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The neonicotinoid pesticide imidacloprid and the dithiocarbamate fungicide mancozeb disrupt the pituitary–thyroid axis of a wildlife bird

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HIGHLIGHTS

- Imidacloprid and mancozeb disrupt the pituitary–thyroid axis of the bird, Red Munia.
- Weight and histopathology of the thyroid gland reflected substantial thyrotoxicity.
- Altered plasma TSH, T4 and T3 revealed disruption of the pituitary–thyroid axis.
- Disruption was more in breeding phase than pre-breeding phase of reproductive cycle.
- This wildlife avian species is more prone to imidacloprid toxicity than mancozeb.

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ABSTRACT

Thyroid is an important homeostatic regulator of metabolic activities as well as endocrine mechanisms including those of reproduction. Present investigation elucidated the thyroid disrupting potential of a neonicotinoid imidacloprid and a dithiocarbamate mancozeb in a seasonally breeding wildlife bird, Red Munia (*Amandava amandava*) who is vulnerable to these two pesticides through diet (seed grains and small insects). Adult male birds were exposed to 0.5% LD_{50} mg kg^{-1} bw d^{-1} of both the pesticides through food for 30 days during the preparatory and breeding phases. Weight, volume and histopathology of thyroid gland were distinctly altered. Disruption of thyroid follicles reflected in nucleus-to-cytoplasm ratio (N/C) in epithelial and stromal cells, epithelial cell hypertrophy and altered colloid volume. Impairment of thyroid axis was pesticide and phase specific as evident from the plasma levels of thyroid (T4 and T3) and pituitary (TSH) hormones. In preparatory phase, plasma TSH was increased in response to decrease of T4 on mancozeb exposure showing responsiveness of the hypothalamic–pituitary–thyroid (HPT) axis to feedback regulation. On imidacloprid exposure, however, plasma levels of both T4 and TSH were decreased indicating non-functioning of negative feedback mechanism. Increased plasma T3 in response to both the pesticides exposure might be due to synthesis from non-thyroidal source(s) in a compensatory response to decrease level of T4. In breeding phase, impairment of HPT axis was more pronounced as plasma T4, T3 and TSH were significantly decreased in response to both mancozeb and imidacloprid. Thus, low dose pesticide exposure could affect the thyroid homeostasis and reproduction.

1. Introduction

Neonicotinoids and dithiocarbamates are widely used worldwide as insecticides and fungicides respectively (World Health Organization, 1988; Jeschke et al., 2011). The pesticides (and their metabolites) of these two groups are thus prevalent in the environment and can be ingested, inhaled and/or absorbed transdermally by non-target organisms, making them susceptible to their toxic effects. Toxicological informations on both the groups of pesticides provide neurotoxic (Kimura-Kuroda et al., 2012; Overgaard et al., 2013), immunotoxic (Corsini et al., 2006; Mondal et al., 2009), developmental and teratogenic (NTP, 1992) effects in both low and high doses. Endocrine toxicity/disruptive effects of neonicotinoids and dithiocarbamates also have been demonstrated. Dithiocarbamates (mancozeb, maneb, zineb etc.) are potent thyroid disruptors. Maneb and zineb are reported to disrupt the hypothalamic–pituitary–thyroid (HPT) axis in rats by affecting the hypothalamic thyrotropin-releasing hormone/TRH and pituitary thyroid-stimulating hormone/TSH (Laisi et al., 1985). Mancozeb/

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MCZ specifically inhibits enzyme thyroid peroxidase, affects the weight and histopathology of thyroid gland as well as cause the hypothyroidism on acute high dose exposures to laboratory rodents (Ksheerasagar and Kaliwal, 2003; Axelstad et al., 2011). Ethylene thiourea (ETU) is the principal metabolic by-product of dithiocarbamate pesticides which exerts various toxic effects including thyroid disruption (Axelstad et al., 2011). Neonicotinoids specifically act as insect-nicotinic acetylcholine receptor (nAChR) inhibitor (Palmer et al., 2013). Imidacloprid/IMI, one of the neonicotinoid insecticides, was shown to have affinity for mammalian nAChR and has been elucidated toxic to mammals (Kimura-Kuroda et al., 2012). IMI is reported to cause the thyroid lesions on acute high dose exposure in laboratory rodents (Zaror et al., 2010) however thyroid disrupting potential of neonicotinoids has not been explored thoroughly. Thyroid gland, through its hormones (THs: thyroxine/T4 and triiodothyronine/T3), not only regulates metabolic activities but also maintains reproductive homeostasis (McNabb, 2007; Nakao et al., 2008; Wagner et al., 2008) in a variety of animals. Circulating concentration of THs is regulated by HPT axis through a negative feedback response. THs up regulate the gonadal growth/development and reproductive axis (Nakao et al., 2008; Wagner et al., 2008) in mammals. In birds, positive regulation though has been reported for temperate zone birds, such as Japanese quail (Yoshimura et al., 2003); there are varied reports on thyroid regulation of reproductive axis in tropical zone birds, such as estrildid finches (Dawson and Thapliyal, 2001). Both dithiocarbamates and neonicotinoids are reported to disrupt the reproductive (Anway et al., 2005; Bal et al., 2012) and metabolic (Bhaskar and Mohanty, 2014) functions in mammals under laboratory conditions through disruption of thyroid axis.

