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PESTICIDE RESIDUE ANLYSIS IN EXCRETA OF SPOTTED OWLET *ATHENE BRAMA* AND BARN OWL *TYTO ALBA*

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ABSTRACT

The continuous use of pesticides to improve agriculture has not only affected the crop but also altered the food chain and worst affected the non-target organisms. Birds utilizing agricultural landscapes for feeding get exposed to these chemicals through ingestion of sprayed soils, treated granules or seeds and prey items. Raptors like owls are the most likely victims of pesticide exposure as they are at the top level of food chain. In present study, excreta samples of barn owl and spotted owlet were collected from three locations and tested for presence of pesticide residues using gas chromatography. The organophosphate residues of dichlorvos, monocrotophos, phorate, malathion, quinalphos, profenophos, ethion were not reported in excreta of both owl species but the residues of chlorpyriphos (0.037 ppm) were detected in spotted owlet samples collected from village Barnhara only. The pyrethroid residues cyhalothrin, permethrin, cypermethrin were also found to be absent or below detectable limit in samples collected from different locations. The feeding habits of birds attribute to the level of contamination in predatory birds. Although the level of chlorpyriphos residues excreted out from the body of spotted owlet does not reflect the actual level of exposure to this bird but gives an indication of their being at the risk of exposure in the environment.

Key words: Pesticides, excreta, contamination, food chain, predatory bird, environment, residues, feeding habits, agricultural landscapes, exposure, spotted owl, barn owl

Pesticides are the chemicals that are released intentionally into the environment to suppress the pests and protect agricultural and industrial products. Increasing demand of continuous food supply has resulted in excessive use of these chemicals. However, most of these do not specifically target a particular pest but also affect the non-target species. They can have short term harmful effects on the organisms facing direct exposure or long-term effects by entering the food chain. Birds are highly migratory species, so it is impossible to exclude them from areas treated with more pesticides. Avian species dependent on agricultural landscapes for food may get exposed to pesticides through consumption of treated seeds, granules, or sprayed soils and prey items (Eng et al., 2017). Agricultural areas in India probably experience the heaviest and indiscriminate use of chemical pesticides leading to direct and indirect mortality of predatory and frugivorous birds (Dhindsa et al., 1986). A visible decline has been witnessed in abundance of birds as a result of alteration and reduction in their feeding, nesting and breeding grounds (Isaksson, 2018) Agroecosystem of Punjab, for the many past decades, has been subjected to tremendous change due to deforestation, intensive agriculture and excessive use of pesticides along with urbanization and industrial growth which in turn affected the avian community resulting in the reshuffling of many species of birds.

Pesticides become lethal at higher concentrations and result into density-dependent indirect effects (Fleeger et al., 2003). At sub-lethal concentrations, they show alterations in bird's morphology, physiology, hormones, reproduction, neurotransmitters, immune response and behavior including predator detection and swimming ability (Abrams, 1995). These chemicals can disrupt the central nervous systems, alter behaviour, cause endocrine system dysfunctions, affect immune systems, and inhibit growth in living organisms (Mitra et al., 2011). The growing concern has recently focused on the indirect effects of pesticides on aves besides their lethal and sub lethal effects. These effects act mainly by reducing food supplies (weeds, invertebrates), especially during breeding or winter seasons. Insecticides and acaricides primarily affect bird populations by reducing the availability of their arthropod prey. Still, the consumption of contaminated prey, e.g. ants contaminated with DDT, locust contaminated with fenitrothion may cause the deaths of insectivorous birds through acute poisoning or causes sub-lethal effects which will affect their behaviour or breeding success. Birds and mammals are not able to excrete easily the metabolites of organochlorine pesticides due to their lipophilic nature which results into their accumulation in adipose tissues and biological magnification at the higher trophic level (Mitra and Maitra, 2018). When it comes to birds of higher levels in the food chain, like raptors they develop abnormalities like shell-less eggs or sometimes thinner eggs hells. This, in turn, affects the different birds' reproductive capacity (Vishnudas, 2007). Birds of prey like owls, being at the top of food chain are the most likely victims of pesticide and chemical contamination. Like other predatory birds, owls are adversely affected by indiscriminate use of pesticides in modern agriculture. The population of these owl species therefore seems to be declining in many agricultural areas like Punjab, indicating a collapsing ecosystem.

The assessment of the exposure of birds to toxic compounds needs some non-invasive methods. Moreover, the capturing and killing of birds is legally banned by Govt. of India according to the Wildlife Protection Act 1972; therefore any analytical studies on the tissues and eggs of these organisms are beyond the reach of scientists working in this area. Thus, excreta of bird are an alternative source, which if analyzed can assess the harmful impact of environmental contaminants on these organisms and in our previous studies bird excrements have been used successfully as non-destructive indicators of chemical contamination in birds' diet (Sharma and Vashishat, 2017; Gaba and Vashishat, 2018). Therefore, keeping in view the beneficial role of spotted owlet and barn owl in agricultural areas, the present study was carried out to provide information on the level of their exposure to environmental contaminants through analysis of excreta.

