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Basant Kumar Bhinchhar
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Vinod Kumar Paswan
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Satya Prakash Yadav
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Prity Singh
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Basant Kumar Bhinchhar
Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Levels of Aldrin and Endosulfan pesticide residues in green fodders of Varanasi district, Uttar Pradesh, India

Basant Kumar Bhinchhar, Vinod Kumar Paswan, Satya Prakash Yadav and Prity Singh

Abstract
The present study was conducted to find out the organochlorine pesticide (OCP) aldrin and endosulfan residues in fodder samples in Varanasi District, Uttar Pradesh, India. Green fodder samples collected from the four block of Varanasi in which five villages selected evenly distributed from each block, were analyzed by gas chromatography-mass spectrometry (GC-MS) for OCP residues. Aldrin concentration (mg/kg) in green fodder obtained from different blocks were 0.00198 (Baragaon block), 0.00092 (Cholapur), 0.00133 (Araziline) and 0.00198 (Harhua). In Baragaon, Cholapur, Araziline and Harhua block 66.66%, 33.33%, 60% and 40 % samples, respectively were contaminated with endosulfan. The mean level of endosulfan concentration was found to be 0.00214 mg/kg throughout the year. In green fodder samples maximum concentration was found in winter (0.00296 mg/kg) and minimum was found in rainy with mean residues level of 0.00123 mg/kg.

Keywords: organochlorine, pesticide, aldrin, endosulfan, fodder

Introduction
Nowadays more than 800 different kinds of pesticides are used for control of insects, rodents, fungi and unwanted plants in the process of agricultural production. Although most of them leave the products or degrade in soil, water and atmosphere, these chemicals can be transferred to human via food chain (Benbrook, C.M. 2002) [4]. Average national consumption of pesticides is low but some states/union territories like Haryana, Punjab, Delhi and Pondicherry consume over 800 g/ha. States of Uttar Pradesh, Andhra Pradesh, Gujarat, Tamil Nadu, Kerala, Kamataka and West Bengal also have higher consumption of pesticides (Agnihotri, 2000) [1]. Animal feed and fodder containing pesticides is the main source for accumulation of these residues in tissues of animals, although inhalation of polluted air and absorption through intact skin also may take place. OCP's are lipophillic in nature, so after ingestion through contaminated feed and fodder, they accumulate in fat and usually do not get converted to the water-soluble metabolites inside the animal body. As their concentration exceeds a certain threshold level, pesticides are translocated to mammary glands and are secreted in milk as residues. Pesticide residues in feed and fodders may transfer into herbivores through food chain of the animals (Prassad, K.S.N. and Chhabra, A. 2001) [14]. Endosulfan, marketed as thiodan, is widely used in cotton growing areas, on vegetable farms, and on coffee plantations (Ntow *et al.*, 2006) [12]. Organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), lindane and endosulfan are also employed to control ectoparasites of farm animals and pets in Ghana (Awumbila and Bokuma, 1994) [2]. Most organochlorine insecticides, except lindane are very stable solids with limited vapour pressure and very low water solubility. They are highly lipophilic and resistant to microbial degradation (UNEP, 2001; Kaushik and Kaushik, 2007) [15, 9]. As these chemicals are inherently toxic to living organisms, the presence of their residues in food items is of a major concern to environmental and consumer groups (Willes *et al.*, 1993) [16]. The purpose of this study was to check whether green fodder was contaminated with pesticides or not and what was the limit of contamination with pesticides residues. This study will be helpful for general public and farmers that they should use pesticides with caution.

Material and Methods
Collection of samples
This study was based on 60 green fodder samples collected from four blocks of Varanasi. From each of the 4 blocks 5 villages were selected randomly for sample collection.

From each of the selected 5 villages green fodder samples were collected one time in each season (winter, rainy and summer). Approximately, 250 g of fodder samples were collected in sterilized polyethylene packs, packed and transported to lab. Samples were subjected to analysis within 24 h from their arrival.

Analytical procedure

The samples were extracted in the laboratory of the Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The GC analysis of the extracted samples was done in the same laboratory of the Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Chemicals and reagents

Acetonitrile, acetone, dichloromethane, graphitized carbon black, hexane, magnesium sulfate, silica gel, sodium chloride, sodium sulfate, prostate specific antigen (PSA) of high-performance liquid chromatography residue grade obtained from Merck specialties Pvt. Ltd. Analytical standards with >99% purity were also obtained from Merck specialties Pvt. Ltd during 2015 and stored in deep freeze maintained at −40°C.

