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Monitoring of Insecticide Residues in Cotton Seed in Punjab, India

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Cotton (*Gossypium hirsutum* L.) is an important commercial fibre crop of India and plays a vital role in the agricultural and industrial economy of the country. The pest infestation in cotton causes heavy losses and remains a major bottleneck in its economic cultivation. To reduce the losses caused by insect pests, the main reliance has been on the use of insecticides. Due to the change in pest complex over the years and severe damage caused by American bollworm (*Helicoverpa armigera* Hubner), specifically, the number of sprays have increased from 4-7 in 1980's to around 15-20 in 2000's. The farmers quite often, apply more number of sprays at higher doses, at shorter intervals and sometimes use mixtures of insecticides at their own level (Dhawan 2000). Because of this injudicious and indiscriminate use of insecticides, it is feared that cotton reaching the market might be heavily contaminated with insecticide residues. Therefore, it becomes imperative to estimate the resulting residues of insecticides in cotton seed which is an important source of edible oil. So far, the residue data have been obtained by conducting supervised field trials with some of the commonly used insecticides. However, there is no information available in India regarding the status of contamination of marketed cotton seed. Thus, the present investigations were undertaken to study the status of contamination of cotton seed.

MATERIALS AND METHODS

The samples of seed cotton were collected from five locations in Punjab, viz., Abohar, Bathinda, Malout, Mansa and Muktsar which represent the cotton growing areas of Punjab. In these areas, cotton is grown as a major cash crop. From each location, five samples (weighing about 1 kg) were collected each at the first pick and second pick, i.e. ten days after the first pick. The samples were air dried, delinted and the cotton seed samples, thus, obtained were processed and analyzed at the Pesticide Residue Analysis Laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana.

The method described by Singh et al. (2001) was used with slight modifications for extraction, clean up and final estimation of organochlorine, organophosphate and synthetic pyrethroid insecticides in cotton seed. A finely grounded representative sample of cotton seed (5 g) was extracted for 8 hours with 400 mL of hexane : acetone (1: 1, v/v) using Soxhlet apparatus. The extract was cooled,

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concentrated and oil thus obtained was dissolved into 40 mL hexane and partitioned thrice into acetonitrile using 40 mL each time. The combined acetonitrile fractions were diluted four times with 5 per cent aqueous sodium chloride solution and then partitioned first into 100 and 50 mL of dichloromethane and then into 100 and 50 mL of hexane. The combined dichloromethane and hexane fraction was concentrated to 2-3 mL *under vacuum* at a temperature below 30°C. After concentration, the residues were transferred into acetone and cleanedup on a column of silica gel (60-120 mesh) which was activated for 2 hours at 110°C. The extract of cotton seed was mixed thoroughly with a mixture of silica gel (20 g), anhydrous sodium sulphate (10 g) and activated charcoal (1 g) to obtain a free flowing powder. The adsorbent was transferred to a glass column (60 cm x 2 cm i.d.) by making a slurry with dichloromethane. The column was stoppered when about 50 ml dichloromethane remained above the surface of the adsorbent and allowed to stand for 30 minutes. The extract was eluted with 150 mL freshly prepared solvent mixture of dichloromethane: acetone (1: 1, v/v). The eluate, thus, obtained was concentrated to near dryness in a rotary evaporator *under vacuum* and then transferred to 4- 5 mL of acetone for further analysis.

The estimation of residues of different insecticides was carried out on gas- liquid chromatograph (GLC) equipped with ^{63}Ni electron capture detector (ECD) and nitrogen- phosphorus detector (NPD). A pyrex glass column (2 m x 2 mm i. d.) packed with ready- to- use 1.5% SP-2250 + 1.95% SP 2401 on 80- 100 mesh Supelcoport was used for the estimation of organochlorines and synthetic pyrethroids while a glass column of similar dimensions packed with 3% OV- 101 on chromosorb WHP was used for the estimation of organophosphate insecticides. The operating conditions of GLC were as follows: column (oven) temperature- 210°C, injector temperature- 250°C, detector temperature- 280°C and carrier gas (nitrogen) flow rate was 40 mL per minute. The cleanedup extracts of 5 g seed when concentrated to 2.5 mL, out of which 2μL (4 mg plant equivalent) when injected into the GLC did not give any interference peak in the region of the compounds detected. Under the operating conditions, 0.2 ng of endosulfan and chlorpyriphos, 5 ng of ethion and 0.2 ng of each synthetic pyrethroid gave about half scale deflections. Based on twice the noise level, the limits of determination of different insecticides were worked out to be 0.01 mg kg^{-1} for organochlorines and synthetic pyrethroids and 0.08 mg kg^{-1} for organophosphates except chlorpyriphos where this limit was 0.01 mg kg^{-1}. The average recovery values obtained following the above method from samples of cotton seed fortified at 0.5 and 1.0 mg kg^{-1} before extraction ranged from 82 and 86.6, 82 and 81.3, 80 and 81 and 80 and 86 per cent for endosulfan, chlorpyriphos, ethion and cypermethrin, respectively.