MATERIALS AND METHODS

The collection of dry excreta samples of spotted owlet and barn owl was done from their from the roosting and nesting sites at three locations of district Ludhiana i.e. location I- village Barnhara situated near Buddha Nullah, location II- village Ladhowal situated near river Sutlej and location III- agricultural field areas of Punjab Agricultural University. Acetone, dichloromethane, hexane, sodium sulphate and activated charcoal (LR grade) were redistilled in all glass apparatus. The analytical technical grade pesticide standards i.e. heptachlor, quinalphos, chlorpyriphos and triazophos were used and procured from Sigma-Aldrich (USA). Five gram pooled excreta sample of each species was weighed and dipped in 50 ml acetone overnight. The extracts were filtered with rinsings of acetone in funnel containing 250 ml of 5% sodium chloride solution followed by addition of 75 ml of dichoromethane to collect lower layer. Second rinsing was given with 75 ml of hexane to collect upper non aqueous layer. The different fractions were combined and then treated with 100 mg of activated charcoal (in powder form) for about 2-3 hours at room temperature. The clear extracts so obtained were filtered and concentrated in a rotary evaporator to 15 ml. The extracts were cleaned up by using the column chromatography with the help of silica gel as an adsorbent which was activated at 110º C for 2 hours before use. A glass column was packed with activated silica gel (made of 20g silica +1g charcoal) in between the two small layers of anhydrous sodium sulfate supported on a plug of glass wool. The column was pre-washed with dichloromethane and the extract was poured over it. The extract was eluted with a freshly prepared solvent mixture of dichloromethane-acetone (1:1). The elute was concentrated to near dryness in a rotary evaporator under vacuum and then transferred to 5 ml acetone for further analysis.

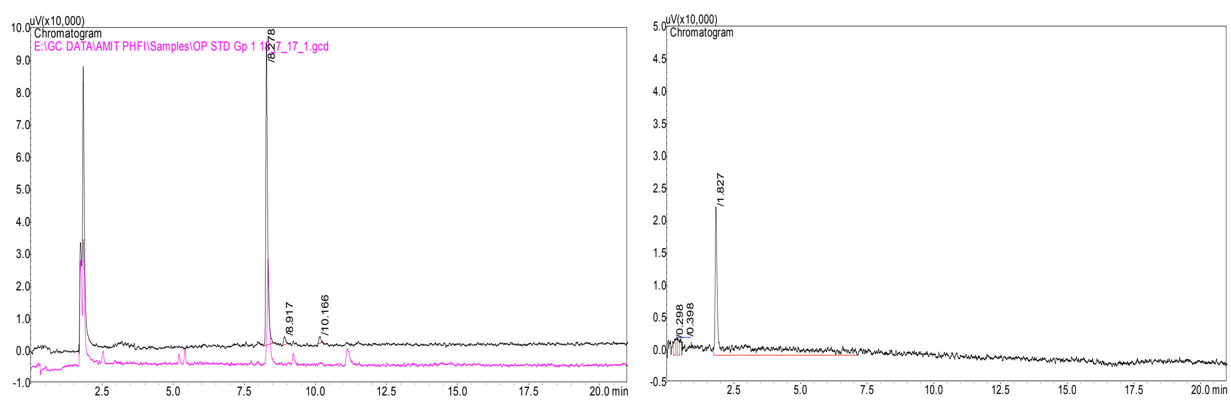
The pesticide sample extracts were analyzed using gas chromatograph (GC) equipped with electron capture detector (Agilent 7890B System) and flame thermionic detector (Shimadzu model 2010). For electron capture detector the initial temperature of GC oven was set at 170º C for 13 min followed 20 by increase in temperature to 270º C at the rate of 3º C per minute with hold time for 20 min. The initial oven temperature for flame thermionic detector was set at 150º C with hold time for 5 min followed by increase in temperature to 220º C at the rate of 10º C per minute with hold time for 5 min, and the final temperature was made up to 250º C at the rate of 5º C per minute with hold time for 13 min. The injection port temperature was set at 280º C whereas the temperature of both the detectors was adjusted at 310º C. Nitrogen was used as carrier gas while hydrogen and air were used for flame formation. Calibration curves for all standards of organochlorines (OCs), organophosphates (OPs) and synthetic pyrethroids (SPs) were drawn for concentration versus area of the peak and the correlation coefficients (r2) were determined near to 0.99. The residues were quantified by using the standard formula (Van Coot et al., 2018).

