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Determination of Cypermethrin, Chlorpyrifos and Diazinon Residues in Tomato and Reduction of Cypermethrin Residues in Tomato Using Rice Bran

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Abstract The present study was an attempt to address the health hazard which posed by the ingestation of pesticide residues contained in tomato. Fourteen tomato samples were randomly selected from different shops of local markets of Savar Upazila, Bangladesh and analyzed to identify the level of widely applied cypermethrin, chlorpyrifos and diazinon residues by using high performance liquid chromatography (HPLC) technique. It was found that out of 14 samples, five were found contaminated with cypermethrin and one with chlorpyrifos, but none residue of diazinon was detected in any of the tested samples. Only 7% of the samples were found contaminated with cypermethrin insecticide residue which was above the maximum residue level (MRL) adopted by the FAO/WHO Codex Alimentarius Commission (CAC), although cypermethrin (0.065 ± 0.07mg/kg) and chlorpyrifos (0.024 ± 0.041mg/kg) residues were detected in samples. Rice bran rubbing, a cheap decontamination technique, was used to treat applied cypermethrin residues. 0.45 mg/kg of cypermethrin spiked tomato samples were rubbed by rice bran paste for 5, 10 and 15min. Two experiments were conducted for assessing the efficiency of the adsorbent. Rubbing with rice bran paste for 10min, removed 97.73% and 97.4% in both trails, and whereas cypermethrin residue was not detected in tomato samples treated for 15 min. The removal efficiency was 97.56 ± 0.22% with the adsorbent rice bran rubbing for 10min. The result showed that cypermethrin residues of tomato sample could easily be decontaminated through absorption of rice bran.

Keywords: *solanaceae, lycopersicum esculentum, pesticides, contamination, HPLC*

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae and is one of the most popular in healthy diets, fulfilling the nutritional requirements of Bangladesh´s consumers [1]. It is one of the most consumable vegetables in winter season [2] which contains a substantial quantity of vitamins A and C as well has a medicinal value [3]. In Bangladesh, tomato cultivation covers a 6.10% area both in winter and summer seasons due to its adaptability to a wide range of soil and climate [4]. Due to its high profit, farmers have become more interested to cultivate tomato in recent years. Tomato is consumed as cooked and uncooked manners [5].

Tomato production has enhanced the livelihood standard of rural farmers due to its good production yield, commercial use and high rate of consumption [6]. This vegetable is affected by many pests due to favorable weather conditions during winter season. Farmers applied excessive amounts of different types of pesticides to protect their crops. Cypermethrin, is one of the most common pesticides, which is used on tomato and other vegetables frequently. Although it is a profitable and delicious vegetable, it is highly susceptible to pests and farmers have no other option except application of pesticides to protect this crop [7].

Pesticides, mainly DDT and BHC, were introduced in Bangladesh in 1957 and distributed by the Government to the farmers. Later the Government of Bangladesh promoted the use of pesticides to boost the agricultural production to ensure food security [8]. Currently, 84 pesticides with 242 trade names are registered in this country [9]. Organophosphates (i.e. chlorpyrifos, diazinon, etc.), pyrethroids (i.e. cypermethrin, fenvalerate, etc.) and carbamates (i.e. carbofuran, aldicarb, carbaryl, etc.) pesticides are widely applied in vegetables including tomatoes [7].

Pesticides are sprayed directly on the plants, which are able to persistent for a long time in vegetables [10]. Selling of vegetables after 1-2 days of spraying pesticides is a normal practice in most of the areas of Bangladesh [7]. Consumers are exposed to harmful chemicals by ingesting directly raw or half-cooked contaminated tomatoes. There are several studies which addressed the harmful effects of pesticides in humans. Pesticides´ residues react with the enzyme acetyl cholinesterase, which causes cancer,

teratogenesis, genetic damage and suppression of the immune system [11]. Also, children become more susceptible to pesticides because they have less developed the immune system and higher rates of metabolism [9]. Red eyes, headache and chronic cough are common health problems of farmers of Bangladesh, who are exposed to pesticides during their work [12,13]. The indiscriminated and overuse of harmful pesticides have contaminated the water system and ultimately entered into the food chain which cause severe damage to humans health, fishes and many other animals, conducting to death [14].

