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Pesticide Residues in Tobacco Leaves from the Kushtia District in Bangladesh

Mohammad Abdur Rahman · Alamgir Zaman Chowdhury · Mohammed Moniruzzaman · Siew Hua Gan · Mohammad Nazrul Islam · Zeenath Fardous · Mohammad Khorshed Alam

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Abstract In this study, tobacco leaf samples (n = 26) were collected from different areas in Kushtia to determine the presence of residues of cypermethrin, diazinon, heptachlor, methoxychlor dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethan (DDD) and dichlorodiphenyldichloroethylene (DDE). The analysis was conducted by a High Performance Liquid Chromatography (HPLC) system that was equipped with a photodiode array detector. Both cypermethrin and diazinon were detected in tobacco samples from six districts, namely, Mirzapur, Shahebnagar, Kodalipara, Pragpur, Farakpur and Taragunia. The highest concentration of cypermethrin was found in Kodalipara (2.00 ppm) while the highest concentration of diazinon was detected in a sample from Pragpur (0.15 ppm). The pesticide DDT was only detected in the sample from Pragpur at 4.00 ppm. This is the first study in Bangladesh that reports pesticide residue concentrations in tobacco leaf samples.

Keywords Cypermethrin · Diazinon · Pesticide · DDT · DDD · DDE · Tobacco · HPLC

M. A. Rahman · A. Z. Chowdhury · M. Moniruzzaman · M. N. Islam · Z. Fardous · M. K. Alam
Agrochemicals and Environmental Research Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Ganakbari, Savar, Dhaka 1349, Bangladesh

M. Moniruzzaman
Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

S. H. Gan (✉)
Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
e-mail: shgan@kck.usm.my

Bangladesh is predominantly an agricultural country, with over 70 % of its population dependent on farming and approximately 23 % of its gross domestic product derived from this sector (Akhter 2011). Together with jute, cotton, sugarcane, tea and betel leaf, tobacco has been listed as one of the six major cash crops in Bangladesh (Akhter 2011). Tobacco (*Nicotiana tabacum L.)* from the family *solanaceae* grows in drylands, is water demanding and consumes large quantities of fertilizers and pesticides.

Pesticide residues can remain on tobacco leaves after processing tobacco into its manufactured forms. Furthermore, and compared with other crops, tobacco leaves have a greater surface to weight ratio (Drossopoulos et al. 1998), and with excessive use of pesticides, pesticide residues may accumulate in the leaf tissue. When tobacco is smoked, the residues are burnt together with the tobacco and both active and passive smokers inhale the mixture. A study to determine the chlorinated pesticide residue levels in mainstream smoke reported that the transfer rate of pesticides from tobacco into smoke averaged approximately 12 % of that in tobacco before combustion (Clapp et al. 1972). Another study reported that dichlorodiphenyltrichloroethane (DDT) and its derivatives transfer into mainstream smoke at up to 20 % of the residue in unsmoked tobacco (NHMRC 1982). These findings indicate that pesticide residues in tobacco are likely to increase the known adverse effects of inhaling tobacco smoke.

Tobacco is mainly cultivated in different locations of Khagrachari, Bandarban, Chittagong Hill tracks and Kushtia. A study conducted by the Policy Research for Development Alternatives (UBINIG) in three different districts indicated that Kushtia has 53.33 % of the total farm holdings of tobacco, followed by Cox's Bazar (36.04 %) and Bandarban (10.41 %) (Akhter 2011). Tobacco is prone to many diseases and therefore requires chemical treatments with up to 16 applications of pesticides, including those that are banned for use, such as aldrin, dieldrin and DDT (Khan 2007). As a result, farmers have increased pressure to use different types of pesticides to produce in large quantities. Farmers who may not be aware of the health effects and proper safety protocols for handling pesticides can be exposed to their hazardous effects.