RESULTS AND DISCUSSION

The 5 g of dry excreta samples of spotted owlet and barn owl were collected and pooled from each site. The samples were then analyzed for detection of pesticide residues like Organophosphates and synthetic pyrethroids. The OP residues of Dichlorvos, Monocrotophos, Phorate, Malathion, Quinalphos, Profenophos, Ethion were found to be absent in excreta of both owl species but the residues of chlorpyriphos were detected in samples of spotted owlet only collected from village Barnhara. Other OP residues were not detected which may be because of low biomagnification properties in comparison to other pesticides like OCs. Jayakumar et al (2020) studied the presence of OCs with highest accumulation of hexachlorocyclohexane in tissues of colonial nesting birds in sanctuary of Tamil Nadu, India. Further, Dhananjayan et al (2020) observed OC residues in abandoned eggs of 22 terrestrial avian species from Tamil Nadu, India. The OPs and carbamates which are commonly used pesticides throughout the world have low bioaccumulating capacity in food chain and less persistence. This may be a reason for non-detection of many of the organophosphates in present study. The OPs, until activated in the liver by microsomal oxidation enzymes do not become potent inhibitors of cholinesterase. They are generally less toxic than other pesticide groups. Birds are particularly sensitive to the toxic effects of organophosphorus and carbamate pesticides (Hill, 1995). Isenring (2010) reported OPs namely chlorpyrifos, diazinon, isofenphos, malathion, mevinphos, phorate and carbamates namely aldicarb, bendiocarb, carbofuran as a cause of fatal bird poisoning. The pyrethroid residues Cyhalothrin, Permethrin, Cypermethrin were also found to be absent in samples collected from different locations. The synthetic pyrethroids are one of the least toxic insecticides. The different routes of exposure to different chemical pollutants can decrease the capacity of avian species to excrete these chemical residues through excreta. The residues of only chlorpyriphos i.e. 0.037 pm were detected in samples of spotted owlet collected from village barnhara (Fig. 1a) however, no such pesticide residue was observed in excreta samples of barn owl (Fig. 1b).

Although the level of chlorpyriphos residues excreted out from the body of spotted owlet does not reflect the actual level of exposure to this bird but gives an indication of their being at the risk of exposure in the environment. Birds in agricultural environments are commonly exposed to the insecticides, mainly through ingestion of invertebrates after insecticide application (Crisol-Martínez et al., 2016). The reason for its presence may be that the chlorpyriphos, because of its insecticidal property, is used for the control of ticks in cattle and buffaloes. It is also sprayed in crop fields for control of insect pests. In a study conducted by Malhotra and Singla (2018) on analysis of regurgitated pellets of spotted owlet from Punjab, India the diet of spotted owlet mainly consisted of insects i.e. 53.8% which indicates the entry of pesticide through food chain. However, the diet of bam owl consisted only of vertebrates, in which 88 per cent were rodents alone (Malhotra and Singla, 2017). The absence of insects in the diet of barn owl may be a reason behind the non-detection of pesticide residues in excreta of this bird.

Chlorpyriphos, an OP, inhibits acetyl choline esterase enzyme in a way that has cross generational implications (Anway et al., 2005) and it severely affects birds (Mitra et al., 2011). In order to monitor the pesticide residue in birds, the metabolic studies are required not only to identify the primary metabolites but also their accumulation and distribution within the body (Katagi and Fujisawa, 2021). Eng et al. (2017) have reported the impairment of migration



(a) (b)

Fig. 1. Chromatogram for pesticide residue analysis of excreta of a) Spotted owlet from village Barnhara; b) Barn owl from village Ladhowal

ability in white-crowned sparrow, a seed eating bird, after exposure to low (10% LD_{50}) and high dose (25% LD_{50}) of chlorpyriphos. The non detection of the other pesticide residues in excreta samples revealed that the residues of OPs and pyrethroids can be absent in faecal samples. The feeding habits of carnivorous birds actually attribute to the level of contamination in predatory birds (Van Drooge et al., 2008; Gupta et al., 2017). In general, birds that eat other birds, or fish, have higher residues than those that eat seeds and vegetation. Birds of prey were primarily affected; exceptions apparently are the result of lesser exposure because of different food habits. The detection of chlorpyriphos in the excreta of Spotted Owlet highlights its potential as bioindicator of pesticide contamination in birds of prey. Furthermore, the present study can serve as baseline for future research in general as well as control values during the analysis of samples obtained from birds in the event of suspected pesticide poisoning.

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AUTHOR'S CONTRIBUTION STATEMENT

NV conceived and designed research. YG conducted the experiments and collected data. NV and YG interpreted the results and wrote the manuscript. Both authors read and approved the manuscript.

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