The United Nation's Food and Agriculture Organization (FAO), and the World Health Organization (WHO) have established the Codex Alimentarius Commission (CAC) to monitor and to set up Minimal Residue Limits (MRL) for pesticides in different vegetables. There are limited studies and initiatives taken to assess MRL at local markets in developing countries [11]. Furthermore, lack of awareness, literacy and poverty expose the general population to pesticides exposure.

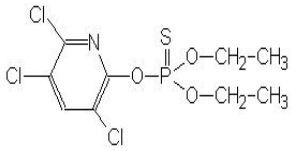
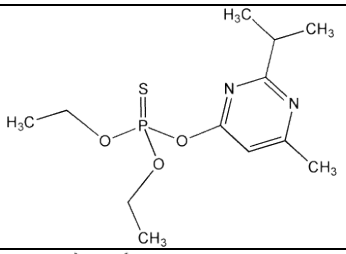
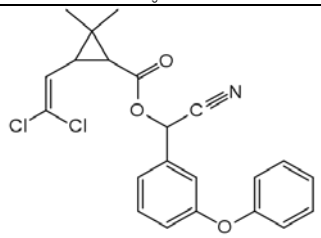
A very limited work has been done about pesticides residues in tomatoes and related studies about the decontamination of pesticides residues in tomatoes are scarce. It is necessary to have cheap and effective decontamination techniques and methods to reduce pesticides´ contamination in vegetable samples for developing countries. Rice bran is a byproduct obtained by polishing brown rice, which is cheap, adsorbent and available all over the world [15]. It is necessary to know about the present scenario of pesticides´ residues in tomatoes, which will help to improve the environment quality and to minimize the potential health risk. This study is an attempt to provide information about pesticides residues in tomatoes and about the decontamination of cypermethrin in them by using rice bran paste.

2. Materials and Methods

2.1. Pesticides and Reagents

Reference grade chlorpyrifos, diazinon and cypermethrin (Table 1), were obtained from Dr. Ehrenstorfer GmbH, D-86199 Augsburg, Germany. The reagents florisil (Magnesium silicate, Sigma, USA, mesh 60-100, Active at 1200ºF), n-hexane, Merck, Germany) and dichloromethane (Liquid chromatography grade, Merck, Germany), were procured and distilled before use.

Table 1. Pesticides used and minimal residue limits allowed in tomato

Common name of pesticides	Chemical name	Structural formula	Empirical formula	MRL (ppm) in tomato [16,17,18]
Chlorpyrifos	O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate		$C_9H_{11}C_{B}NO_3PS$	0.5
Diazinon	O, O-diethylO-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl) phosphorothioate.		$C_{12}H_{21}N_2O_3PS$	0.5
Cypermethrin	RS9-alpha-cyano-3-phenoxybenzyl (1RS) cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylate		$C_{22}H_{19}Cl_2NO_3$	0.5

MRL = maximum residue level

2.2. Sample Collection, Preservation and Pre-Extraction Processing

Tomato samples were obtained from 14 different vendors of local markets of Savar Upazila under Dhaka district, Bangladesh. Separated plastic bags in icebox were used during the sampling collection and given identification (I.D.) (TS-1 to TS-14). The samples were transferred to the laboratory as early as possible for analyzing and then refrigerated for further processing. Samples of each group were taken out from the refrigerator and normalized to ambient temperature, which is prior to extraction then chopped into small pieces and mixed well.