Method validation

The AOAC official method 2007.01 with slight modifications was validated for the estimation of the limit of quantification (LOQ) of organochlorine in fodder. Fodder samples were chopped, and 7.5 g of sample was taken into 50 ml centrifuge tubes and 30 ml of acetonitrile was added and shaken well. The sample was homogenized at 14000-15000 RPM for 2-3 min then 3 g of sodium chloride was added, mixed well by shaking gently then it was centrifuged at 2500-3000 RPM for 3 min to separate the organic layer, approx. 16 ml of organic layer was taken into a test tube and 9 g of anhydrous sodium sulfate was added to remove moisture (Benbrook, C.M. 2002) [4]. Taken about 0.4 g PSA sorbent and 1.2 g anhydrous magnesium sulfate into 15 ml centrifuge tubes. The 8 ml of organic layer extract was transferred into this 15 ml centrifuge tube, capped and vortex for 30 s, then tubes were centrifuged at 2500-3000 RPM for 5 min then 2 ml of extract was transferred into test tubes and the solvent (acetonitrile) was evaporated turbovap concentrator for GC analysis. Gas Chromatograph Mass Spectrophotometer was standardized by giving several injection of standard (working standards prepared) simultaneously. A volume of 1 ml sample was injected into the GC; peaks were identified by comparing their retention times with those of standards under the same injection conditions.

Results and Discussion

Out of total 60 fodder samples collected in different seasons from four block of Varanasi, 28(46.66%) samples contained aldrin and 30(50%) samples contain endosulfan residues. About 46.66% samples from Baragaon block were positive for aldrin with mean concentration of 0.00198 mg/kg. Aldrin concentration (mg/kg) in green fodder obtained from different blocks were 0.00198 (Baragaon block), 0.00092 (Cholapur), 0.00133 (Araziline) and 0.00198 (Harhua) and this has been graphically represent in fig 2. In our observation, the maximum (0.00198 mg/kg) aldrin concentration was found in Baragaon (Block-1) and Harhua (Block-4) with same concentration while the minimum (0.00092 mg/kg) aldrin level was found in Cholapur (Block-2). The amount of sample of aldrin concentration found in green fodder collected from different blocks of Varanasi district has been depicted in table 2. In green fodder samples we observed from table 1 that aldrin concentrations for different season was found to be significant (P value=0.007; P≤0.05), whereas aldrin concentrations for different block was found to be non significant. (P value=0.516; P≥0.05). The mean result concentration obtained in the study was similar to the result of Panseri *et al.*, (2013) [13] whereas it is below to the result obtained by Nagra (2006) [11] in Punjab that is 0.03ppm.

Out of 60 samples 30 samples (50%) were contaminated with endosulfan in different blocks. In Baragaon, Cholapur, Araziline and Harhua block 66.66%, 33.33%, 60% and 40 % samples, respectively were contaminated with endosulfan. From the table 1, it has been observed that the mean level of endosulfan concentration was found to be 0.00214 mg/kg throughout the year. In green fodder samples maximum concentration was found in winter (0.00296 mg/kg) and minimum was found in rainy with mean residues level of 0.00123 mg/kg. Endosulfan concentration (mg/kg) in green fodder obtained from different locations were 0.00333 (Baragaon), 0.00074 (Cholapur), 0.00244 (Araziline), 0.00204 (Harhua) and this has been depicted in the table 2. In our observation, the maximum (0.00333 mg/kg) endosulfan concentration in green fodder was found in Baragaon Block while the minimum (0.00074 mg/kg) endosulfan level was found in the Cholapur Block. The amount of endosulfan concentration found in green fodder samples collected from different blocks of Varanasi district has been graphically depicted in the fig. 2. The table 1 reveals that the mean difference of endosulfan during all three seasons were found to be non-significant (P value=0.137; P≥0.05).The mean difference of endosulfan for different blocks were also found to be non-significant (P value=0.073; P≥0.05). In this study the endosulfan concentration in the fodder samples is in accordance with some previous studies, in which, analysis of 104 feedstuffs performed in 2003-2004 in Germany revealed the presence of endosulfan in two samples only at a concentration of 7 µg/kg. In all other samples including crops like maize, oilseeds, mineral feeds for ruminants, pigs, horses and pets no endosulfan could be detected at a limit of detection of 1 µg/kg (EFSA, 2005). Another survey of pesticide residues in animal feed ingredients had been conducted in the UK, where 151 samples of cereals (barley grain and malting, wheat grain, maize gluten and distillers), fodder (barley and wheat straw grass and maize), seeds (rapeseed, cotton seed, sunflower seed) and soybean meal were analyzed. None of these samples contained endosulfan at a concentration over the detection limits (50 µg /kg) (MAFF-UK, 1998) [10]. Result of present study is also similar to the study done by Bedi J S *et al*., (2015) [3] they found that concentration of endosulfan was 5.20 ng/g, 5.49 ng/g, 4.35 ng/g, 5.84 ng/g and 7.84 ng/g in zone-1, zone-2, zone-3, zone-4 and zone-5 respectively. In this study the endosulfan concentration in the fodder samples samples is in contrast with some previous studies, in which endosulfan residues has been reported in different green fodders up to a concentration of 6 mg/kg (Dikshit *et al*., 1989; Prassad and Chhabra, 2001; Kang *et al*., 2002; Imrankham *et al*., 2003; Deka *et al*., 2004) [6 14, 8, 7, 5]. However, presence of endosulfan in fodder samples in present study could be due to the major consumption of these pesticides in agriculture sector in past years.

