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Occurrence of pesticide residues in fodder from Punjab (India): Temporal and spatial variation

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Once the milch animals get exposed to pesticides through the consumption of contaminated feed, fodder and dermal treatment for ectoparasite control, these directly affect animal health and indirectly human health through consumption of contaminated food of animal origin. Animal fodder crops require less chemical sprays, but due to mixed agriculture practices, these crops get contamination while nearby grown crops are being sprayed with pesticides. Reports on contamination of animal feed and fodder with pesticide residues are mainly confined to organochlorines pesticides (Sharma *et al.* 2005). However, with the imposition of ban/restriction on use of HCH and DDT and, changing consumption pattern of pesticides, the present investigation was undertaken to analyse organochlorine pesticides (OCPs), organophosphates (OPs) and synthetic pyrethroids (SPs) in fodder samples.

Fodder samples (500 g) comprising 19 of bajra (*Pennisetum glaucum*), 38 of berseem (*Trifolium alexandrinum*), 23 of maize (*Zea mays*) and 26 of oat (*Avena sativa*) were collected from randomly selected dairy farms and transferred to laboratory. To investigate the spatial variation for occurrence of pesticide residues, these samples were collected from Amritsar, Bathinda, Ludhiana and Mansa districts of Punjab. The extraction and clean-up of the fodder samples was performed by adopting the method described by Kang *et al.* (2002) with slight modifications.

Estimation of pesticide residues was done in gas chromatograph (GC) equipped with electron capture detector for OCPs and SPs and flame thermionic detector for OPs pesticide residues. The GC oven temperature for electron capture detector was programmed with initial temperature of 170°C which was raised to 270°C, while for flame thermionic detector, initial oven temperature was set at 150°C and ramped to 250°C. The temperature of injection port was set at 280°C, whereas temperature of both the detectors was adjusted at 310°C. In present study technical grade standards of OCPs (α-, β-, γ-, δ-HCH, *p,p'* DDT, *p,p'* DDE, *o,p'* DDT, *o,p'* DDE, *p,p'* DDD, aldrin, dieldrin, endrin, heptachlor, α-, β-, endosulfan, endosulfan sulphate), OPs (dimethoate, monocrotophos, malthion, parathion, chlorpyrifos, ethion, fenamiphos, phosalone, triazophos) and SPs (cypermethrin, cyhalothrin, cyfluthrin, deltamethrin, permethrin, fenvalerate) were procured commercially. One way ANOVA statistical test was applied to find out the significant difference between presence of pesticide residue levels in different fodder samples and different districts of sample collection.

Reagent blank samples were run every 6 samples to check the interference or contamination from solvents and glassware. The mean recovery values of spiked samples based on external standard were in the range of 75.2 to 93.4%. The calculated concentrations of residues in samples were not corrected for recovery. The limits of detections were calculated from the detector response using a signal to noise ratio of 3 and the values were varied from 0.3–4.0 ng/g for OCPs, 1.0–8.0 ng/g for SPs and 2.5–13 ng/g for OPs.

The analysis of fodder samples revealed the presence of HCH, DDT, endosulfan, cypermethrin, deltamethrin, chlorpyrifos and monocrotophos residues (Table 1). Cypermethrin and deltamethrin were predominant and determined in 55 and 62% of the total fodder samples, respectively. The total pesticide residues load in fodder samples depicted the contribution of 38% each by cypermethrin and deltamethrin. Residues of total endosulfan were detected in 55% of the samples which accounted for 17% of total pesticide load. β- and γ-HCH isomers of HCH contributed to only 4%, while *p,p'* DDE and *p,p'* DDD accounted for 2% of total pesticide load in fodder samples collected from Punjab. Cyfluthrin, monocrotofos and chlorpyrifos residues were observed with detection frequency of 8, 10 and 33%, respectively, and they contributed to 1% of total pesticide load. The comparative evaluation of pesticides residues detected in different types

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Table 1. Levels of pesticide residues (ng/g) in different types of fodder samples collected from Punjab