2.3. Preparation of Rice Bran Paste and Tomato Samples Spiked with Cypermethrin

Rice bran and tomato samples were collected from local markets. Two hundred grams (dry weight basis) of rice bran were mixed with 200 mL of distilled water to process the rice bran paste according to Adachi and Okano´s method [15]. Four tomato samples, 50g each, were taken for the experiment. Tomato samples were spiked uniformly with cypermethrin (0.45mg/kg). Spiked tomato samples were rubbed with rice bran paste between 15 and 30 min. Samples spiked with pesticides were divided into four groups: (I) rubbed with no rice bran (control); (II)

rubbed with rice bran for 5 min; (III) rubbed with rice bran for 10 min; (IV) rubbed with rice bran for 15 min.

2.4. Processing of Tomato Samples by Shaker Extraction and Clean up

Fifty grams portion of homogenized tomato samples of each group was weighted (KEEN ABS 80-4, Germany) separately for subsequent experiment. The representative tomato samples were blended using a mortar-pestle, taken in a conical flask and extracted with 150mL solvent (135mL double distilled n-hexane and 15mL dichloromethane) using a shaker (MAX, 4000, Guyson Corporation, USA) for 16h at 150rpm. It was then kept standing for 10-15min for settle it down and the combined n-hexane and dichloromethane extractor was treated with 10g of anhydrous sodium sulfate to remove traces of water. Then the upper solvent layer was poured in another rounded bottom flask. The collected extract was then concentrated under reduced pressure using a rotary vacuum evaporator (Rotavapor, R-215, BUCHI, Switzerland) and rinsed with double distilled n-hexane. The extract was passed through a column (10mm ID) with florisil (60-100 mesh). This extract was finally eluted with 100mL of dichloromethane 10% in double distilled n-hexane and again concentrated with the rotary vacuum evaporator to 2mL for high performance liquid chromatography (HPLC) analysis.

2.5. Sample Analyzing Instrument and Chromatographic Condition

A HPLC (SHIMADZU, Japan) mode equipped with SPD-M 10 Avp, SCL-10 Avp, CTO-10 Acvp, LC-10 Advp, DGU-14A; C_{18} Reverse Phase Alltech analytical column (4.6 × 250mm) was used to analyze the tomato samples. The cleaned up tomato extracts, prior to analyses by HPLC, were passed through 0.45µm nylon (Alltech Assoc) syringe filters. The details of HPLC analytical conditions are given in Table 2.

2.6. Identification and Quantification Procedures

Retention time (RT) of the pure analytical pesticide standard was used to identify suspected pesticide in tomato samples. The retention feature was changed a little bit for the identification and a 0.05% difference was acceptable [19].

The quantification of the identified pesticides was performed with a freshly prepared standard calibration curve of the relevant (standard) pesticide [20].

2.7. Statistical Analysis

The SPSS software 16.0 was used to calculate mean, standard deviation and 95% confidence interval. To calculate the pesticide residues removal, the following formula was used [15].

$$\textbf{Residualpesticide}(\%) = \frac{\textbf{Initial concentration} - \textbf{Final concentration}}{\textbf{Finalconcentration}} \times 100$$

Table 2. Details of the parameters of HPLC for specific pesticide residues analysis

Parameters	Chlorpyrifos	Cypermethrin	Diazinon
Mobile phase (%)	Acetonitrile 70, distilled water 30	Acetonitrile 70, distilled water 30	Acetonitrile 70, distilled water 30
Detector type	Photo diode array	Photo diode array	Photo diode array
Injection technique	Manual by micro syringe	Manual by micro syringe	Manual by micro syringe
Injection volume	20µL	20µL	20µL
Oven temperature	30°C	30°C	30°C
Pump pressure	112Kgf/min	112Kgf/min	112Kgf/min
Total flow	1mL/min	1mL/min	1mL/min
Run time	10 min	10 min	15 min
Retention time	6.60	5.37 min	9.52
Column (Type)	C_{18} (Nova Pack)	C_{18} (Nova Pack)	C_{18} (Nova Pack)
Absorbance	254nm	210nm	254nm

3. Results and Discussion

3.1. Estimation of Cypermethrin, Chlorpyrifos and Diazinon Residues in Local Tomato Samples

The results showed that the tomato samples collected from the local markets of Savar were contaminated with cypermethrin and chlorpyrifos pesticide residues (Table 3).