Pesticides and fertilizers usually end up in soil and are thus transferred to waterways and food sources. Early exposures to pesticides may increase a person's lifelong risk for cancer and harm the nervous and immune systems. Many of these compounds can cause moderate to severe respiratory and neurological damage or may act as genotoxic and carcinogenic agents (Hayat et al. 2011). Acute exposure to high levels of DDT by any route is reported to lead to neurological effects including excitability, headache, nausea, vomiting and dizziness (ATSDR 2002). The pesticide DDT is toxic not only to the nervous system but also to the liver (ATSDR 2002). Both DDT and its metabolites dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) are xenoestrogens, which influence both normal and neoplastic estrogen-responsive tissues (Muñoz-de-Toro et al. 2006). Accordingly, it has been hypothesized that OCC contribute to the risk of breast cancer.

To date, no data are available pertaining to the levels of pesticide residues in tobacco leaf samples that are cultivated throughout Bangladesh. These data could provide information about the status of the tobacco leaves used and the possible detrimental effects to human health should pesticides be present. In this study, we aim to determine the presence and quantity of some selected organochlorine pesticide residues (DDT and its metabolites DDD and DDE), organophosphate (diazinon) and pyrethroid (cypermethrin) in tobacco leaf samples.

Materials and Methods

Reference grade standards for cypermethrin (99.0 %), diazinon (99.0 %), dichlorodiphenyltrichloroethane (DDT) (99.0 %), dichlorodiphenyldichloroethane (DDD) (99.5 %), dichlorodiphenyldichloroethylene (DDE) (98.5 %), heptachlor (99.50 %) and methoxychlor (99.5 %) were purchased from GmbH (D-86199 Augsburg, Germany). The organic solvents were n-hexane (Merck, Germany) and dichloromethane (BDH, England) and were of analytical grade while acetonitrile (Scharlau, EU) was of HPLC grade.

Tobacco leaf samples (n = 26) were collected from the local area of Kushtia, Bangladesh between 2010 and 2011. The leaves were labeled prior to storage at 4°C and were extracted within 10 days. The tobacco leaves were dried and then blended using an electric blender (Model Classic 5 Speed, Crofion, China). The blended leaves were then weighed using an analytical balance (Crystal-200, GIBERTINI, Italy). Five grams of each blended tobacco leaf samples were transferred into a 250 mL Erlenmeyer flask and then 100 mL of residue grade acetone was added using a multi-Shaker (Max-4000, Guyson Corporation, USA) over 12 h at 150 rpm. The extracts were then concentrated to 5 mL on a rotary vacuum evaporator (Rotavapor-R 215, Buchi, Switzerland).

The samples were cleaned up by following the method described by (Fardous et al. 2007). Briefly, the acetone extract was subjected to clean up using florisil column chromatography. The florisil (60–100 mesh) was activated at 200°C for 6 h and was subsequently deactivated with 2 % distilled water. The top 1.5 cm of the florisil column was packed with anhydrous sodium sulfate. Elution was performed with a solvent mixture of double distilled hexane (65 %) and dichloromethane (35 %) at 5 mL/min. The elute was concentrated using a rotary vacuum evaporator and was transferred to a vial. Solvents were completely removed under a gentle flow of fresh nitrogen. The evaporated sample was dissolved in acetonitrile, and a 1 mL volume was used for the HPLC analysis. The procedure was repeated for all of the 26 extracted tobacco leaf samples.

After the sample clean up, aliquots of the final volume were quantified with a HPLC system (SHIMADZU LC-10 Avp-Series Automated with LC Solution Software LabSolutions (LC solution Release 1.11SP1) that was equipped with a SPD-M 10 Avp outfitted with a photodiode array (PDA) detector. A C18 Reverse Phase Alltech analytical column (5 µm, 250 × 4.6 mm) was used and maintained at 30°C in a column oven. The mobile phase, which was a combination of 70 % ACN and 30 % water, was filtered using a cellulose filter (0.45 µm) prior to use and was allowed to run at 1.2 mL/min. Prior to the HPLC analysis, the samples were passed through 0.45 µm nylon (Alltech Assoc) syringe filters and were manually injected (20 µl) into the HPLC system each time. The identification of the suspected pesticide was performed by comparing peak retention times in samples to those of peaks in the pure analytical standard. Quantification was performed using the method described by (Chowdhury et al. 2012). A typical chromatogram from the analysis is shown in Fig. 1. The calibration curves for cypermethrin, diazinon and DDT were prepared between 0.1 and 5.0 ppm in triplicates.