Pesticide	*Bajra* (n=19)		Berseem (n=38)		Maize (n=23)		Oat (n=26)	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
ß-HCH	nd	nd	2.7±1.0 (15.8)	nd-44.3	3.6±2.0 (17.4)	nd-29.3	5.1±2 (26.9)	nd-22.3
γ-HCH	9.1±4.0 (31.6)	nd-60.2	9.3±3.2 (28.9)	nd-78.8	6.4±2.0 (34.8)	nd-34.4	3.9±3 (23.1)	nd-60.2
Total HCH	9.1±4.0 (31.6)	nd-60.2	12.0±3.2 (44.7)	nd-78.8	10.1±2.0 (47.8)	nd-34.4	9.0±3.0 (42.3)	nd-60.2
p,p' DDE	nd	nd	nd	nd	5.2±4.1 (30.4)	nd-90.2	4.1±2.0 (19.2)	nd-31.4
p,p' DDD*	nd	nd	4.1±2.0 (13.2)	nd-70.2	10.8±4.0 (4.3)	nd-72.3	1.0±1.0 (3.8)	nd-22.1
Total DDT*	nd	nd	4.1±2.0 (34.2)	nd-70.2	16.0±7.0 (34.8)	nd-139.1	5.2±2.1 (23.1)	nd-31.4
ß-endosulfan	4.0±2.0 (20.1)	nd-23.2	1.0±1.0 (10.5)	nd-32.4	1.0±0.4 (13.0)	nd-9.0	0.4±4.0 (3.85)	nd-11.0
Endosulfan sulphate	26.0±12.0 (26.3)	nd-178.3	82.0±22.0 (57.9)	nd-37.9	15.0±5.0 (43.5)	nd-70.0	22.0±12.0 (42.3)	nd-286.2
Endosulfan*	30.0±12.0 (42.1)	nd-178.3	83.0±22.0 (68.4)	nd-37.9	16.0±5.0 (52.2)	nd-70.0	23.0±11.1 (46.1)	nd-286.2
Cyfluthrin	nd	nd	1.0±1.0 (7.9)	nd-15.2	2.0±1.0 (13.0	nd-14.1	1.0±1.0 (7.7)	nd-22.0
Cypermethrin*	130.1±2.5 (68.4)	nd-344.3	64.2±13.0 (44.7)	nd-263.0	144.2±22.0 (78.3)	nd-397.1	86.1±26.0 (38.5)	nd-500.3
Deltamethrin	84.2±21 (52.6)	nd-397.0	105.0±14.0 (42.1)	nd-261.0	101.0±18.0 (69.6)	nd-268.0	91.0±18.0 (53.8)	nd-268.1
Monocrotofos	0.10±0.2 (5.3)	nd-24.1	0.2±0.2 (10.5)	nd-3.2	1.0±0.4 (17.4	nd-10.0	0.1±0.1 (7.7)	nd-2.0
Chlorpyrifos	1.3±0.2 (42.1)	nd-4.0	1.1±0.2 (23.7)	nd-15.1	1.0±0.2 (34.8)	nd-6.0	1.0±0.2 (30.8)	nd-6.0

nd, not detected; n, number of samples. Figures in parenthesis indicates the percentage detection frequency. * significant difference between different green fodders according to one way ANOVA at P<0.05 level of significance.

of fodders indicated the higher accumulation of cypermethrin in maize followed by *bajra*, oats and berseem. However, deltamethrin was predominant in berseem pursued by maize, oats and *bajra*. Statistical significant difference (P<0.05) was observed for residue levels of DDT, endosulfan and cypermethrin residues in different types of fodder with their predominance in maize, berseem and maize, respectively. OPs and SPs are widely used to control insects/pests in a variety of crops including cotton, paddy, wheat, vegetables, sugarcane, mustard, maize, barley, sorghum, fruits and for termite control in soil, buildings in agriculture, homes, communities and dairy farms (Government of India 2009). Pesticides such as cypermethrin, deltamethrin and fenvalerate pesticides are ranked high in farmers' preferences in cotton growing areas of India. Therefore, the increasing use of synthetic pyrethroids may have resulted in their higher occurrence in fodder samples. In addition, deltamethrin and cyperemthrin are also used as ectoparaciticidal in dairy animals, which can also lead to contamination of the feed and fodder if proper precautions are not being followed. Some recent studies in India have also reported the occurrence of cypermethrin, α-cyhalothrin and chlorpyrifos residues in the market vegetables in Mumbai and Punjab (Mandal and Singh 2010, Chandra *et al.* 2010). Earlier, India was one of the foremost producer and consumer of OCPs particularly DDT and HCH, till the ban/restriction on their use in late 1990s. Still, a substantial amount of these chemicals are being used for malaria control and eradication programme. Among OCPs group, endosulfan residues were detected predominantly in fodder samples with endosulfan sulphate as the main contaminant. Prior to ban in year 2012, endosulfan was widely used pesticides on cotton and other crops in Punjab which may have resulted into the contamination of fodder crops. The presence of endosulfan residues have also been reported in fodder and concentrate feed in other parts of India (Sugathy *et al.* 2002, Sharma *et al.* 2005). The temporal variation for the presence of HCH and DDT residues in present study (HCH ranged from: nd-78.2 ng/g; DDT ranged from: nd-139.2 ng.g) with previous studies conducted in this geographical region and other parts of India reflected the significant decline in levels as well as detection frequency. HCH residues were detected with 100% detection frequency and its levels were ranged from 120–4690 ng/g (Gupta *et al.* 2000), while DDT residues ranged from 360–4310 ng/g (Nath *et al.* 2000). Punjab state is known as food bowl of India with intensive agricultural practices, during year 2010, 42264 MT pesticides were used