Table 3. Concentration of pesticide residues found in tomato samples from the local markets of Savar

Sample number	Sample code	Identified cypermethrin concentration (mg/kg)	Identified chlorpyrifos concentration (mg/kg)	Identified diazinon concentration (mg/kg)
1	TS–1	BDL*	BDL	BDL
2	TS–2	BDL	BDL	BDL
3	TS–3	0.27	BDL	BDL
4	TS–4	0.02	BDL	BDL
5	TS–5	BDL	BDL	BDL
6	TS–6	0.55	BDL	BDL
7	TS–7	0.04	BDL	BDL
8	TS–8	0.03	BDL	BDL
9	TS–9	BDL	BDL	BDL
10	TS–10	BDL	BDL	BDL
11	TS–11	BDL	0.34	BDL
12	TS–12	BDL	BDL	BDL
13	TS–13	BDL	BDL	BDL
14	TS–14	BDL	BDL	BDL
Mean		0.065	0.024	0.00
Standard Deviation		0.15	0.087	0.00
95% Confidence Interval		0.078	0.041	0.00

*BDL = Below the detection limits which stand 0.0 for mean calculation. Detection limit - 0.001mg/kg.

It was found that out of 14 tomato samples five were contaminated with cypermethrin residues ranging from 0.002 to 0.55mg/kg, and one sample was contaminated with chlorpyrifos (0.34mg/kg). No sample was found contaminated with diazinon. Average quantities of cypermethrin (0.065 ± 0.07mg/kg) and chlorpyrifos (0.024 ± 0.041mg/kg) were detected in 95% confidence level in the 14 tomato samples. Fifty seven percent of tomato samples were not found contaminated with Cypermethrin, chlorpyrifos or diazinon residues (Figure 1). The presence of chlorpyrifos and cypermethrin was detected in 42.86% tomato samples. Among the contaminated samples, only one was found exceeding the MRL value (0.5mg/kg) established by FAO and WHO [18]. The chromatogram of the standard cypermethrin and presence of this pesticide in tomato samples are showed in Figure 2a and b, respectively. Multiple pesticide residues were not detected in the samples.