Table 1: Aldrin and Endosulfan Residues in Green Fodder during Different Seasons of the Year (mg/Kg).

	Aldrin	Endosulfan
Winter	0.00275[b]	0.00296
Rainy	0.00056[a]	0.00123
Summer	0.00135[a]	0.00222
Overall Mean±SEM	0.00155±0.00029	0.00214±0.00036
P value	0.007	0.137

Table 2: Aldrin and Endosulfan Residues in Different Blocks in Green Fodder (mg/kg).

	Aldrin	Endosulfan
Baragaon	0.00198	0.00333
Cholapur	0.00092	0.00074
Araziline	0.00133	0.00244
Hahua	0.00198	0.00204
Overall Mean±SEM	0.00155±0.00029	0.00214±0.00036
P value	0.516	0.073

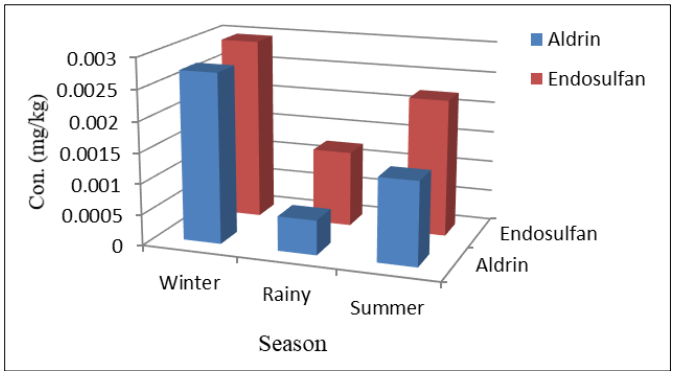

Fig 1: Aldrin and Endosulfan Level in Different Seasons in Fodder

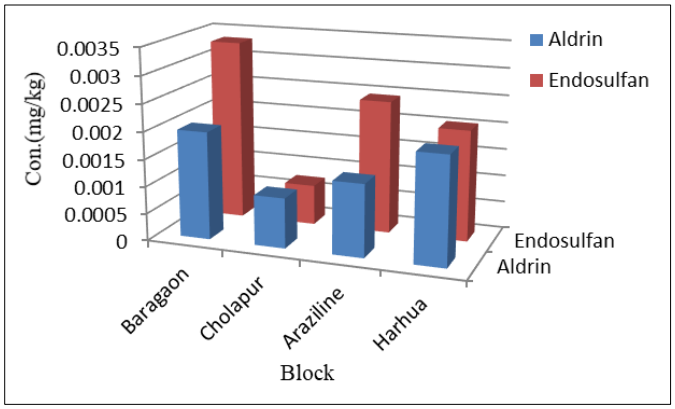

Fig 2: Aldrin and Endosulfan Level in Different Blocks in Green Fodder

Conclusion

It could be concluded that OC pesticide residues were detected in green fodder as they were persistent in nature due to their slow decomposition rate, long half life and high stability in the environment. Baragaon block was highly contaminated with aldrin and endosulfan residues. Owing to effects on human, animal and environmental health of pesticide residues need for education and awareness among farmers about extensive use of pesticide was envisaged.

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