacloprid. Environ Sci Pollut Res Int 30, 80293-80310. https://doi.org/10.1007/s11356-023-28074-y

REED, D.J. 1990. Glutathione: Toxicological Implications. Annual Review of Pharmacology and Toxicology. https://doi.org/10.1146/annurev.pa.30.040190.003131

REGLERO, M.M.; TAGGART, M.A.; MONSALVE-GONZÁLEZ, L.; MATEO, R. 2009. Heavy metal exposure in large game from a lead mining area: Effects on oxidative stress and fatty acid composition in liver. Environmental Pollution 157, 1388-1395. https://doi.org/10.1016/j.envpol.2008.11.036

SASIDHAR, B.N.; ANAND, K.A.; GOPALA, R.A.; AMARAVATHI, P.; HEMANTH, I.; SASIDHAR BABU, N.; KUMAR, A.A.; REDDY, A.G. 2014. Chronic experimental feedong of imidacloprid in-

duced oxidative stress and amelioration with vitamin C and Withania somnifera in layer birds. International Journal of Science, Environment and Technology 3, 1679-1684.

SIES, H. 2020. Chapter 1 Oxidative eustress and oxidative distress: Introductory remarks. In: SIES, H. (Ed.). Oxidative Stress. Academic Press. 3-12 pp. https://doi.org/10.1016/B978-0-12-818606-0.00001-8

SIMON-DELSO, N.; AMARAL-ROGERS, V.; BELZUNCES, L.P.; BONMATIN, J.M.; CHAGNON, M.; DOWNS, C.; FURLAN, L.; GIBBONS, D.W.; GIORIO, C.; GIROLAMI, V.; GOULSON, D.; KREUTZWEISER, D.P.; KRUPKE, C.H.; LIESS, M.; LONG, E.; MCFIELD, M.; MINEAU, P.; MITCHELL, E.A.D.; MORRISSEY, C.A.; NOOME, D.A.; PISA, L.; SETTELE, J.; STARK, J.D.; TAPPARO, A.; VAN DYCK, H.; VAN PRAAGH, J.; VAN DER SLUIJS, J.P.; WHITEHORN, P.R.; WIEMERS, M. 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res 22, 5-34. https://doi.org/10.1007/s11356-014-3470-y

WICKHAM, H. 2016. ggplot2: Elegant Graphics for Data Analysis.

WICKHAM, H.; AVERICK, M.; BRYAN, J.; CHANG, W.; MCGOWAN, L.D.; FRANÇOIS, R.; GROLEMUND, G.; HAYES, A.; HENRY, L.; HESTER, J.; KUHN, M.; PEDERSEN, T.L.; MILLER, E.; BACHE, S.M.; MÜLLER, K.; OOMS, J.; ROBINSON, D.; SEIDEL, D.P.; SPINU, V.; TAKAHASHI, K.; VAUGHAN, D.; WILKE, C.; WOO, K.; YUTANI, H. 2019. Welcome to the tidyverse. Journal of Open Source Software 4, 1686. https://doi.org/10.21105/joss.01686

WICKHAM, H.; FRANÇOIS, R.; HENRY, L.; MÜLLER, K.; VAUGHAN, D. 2023. dplyr: A Grammar of Data Manipulation.

YAGI, K. 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochemical Medicine 15, 212-216. https://doi.org/10.1016/0006-2944(76)90049-1