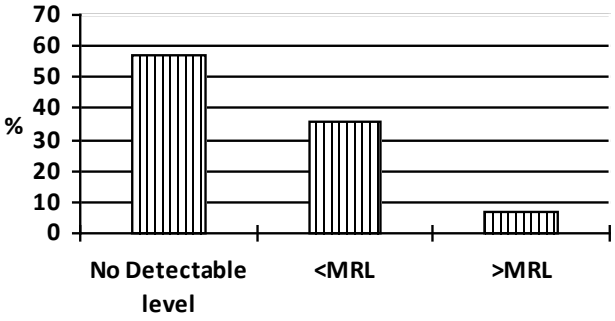


Figure 1. Percentage of contaminated tomato samples

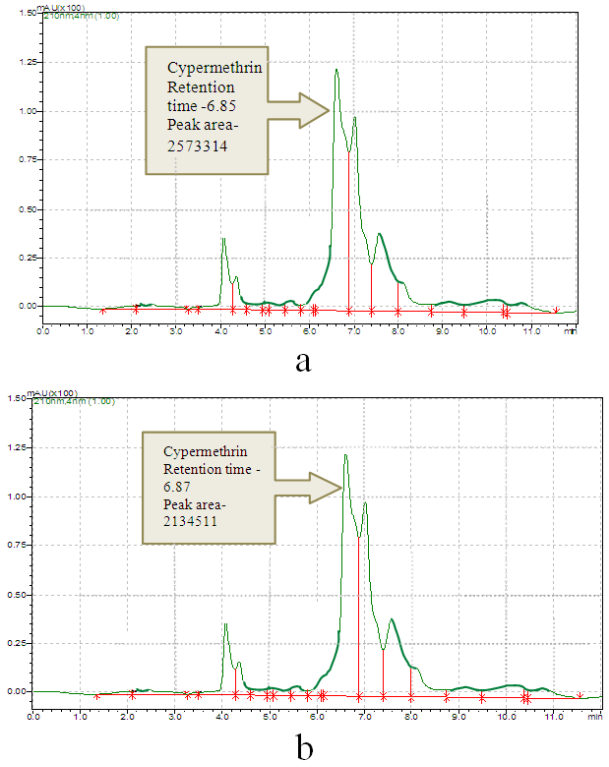


Figure 2. a) Chromatogram of the standard cypermethrin, **b)** Chromatogram of cypermethrin pesticide residue in tomato samples

Tomatoes are usually eaten as uncooked. Pesticides are chemicals that can have health effects to its consumers. The Codex Alimentarius Commission (CAC) of the United Nation's Food and Agriculture Organization and World Health Organization has settled MRL for pesticide residues in different vegetables including tomatoes. In Bangladesh, there are no rules to limit or to ensure the MRL in vegetables for local buyers. This standard limit is strictly followed and leveled well in the vegetables container boxes, exported to foreign countries [11]. This investigation was carried out to know the presence of pesticide residues in tomato samples. As a result, there was only one sample that exceeded the MRL of cypermethrin and most of the tomato samples were not able to cause serious health hazard.

The findings of this study are relevant to a previous investigation carried out in Lahore [5], in which cypermethrin along with other pesticides were analyzed in tomato samples. None of the pesticide residues exceeded the MRL. Similarly, in another investigation carried out in Bangladesh to assess pesticide residues in tomato samples, it was concluded that few of the samples were found contaminated with pesticide residues, whose values were above the MRL [21,22].

Residues of pesticides in food samples of Nepal were studied between 1995 and 2004. One thousand and thirty-four samples of different food commodities were analyzed. Both organophosphate and organochlorine pesticides were detected. Twelve percent of the samples were found contaminated with the organophosphate pesticide; few exceeded the accepted MRL [14]. Fenoll et al. [23] concluded that multiclass pesticide residues were found in pepper and tomato samples in Spain. Concentrations of 0.1mg/kg, 0.2mg/kg and 0.34mg/kg of diazinon, chlorpyrifos and cypermethrin, respectively, were identified in tomato samples. Similarly, multiclass pesticide residues were analyzed and identified in tomato samples in India, finding that some of the samples exceeded the MRL [24,25].

3.2. Reduction of Cypermethrin Residues in Tomato using Rice

Two trails were performed to calculate the change of the concentration of the pesticide residues. The chromatogram of the study revealed the decline in concentration of cypermethrin, which was adsorbed by the of rice bran paste. In both trials the removal efficiency of cypermethrin ranged between 62.23% and 68.11% for 5 min, whereas a range of 97.73% and 97.4% of removal efficiency was observed for 10min rubbing in the first and second trail, respectively (Table 4). A linear relationship was observed between the rubbing time and depletion concentration (Figure 3). The removal decreased rapidly after rubbing with rice paste and no cypermethrin residues were detected after 15min.

Cypermethrin is a synthetic pyrethroid, which has lipophilicity capacity [26]. Pesticide residues are present on the surface of the samples of tomatoes and hence it is very easy to remove by simple washing or rubbing by solid matrix. The removal of chemicals is dependent on the uptake from the intercellular particles called spherosomes [27]. These are found among fungi and plants [28]. Rice bran is the byproduct of polished brown rice. It has small surface area, which works as spherosomes. Thus, it acts as a cheap adsorbent and available all over the world [15].