The percentage recoveries were calculated using the following equation:

Percentage of recovery = $[C_E/C_M \times 100]$, where C_E is the experimental concentration determined from the calibration curve and C_M is the spiked concentration.

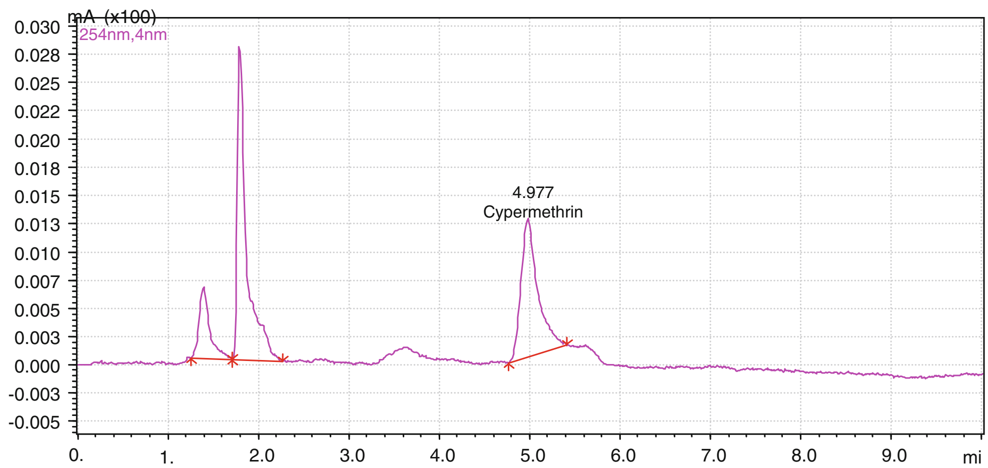

Fig. 1 A typical Chromatogram showing cypermethrin standard peak (RT = 4.97)

Table 1 Percentage recoveries of the analyzed pesticides

Analyte	Stock solution (ppm)	Spiking volume (µl)	Spiking amount in 5 g of leaf sample (µg)	Single spiking level (ppm)	Mean amount (µg)[a]		Mean recovery (%)
					Spiked	Measured	
Cypermethrin	200	25	5	5	5	4.25	85.0
Diazinon	200	25	5	5	5	4.60	92.0
DDT	200	25	5	5	5	4.50	90.0
DDD	200	25	5	5	5	4.35	87.0
DDE	200	25	5	5	5	4.21	84.2
Heptachlor	200	25	5	5	5	4.43	88.6
Methoxychlor	200	25	5	5	5	4.53	91.0

RT retention time

[a] mean values of triplicates

The mean percentage recoveries of cypermethrin, diazinon, DDT, DDD, DDE, heptachlor and methoxychlor in the spiked positive controls of tobacco leaf samples after the florisil clean up were greater than 80 %. This value complies with the FDA guideline (FDA 2001) (Tables 1, 2), indicating that the method is suitable for the analysis. The LOD and LOQs for the pesticides ranged from 0.01 to 0.30 ppm and 0.03 to 0.10 ppm, respectively.