The present investigation demonstrated the effect of dithiocarbamate MCZ and neonicotinoid IMI, two popularly used dithiocarbamates and neonicotinoids respectively, on pituitary–thyroid axis of a seasonally breeding wildlife avian species, Red Munia (*Amandava amandava*). Specific action of MCZ and IMI, as fungicide and insecticide respectively, demands their simultaneous use in agricultural fields making the wildlife birds vulnerable to their exposure. The bird of the present investigation feeds on seed grains as well as small insects, and thus, is susceptible to both MCZ and IMI through dietary exposure. In view of lack of informations on MCZ and IMI induced disruption of HPT axis of wildlife avian species, a comparative study of dithiocarbamate and neonicotinoid pesticides was conducted during two of the important stages of reproductive cycle i.e. pre-breeding/preparatory and breeding stages using environmentally relevant/low dose. The disruption of thyroid physiology was evaluated through assessment of the various end points/biomarkers of thyroid health (thyroid weight & volume, follicles & colloids, epithelial cell height & nucleus size and their N/C) and alterations in circulating concentration of hormones of pituitary–thyroid axis (TSH, T4 and T3).

2. Materials and method

2.1. Experimental design

Male birds were captured from around Allahabad (25°27′N 81°44′E), UP, India, from a particular forest area away from agricultural croplands (to avoid background exposures to pesticides) in the beginning of preparatory (first week of July) and breeding (first week of September) phases of the reproductive cycle. Preparatory phase of the reproductive cycle is the pre-breeding transition stage in which a severe cellular/tissue differentiation, remodeling and development take place in gonads before entering into active breeding phase. The preparatory phase is characterized by a complex of developing secondary sexual characters as well as sexually motivated behaviors (Sharp, 1996). Birds were acclimatized in open air aviaries under natural conditions of temperature, humidity and photoperiod for 10 days (d). Food (grinded wheat grains, grown without any background chemical exposure/organic; available commertially) and water were given *ad libitum*. Food intake of individual bird was maintained.

Acclimatized male birds (body weight/bw 8.5 ± 0.5 gm) were divided randomly and maintained in three groups (n = 8/group): MCZ-exposed group, IMI-exposed-group and control. Low dose (0.5% of median LD_{50}) of commercial pesticides MCZ (75%w/w, Uthane M-45) and IMI (17.80% w/w, confidor) were given to exposure groups through diet using soy oil as vehicle. Control birds were given food with vehicle. Food was mixed (coated) with pesticides using vehicle and kept overnight. Two sets of the experiment were executed in preparatory (mid July-mid August) and breeding (phase of active mating and courtship; mid September-mid October) phases respectively and were exposed for 30 d in both sets. All the birds (pesticides-exposed as well as control) in each set of experiment were euthanized at the end of experiment. Dietary median LD_{50} of MCZ for bird (860 mg kg^{-1} bw d^{-1}) was taken as the reference dose (Health and Consumer Protection Directorate-General, European Commission, 2009). For IMI, chronic median LD_{50} of Japanese quail (31 mg kg^{-1} bw d^{-1}) was taken as the reference dose (Lopez-Antia et al., 2012). Body weight was recorded every alternate day. Precision of pesticide-dose intake by each bird was maintained by exposing them to the decided dose through calculated amount of food taken by birds during first 2 h of feeding (7:00–9:00 A.M.) each day. The test dose (0.5% of LD_{50}) was considered as environmentally relevant. Studies have reported the environmental concentrations of imidacloprid (Blacquie're et al., 2012) and mancozeb (Koppad and Umarbhadsha, 2006; Adamski et al., 2009) in invertebrates, seeds/grains and crop fields which is equivalent to our test dose, however, the precise biomonitoring data on test compounds are not available for birds.

2.2. Plasma sampling and hormonal assay

Birds were terminated by decapitation, blood was collected by cardiac puncture in 0.1% EDTA treated vials, centrifuged at 2500 r.p.m. for 15 min for separation of plasma and pooled. ELISA assay for TSH, T4 and T3 hormones was carried out immediately without storing/freezing the plasma. ELISA kits were used for measuring plasma conc. of TSH (SmarTest Diagnostics, Israel), T3 and T4 (LDN GmbH & Co. KG, Germany). Interassay and intraassay coefficient of variations (%) were <10% for T3 (7.6 & 7.0), T4 (8.1 & 3.9) and TSH (7.6 & 4.6) respectively. Samples were run in duplicate and optical density was measured by Bio-Rad iMark microplate reader (USA).

2.3. Thyroid histopathology

Thyroids were quickly dissected out, blotted and weighed before fixation in Bouin's fixative for overnight followed by washing and paraffin embedding according the standard protocol of our laboratory (Mishra and Mohanty, 2010). Thyroid sections were cut serially (5–6 μm), stretched on sterilized glass slides and stained with eosin-haematoxylene stain. Microphotography was done using Leica DM 2500 (Germany) light microscope. Every fifth serial section out of total 120–150 sections (per bird) was analyzed for histomorphometric analysis of epithelial cell heights & their nuclei, number of follicles filled with colloids and volume of thyroid gland (mentioned below) using ImageJ 1.32j (Image analysis software package, NIH, Bethesda, MD). Each of the histology measures for the thyroid gland was conducted in replicates and 32 replicates were conducted per bird.