RESULTS AND DISCUSSION

The insecticides detected in cotton seed samples included endosulfan among the organochlorines, chlorpyriphos and ethion among the organophosphates and

cypermethrin among the synthetic pyrethroids. The cotton seed samples collected at the first pick from Abohar were found to be contaminated with ethion and chlorpyriphos with the average residue values for the five samples being 0.018 and 0.014 mg kg^{-1}, respectively. Only chlorpyriphos was detected in the samples collected from Bathinda and the range of residues was BDL-0.081 mg kg^{-1}. One sample exceeded the MRL of 0.05 mg kg^{-1} prescribed for chlorpyriphos (Anonymous 1999). Among the cotton seed samples collected from Malout, endosulfan and chlorpyriphos were detected with the range of residues being BDL-0.022 and BDL-0.012 mg kg^{-1}, respectively for the two insecticides. The samples collected from Mansa also showed the presence of these two insecticides with the levels ranging from BDL-0.037 and BDL-0.017 mg kg^{-1}, respectively. Among the cotton seed samples collected from Muktsar, endosulfan and ethion were detected with the mean residue levels being 0.004 and 0.049 mg kg^{-1}, respectively for the two insecticides. Cypermethrin was not detected in any of the samples collected from the first pick (Table 1).

The cotton seed samples collected at the second pick from Abohar were found to be contaminated with endosulfan and ethion with the mean residue levels being 0.013 and 0.047 mg kg^{-1}, respectively. Only cypermethrin was detected in the samples collected from Bathinda and the range of residues was BDL-0.068 mg kg^{-1}. The cotton seed samples collected from Malout were found to contain residues of endosulfan, ethion, chlorpyriphos and cypermethrin with the mean residue levels being 0.006, 0.037, 0.010 and 0.033 mg kg^{-1}, respectively for the four insecticides. The samples collected from Mansa revealed the presence of ethion, chlorpyriphos and cypermethrin with the levels ranging from BDL-0.220, BDL-0.054 and BDL-0.059 mg kg^{-1}, respectively. One sample had chlorpyriphos residues above the MRL of 0.05 mg kg^{-1}. The cotton seed samples collected from Muktsar showed the presence of endosulfan, ethion and cypermethrin with the mean residue levels being 0.004, 0.041 and 0.049 mg kg^{-1}, respectively. One sample contained cypermethrin residues above the MRL of 0.2 mg kg^{-1} (Table 2).

These results revealed that 26 per cent of the 50 cotton seed samples analyzed were found to be contaminated with the residues of chlorpyriphos. Out of these, two samples had chlorpyriphos residues above the MRL of 0.05 mg kg^{-1}. Endosulfan was detected in 22 per cent of the samples but the residue levels were below the MRL of 1.0 mg kg^{-1}. 16 per cent of the samples were contaminated with ethion but the residue levels were found to be below the MRL of 0.5 mg kg^{-1}. Cypermethrin was found to be present in six samples with only one sample exceeding the MRL of 0.2 mg kg^{-1}(Fig. 1).

The presence of insecticide residues in cotton seed in both the picks could be due to the fact that the farmers continue the application of insecticides even after the first pick as against the recommendation to stop the sprays two weeks before the first pick (Anonymous 2003).

Table 1. Insecticide residues (mg kg^{-1}) in cotton seed samples collected at first pick from different locations.

Insecticide(s)	Location(s)				
	Abohar	Bathinda	Malout	Mansa	Muktsar
Organochlorines					
Endosulfan	BDL	BDL	0.007 (BDL-0.022)	0.012 (BDL- 0.037)	0.004 (BDL- 0.019)
Organophosphates					
Ethion	0.018 (BDL- 0.092)	BDL	BDL	BDL	0.049 (BDL- 0.246)
Chlorpyriphos	0.014 (BDL- 0.036)	0.019 (BDL- 0.081)	0.002 (BDL- 0.012)	0.003 (BDL- 0.017)	BDL

Table 2. Insecticide residues (mg kg^{-1}) in cotton seed samples collected at second pick from different locations.