Results and Discussion

This is the first study to report the presence and levels of pesticide residues in tobacco leaf samples from Bangladesh. Cypermethrin and diazinon were found in six tobacco samples from Mirzapur, Shahebnagar, Kodalipara, Pragpur, Farakpur and Taragunia. The highest concentration of cypermethrin was found in Kodalipara while the highest concentration of diazinon was detected in a sample from Pragpur. The amount of pesticide residue that is detected in tobacco leaves is an indication of the amount of pesticide applied, the time elapsed since the application and the speed with which the pesticides dissipated into the environment. Cypermethrin and diazinon were detected in six tobacco samples from Mirzapur, Shahebnagar, Kodalipara, Pragpur, Farakpur and Taragunia (Table 2). The highest concentration of diazinon was from Pragpur, at 2.00 ppm (Fig. 2), while the highest concentration of cypermethrin was from Kodalipara, at 0.12 ppm (Table 2). The pesticide DDT was only detected in one sample from Pragpur, at 4.00 ppm (Fig. 2).

The maximum levels of cypermethrin residues in tobacco leaves are neither available nor reported in other countries for comparison. In Italy and Spain, pesticide residues are generally stipulated not to exceed the limit of detection, which is generally between 0.01 and 0.05 ppm

Table 2 Levels of cypermethrin, diazinon, DDT, DDD, DDE, heptachlor and methoxychlor in tobacco leaf samples collected from different locations of Kushtia district, Bangladesh

Sample Code	Collection Area	Mean value[a] (ppm)						
		Cypermethrin	Diazinon	DDT	DDD	DDE	Hepta-chlor	Methoxy-chlor
BD 01	Bharamara	ND	ND	ND	ND	ND	ND	ND
BD 02	Dharampur	ND	ND	ND	ND	ND	ND	ND
BD 03	Amlasadarpr	ND	ND	ND	ND	ND	ND	ND
BD 04	Sholodag	ND	ND	ND	ND	ND	ND	ND
BD 05	Kholishakuni	ND	ND	ND	ND	ND	ND	ND
BD 06	Rifiatpur	ND	ND	ND	ND	ND	ND	ND
BD 07	Boholbaria	ND	ND	ND	ND	ND	ND	ND
BD 08	Bittipara	ND	ND	ND	ND	ND	ND	ND
BD 09	Bahadurpur	ND	ND	ND	ND	ND	ND	ND
BD 10	Mirzapur	**1.72**	**0.11**	ND	ND	ND	ND	ND
BD 11	Shahebnagar	**0.62**	**0.10**	ND	ND	ND	ND	ND
BD 12	Kodalipara	**2.0**	**0.12**	ND	ND	ND	ND	ND
BD 13	Daulatpur	ND	ND	ND	ND	ND	ND	ND
BD 14	Mothurapur	ND	ND	ND	ND	ND	ND	ND
BD 15	Pragpur	**1.45**	**0.15**	**4.0**	ND	ND	ND	ND
BD 16	Hosenabad	ND	ND	ND	ND	ND	ND	ND
BD 17	Boiragirchar	ND	ND	ND	ND	ND	ND	ND
BD 18	Philipnagarchar	ND	ND	ND	ND	ND	ND	ND
BD 19	Farakpur	**1.41**	**0.09**	ND	ND	ND	ND	ND
BD 20	Allardarga	ND	ND	ND	ND	ND	ND	ND
BD 21	Taragunia	**0.84**	**0.06**	ND	ND	ND	ND	ND
BD 22	Majdiarchar	ND	ND	ND	ND	ND	ND	ND
BD 23	Gongarampur	ND	ND	ND	ND	ND	ND	ND
BD 24	Kiamari	ND	ND	ND	ND	ND	ND	ND
BD 25	Kazipara	ND	ND	ND	ND	ND	ND	ND
BD 26	Dharmodah	ND	ND	ND	ND	ND	ND	ND