in India and out of this 6500 MT were sprayed in Punjab (Ministry of Statistics and Programme Implementation, Government of India 2012). The spatial variation of pesticide residues occurrence in fodder from four districts of Punjab, viz. Amritsar and Ludhiana (medium pesticide use area of Punjab) and Bathinda and Mansa (high pesticide use area) indicated contamination with either one or more pesticides. In Amritsar and Ludhiana deltamethrin was predominant, while in Bathinda and Mansa residues of cypermethrin were utmost in fodder samples.

There was significant difference ($P<0.05$) in residue levels of endosulfan between different districts with maximum levels reported in Amritsar (59.0 ng/g) followed by Bathinda (42.0 ng/g) Ludhiana (16.0 ng/g) and Mansa (15.1 ng/g). Similarly, significant difference was observed for total DDT residues between four districts with highest levels in Mansa district at mean levels of 10.0 ng/g, while in Amritsar, Bathinda and Ludhiana the mean residues levels were 4.1, 5.0 and 6.0 ng/g, respectively. The total mean levels of all pesticide residues in each district indicated, Mansa as the most contaminated district with total pesticide residues load in fodder samples at value of 316.1 ng followed by Bathinda (286.3 ng/g), Amritsar (236.7 ng/g) and Ludhiana (169.6 ng/g). The increased levels of contamination in Mansa and Bathinda districts were expected as these districts fall under high pesticide use area where cotton is the main crop and out of total pesticides consumption in Punjab, >90% of pesticides are being used in cultivation of cotton, rice and vegetables (Singh 2002). The maximum residue limit (MRL) or tolerance limit of pesticides in animal fodder materials have not been set in India. Therefore, based the limiting value (concentration of a pesticide in feed/fodder if fed to lactating animals daily, the likely residues in milk will be less than their MRL values) of a pesticide in feed derived on the basis of its legal permissible limit in milk and its rate of transference from feed to milk have been recommended. In present study we compared present data for β-HCH, γ-HCH, and total DDT with their limiting values of 20, 120 and 100 ng/g, respectively (Kang *et al.* 2002). It was observed that 2.6%, 4.3% and 3.8% samples of berseem, maize and oats, respectively exceeded the limiting value for γ-HCH, while 8.7% maize samples violated the limiting value of total DDT. In this study, levels of pesticide residues showed a substantial decline in total HCH and total DDT residue levels in animal fodder with respect to earlier reports, only few samples exceeded the prescribed limiting values. In view of the changing consumption pattern of pesticides, occurrence of OPs and SPs residues in animal feed and fodder should not be ignored in future studies. There is also need of area confinement for fodder crops as the pesticide residues observed in green fodder are meant for other crops.

SUMMARY

Punjab State of India is increasingly under focus for the indiscriminate use of pesticides in agricultural practices and occurrence of their residues in food of plant and animal origin. This study investigates the occurrence of organochlorines, organophosphates and synthetic pyrethroids residues in animal fodder samples collected from four districts of Punjab. The gas chromatographic analysis revealed the predominant presence of cypermethrin, deltametherin, chlorpyrifos and endosulfan residues reflecting their increased use for pest control. Though few fodder samples exceeded the limiting value of HCH and DDT residues, however the temporal decrease in detection frequency and low concentrations indicated the effectiveness of imposition of ban/restriction on their use in agriculture. The spatial variation for pesticide residues occurrence imitated that cotton-growing area of Punjab is comparatively more contaminated than other regions.

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