Insecticide(s)	Location(s)				
	Abohar	Bathinda	Malout	Mansa	Muktsar
Organochlorines					
Endosulfan	0.013 (BDL- 0.043)	BDL	0.006 (BDL-0.019)	BDL	0.004 (BDL- 0.011)
Organophosphates					
Ethion	0.047 (BDL- 0.151)	BDL	0.037 (BDL- 0.185)	0.066 (BDL- 0.220)	0.041 (BDL- 0.207)
Chlorpyriphos	BDL	BDL	0.010 (BDL- 0.038)	0.024 (BDL- 0.054)	BDL
Synthetic pyrethroids					
Cypermethrin	BDL	0.014 (BDL- 0.068)	0.033 (BDL- 0.089)	0.023 (BDL- 0.059)	0.049 (BDL- 0.247)

BDL- Below Detectable Level

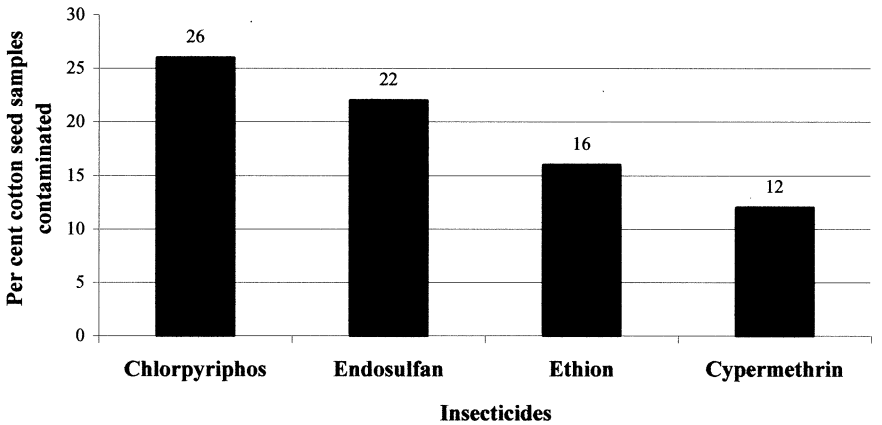

Figure 1. Percent contamination of cotton seed samples with different insecticides in Punjab.

The trend observed in the present studies for dissipation of various insecticides in cotton seed appears to be in conformity with the earlier work reported by various workers. Parveen et al. (1996) in Pakistan reported contamination in 73.6 per cent of the 250 samples of cotton seed analyzed with 24 different pesticides/metabolites. The results also indicated that out of 24 pesticides, 9 were organochlorines, 8 organophosphorous and 7 synthetic pyrethroid compounds. 41 per cent samples exceeded the prescribed maximum residue limits. The most frequently occurring pesticides were cyhalothrin, dimethoate, DDT and its metabolites, endosulfan and monocrotophos. Singh et al. (1990) had reported the residues of cypermethrin at 0.05 and 0.08 mg kg^{-1} in cotton seed samples collected at first picking following its application @ 50 and 100 g a.i. ha^{-1}. However, Raj et al. (1990) reported that the residues of cypermethrin applied @ 50 and 100 g a.i. ha^{-1} were 0.017 and 0.022 mg kg^{-1} in cotton seed. Endosulfan when applied @ 700 and 1400 g a.i. ha^{-1} left residues at 0.003 and 0.011 mg kg^{-1} in cotton seed collected at second picking.

The residues of endosulfan applied @ 700 and 1400 g a.i. ha^{-1} were found to be 0.04 and 0.05 mg kg^{-1} in cotton seed collected at first picking at the two dosages, respectively (Battu et al.1992). Chinniah et al. (1999) reported that the residues of chlorpyriphos applied @ 400 and 800 g a.i. ha^{-1} were 0.014 and 0.069 mg kg^{-1} in cotton seed from the two dosages, respectively. However, Singh et al. (2001) reported that the average residues of cypermethrin applied @ 50 and 100 g a.i. ha^{-1} and that of ethion applied @ 400, 800 and 1600 g a.i. ha^{-1} were below their detectable levels in cotton seed samples collected at first pick.

The occurrence of residues in cotton seed samples collected from the farmers' fields reflects the injudicious and overuse of insecticides by the cotton growers.

The results of these studies strongly warrant strict regulation of insecticide usage and proper education to the farmers to obtain cotton of acceptable quality from the consumer safety and international trade point of view and also safeguarding the environmental health.

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