All samples are measured in triplicate

BD tobacco leaf sample, *ND* not detected

[a] Mean value of the triplicate measurements

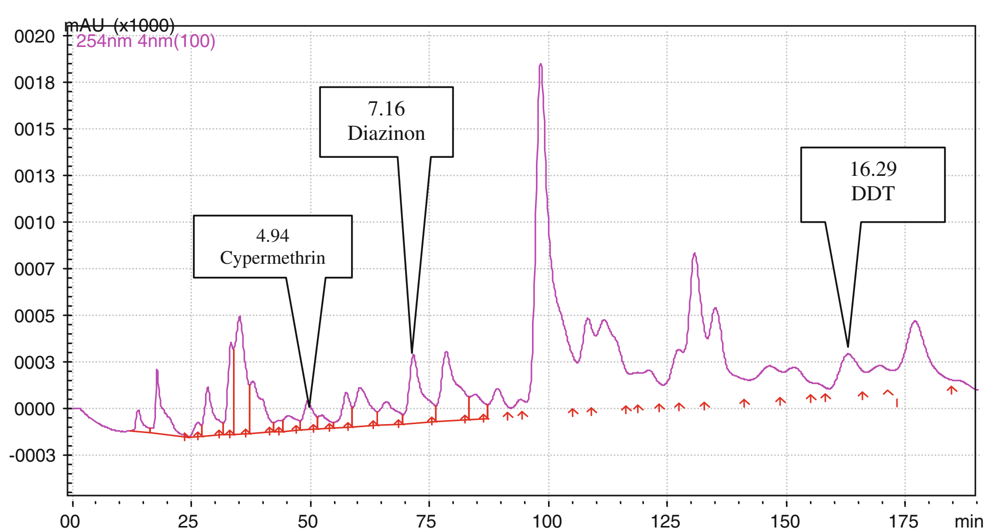

Fig. 2 A chromatogram for a tobacco leaf sample from Pragpur showing the presence of cypermethrin (RT = 4.94), diazinon (RT = 7.16) and DDT (RT = 16.29)

(GAO 2003). The levels of cypermethrin detected in our study, which were between 0.62 and 2.00 ppm, are higher than these recommended values. In Bangladesh, the types of pesticides that are legally allowed for use on tobacco are unknown. The US General Accounting Office reported that 17 out of the 73 pesticides that were commonly used on tobacco in the 1990s in US belong to three chemical classes (organochlorine, organophosphate and carbamates), and at high doses, these pesticides are known to cause adverse effects to human health, including death (GAO 2003). Diazinon is one of the pesticides that falls within this category. Even though the EPA has concluded that most of the 17 pesticides do not cause birth defects, the EPA has also concluded that five of these pesticides, and a by-product of another, may cause cancer (GAO 2003).

In the USA, diazinon is no longer approved for use on tobacco. However, diazinon was detected on six of the tobacco samples in this study, indicating that it is still used in Bangladesh. The highest level of diazinon was found in the sample collected from Kodalipara, at 0.15 ppm. However, this level is below the residue limits at 1.00 ppm for Germany, Italy and Spain (GAO 2003). There are no reported residue levels for diazinon in the US because its use has been discontinued. Even though the largest agricultural use of DDT has been reported for cotton and fish (Environment and Social Development Organization, ESDO; Bangladesh, November 2005), DDT may also be used in a tobacco leaf storehouses to control pests, and in such a way, DDT may contaminate tobacco leaves. In the 1980s, high quantities of organochlorines were used in both agriculture and public health before organochlorines were banned in 1993, exempting heptachlor (Matin et al. 1998). An estimated 100–300 tons of formulated organochlorines, particularly DDT and lindane, were used annually between 1990 and 1993 in Bangladesh to control malaria (Matin et al. 1998). A report by the (Environment and Social Development Organization, ESDO; Bangladesh, November 2005) states that some of the persistent organic pollutants, such as heptachlor, dieldrin, DDT and chlordane, are still in use in different parts of Bangladesh, where they are considered essential for ensuring agricultural production and public health.