Table 4. Cypermethrin treated with the rice bran paste for various time intervals

Minute	Cypermethrin concentration (1st trail)		Removal efficiency (%)	Cypermethrin concentration (2nd trail)		Removal efficiency (%)	Average removal efficiency (%)
	Before treatment (mg/kg)	After treatment (mg/kg)		Before treatment (mg/kg)	After treatment (mg/kg)		
5	0.45	0.1712	62.23	0.45	0.1435	68.11	65.17±5.76
10	0.45	0.0102	97.73	0.45	0.0117	97.4	97.56±0.22
15	0.45	BDL	-	0.45	BDL	-	-

Note: BDL - Below detection level, which is 0.001mg/kg.

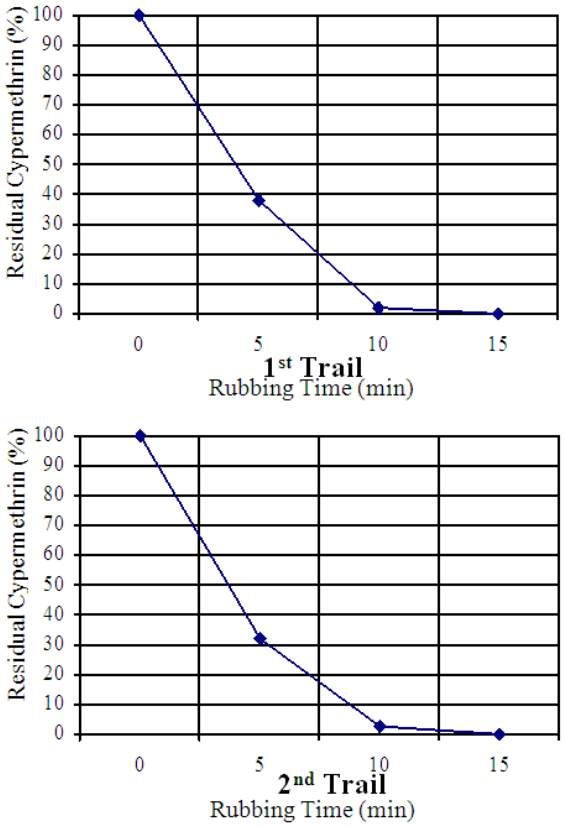


Figure 3. Removal efficiency of cypermethrin by rice bran paste in both trials

The present investigation demonstrated that the rice bran paste is a good adsorbent and able to cause decontamination of tomato samples. The findings of the study are relevant to a previous investigation [15], in which chlorothalonil and tetradifon were successfully removed by rice bran past. Over 85% removal efficiency was observed for both pesticides [15]. Adachi et al. [26] observed almost 88% removal of cypermethrin residue by rice bran paste. The investigation addressed that this adsorbent has good efficiency to remove various pesticides residues. The mechanism of removal through intercellular place of rice bran was identified by them.

The concentration of pretilachlor and esprocarb also were reduced by rubbing the rice bran paste in vegetable samples [29]. The results showed that the rice bran was an efficient method for treatment of acute pesticide poisoning in vegetables samples. Similar studies were conducted by Kesarwani et al. [30], to decontaminate vegetables from pesticide residues and observed the good adsorbent properties of rice bran. In Iran, a similar adsorbent was used to remove surfactant through the mechanism of physicochemical [31].

4. Conclusion

The present study was an attempt to identify and to quantify the pesticide residues in tomato samples of local markets at Savar, Bangladesh. The study demonstrated that most of the tomato samples of local markets of Savar not contained residues of the monitored pesticides above the MRL, although some residues were detected in certain samples only. Cypermethrin was detected widely in comparison to organophosphate pesticides. Contaminated tomato samples excluding one sample was contaminated with chlorpyrifos and cypermethrin in low concentrations, which do not pose serious health risk.

The results confirmed that the applied pesticides remain present on the surface of tomatoes for a certain time. Rice bran paste was a good adsorbent. The findings revealed that this adsorbent removed 97% of cypermethrin residue by rubbing with rice bran paste for 10min. Therefore, processing tomatoes with rice bran before reaching the consumers could reduce the health hazards of pesticide residues.

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