The USA Dairy and Tobacco Adjustment Act set maximum allowable concentrations for 15 pesticide residues that are not approved for use in the USA. However, these pesticides are believed to be used in other countries, as allowed under the EPA's guidelines (EPA 1989). For example, the summed maximum allowable limit for residue in tobacco for DDT, tetrachlorodiphenylethane (TDT) and DDE (all of which are chemically related) is 0.4 ppm. In our study, DDT was detected in one sample from Pragpur at 4.0 ppm. This concentration is ten times above the stated combined limits for DDT. The chemical compound DDT is the only pesticide that is currently produced in Bangladesh (Environment and Social Development Organization, ESDO; Bangladesh, November 2005). Furthermore, Pragpur is located close to the border area between India and Bangladesh, which may facilitate the illegal entry of DDT by retailers for disease control and crop production purposes. The illegal trans-boundary entry of pesticides is a common phenomenon in Bangladesh (Environment and Social Development Organization (ESDO) Bangladesh, November 2005). The study also discovered some old stocks of DDT stored at different districts, namely, Rajshahi, Madaripur, Barisal, Pirojpur and Noakhali, totaling to 6,868 kg.

The indiscriminate and overuse of pesticides creates many problems. The use of pesticides has to be controlled to avoid the contamination of food supplies and the environment, but present measures in Bangladesh are inadequate and allow the sale of unregistered pesticides and misuse of pesticides by farmers (Matin et al. 1998). There are also concerns about prevalent pesticide use leading to the development of resistance in mosquitoes and flies, making the control of diseases such as malaria more difficult. For example, the excessive use of DDT and other pesticides has been responsible for the resurgence of malaria and possibly DDT resistant malaria in certain states (Chapin and Wasserstrom 1983). The DDT pesticide is highly toxic, persists in the environment and tends to accumulate in the body tissues of human and animals, especially in fatty tissues.

In this study, we did not detect any DDD, DDE, heptachlor or methoxychlor residue levels on the tobacco. Since DDE and DDD are both metabolites of DDT, they may be below the detection limit of our machine and may be present in amounts that are too small to be detected. No study has assessed the presence of pesticides in human milk samples in Bangladesh. However, the pesticides DDT, chordane and heptachlor have been used for long time in Bangladesh, and both rural and urban women have been exposed to each; thus, the milk samples of such exposed women are very likely to contain residues of these pesticides (Environment and Social Development Organization (ESDO) Bangladesh, November 2005). The presence of pesticides in milk can be further studied.

Even though the EPA regulates the specific pesticides that may be used in tobacco and the amounts to be applied in some countries, the EPA does not regulate pesticide residues that are approved for the use on tobacco. Even though the residue levels on tobacco typically decline as the leaves are harvested, dried and processed or burned (GAO 2003), varying residue levels may remain. A study reported that chlorinated pesticide residue such as DDT in Australian cigarettes ranged between 18.8 and 53.2 ppm while that in Finland ranged between 3.6 and 7.5 ppm (Morris 1972).

The maximum standard for DDT is 7.5 ppm (CEC 1976). Further research should be conducted to determine the levels of potentially harmful pesticide residues in Bangladesh cigarettes, as these findings will reflect the actual harm that both active and passive smokers are exposed to. The EPA recommends studies to be conducted even if the residues are below 0.1 ppm due to concerns about the toxicity of pesticides. Furthermore, little is known directly about the chronic effects of pesticides. The chronic effects of pesticides on human health are a broad area for further study.

This is the first study to report the levels of pesticide residues in tobacco leaf samples in Bangladesh. Cypermethrin and diazinon were found in six tobacco samples from Mirzapur, Shahebnagar, Kodalipara, Pragpur, Farakpur and Taragunia, indicating that these pesticides are still used on tobacco. The levels of cypermethrin are much higher than those reported in some countries, such as Italy and Spain. However, the levels of diazinon are below the maximum levels allowed in other countries. The pesticide DDT was detected in Pragpur at ten times the recommended level. The Bangladesh government should monitor the use of pesticides in tobacco fields.

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