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Effect of post-harvest storage and house hold processing on reduction of human health risk index of pesticide in tomato

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ABSTRACT

Evaluation of post-harvest storage time with different household processes like washing and boiling/cooking on residual levels of cypermethrin, chlorpyrifos and diazinon in tomato was carried out. Three plots of tomato crop were sprayed separately with cypermethrin (10 EC; 20 ml/acre), chlorpyrifos (20 EC; 120 ml/acre) and diazinon (60 EC; 412.8 ml/acre) at a recommended dose. After treatment, the post- harvest tomato samples were subjected to house hold processings (washing and boiling/cooking) at zero day and 3rd day. Pesticide residues were extracted from tomato by solvent partitioning and cleaned up through florisil column chromatography. Cleaned samples were analyzed by HPLC equipped with PDA detector. Both washing and cooking reduced human risk index value ~12%-69% for chlorpyrifos, ~9-43% for cypermethrin and ~47-90% for diazinon on zero days. During 3 day of storage, washing and cooking reduced human risk index value ~26%-84% for chlorpyrifos, ~11-53% for cypermethrin and ~46-95% for diazinon. Moreover, boiling was found more effective than washing in reducing residual levels of pesticides.

KEYWORDS

Pesticide;
Human health risk index;
Household processing;
Post-harvest storage;
HPLC.

INTRODUCTION

Bangladesh is a densely populated agricultural economy based country where agriculture contributes to 23.50% of the gross domestic product and more than 80% of the people depend on agriculture for their livelihood[7]. Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solnaceae and is the most consumable vegetable crop. For better yield and quality, insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage by the farmers/growers[20]. Establishment of food security, in Bangladesh, eager to adopt effective technologies like irrigation, cultivation of high yielding hybrid varieties along with use of chemical fertilizers and pesticides[15]. Unfortunately, indiscriminate use of pesticide by farmers is evidenced due to illiteracy and unconsciousness about the health hazardous effect of pesticides[9]. Indiscriminate use of pesticide cause several problems like toxic residues in fruit and vegetables, insecticide resistance, killing of natural enemies and ultimately pest resurgence. On the other hand, pesticide residues in fruits and vegetables are a major global public health concern due to their negative health effects. In that prospect fresh food item like unprocessed vegetable salads or fruits indeed demand major focus[22]. According to WHO, the rate of death from unintentional chemical poisoning in Bangladesh is 50-100 per million that seems to results from exposure to agricultural toxic chemicals like pesticides[25].

Pesticides lead to headache nausea and hypersensitivity upon acute exposure while chronic exposure increase the risk of reproductive defects, mutagenic and carcinogenic transformation as well as endocrine disruption[2,5]. Immature immune system makes children as more vulnerable group to pesticide poisoning[14]. Viciously, cypermethrin, a synthetic pyrethroid, induce dopaminergic neurodegeneration in chronic postnatal as well as adulthood exposure and developmental delay of fetus upon exposed during pregnancy besides generalized health hazardous effects[6,21]. However, the excessive use of cypermethrin has been documented previously in Bangladesh[3]. In trace amounts, chlorpyrifos has been reported to cause neurological disorders such as attention deficit hyperactivity disorder and a developmental disorder both in fetuses and children[18]. Moreover, these are being used in agricultural crops without maintenance of any pre-harvest time regulation due to availability and consumer value.

Precise evaluation of residual levels of pesticides in food item allows to asses whether pesticide contaminated food items are truly healthy or health hazardous. Generally pesticide residues in agricultural produce can be reduced by several household processing such as washing, cooking etc.[12]. Recurring study attended to report the effect of such household processing in lowering pesticides residual levels world wide. Therefore, the present study was carried out to evaluate the effect of post-harvest storage time in concert with different household processes like washing and cooking on residual levels of chlorpyrifos, cypermethrin and diazinon in tomato.

MATERIALS AND METHODS

Pesticide reference standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and were provided in sealed vials. Florisil (magnesium silicate, 60-100 mesh) was purchased from Sigma, USA. This study was conducted in agricultural field of Pandua under Savar upazila of Dhaka district. Experimental samples were prepared by spraying recommended dose of cypermethrin (10 EC; 20 ml/acre), chlorpyrifos (20 EC; 120 ml/acre) and diazinon (60 EC; 412.8 ml/acre) in three separate plots of tomato as described in TABLE 1.

TABLE 1 : Recommended dose of pesticides per acre, their solubility and MRL values in tomato

Pesticides	Formulation	Active ingredient	Solubility in water at 20 ^{0}C	MRL (mg /kg)
Chlorpyrifos 20 EC	600 ml/acre	120 ml/acre	2 mg/l	0.5
Cypermethrin 10EC	200 ml/acre	20 ml/acre	0.01-0.2 mg/l	0.5
Diazinon 60 EC	688 ml/acre	412.8 ml/acre	40 mg/l	0.01

MRL-Maximum residue level as determined by EC regulation 396/ 2005

Tomato in one plot was remained untreated serving as control. After about 4 hours of spraying, tomato samples were harvested and packed in polyethylene bags and brought to the laboratory for further processing. One group of samples processed with household treatments immediately was considered as zero day samples and the other group was processed after storage of three days. Washing and boiling/cooking were selected as household treatments. During washing, three phases washing at room temperature was carried out with running or standing water at 10 seconds interval (10, 20, 30 seconds). The samples were cooked for three phases with 5 minutes interval (5, 10, 15 minutes) using 100 g sample in water.

Twenty (20) g sample was macerated with 50 ml of ethyl acetate, hexane and acetone (3:1:1). Sodium hydrogen carbonate (5.0 g) and anhydrous sodium sulfate (20.0 g) were added to neutralize pH and to remove water respectively before the addition of 0.05 - 0.10 g AAC for the removal of soluble plant pigments.

The mixture was further macerated at full speed for 3 min using an Ultra-Turrax macerator (IKA-labortechnik, Janke & Kunkel GmBH & Co, Kg, Germany). The samples were then centrifuged for 5 min at 3000 rpm, and the supernatant was transferred to a clean graduated cylinder for volume measurement. The organic extract was concentrated to 5 ml using a vacuum rotary evaporator (Rotavapor-R 215, Buchi, Switzerland) at 250 mbar with a water bath at 45°C. The extraction process was followed by a clean-up step using column chromatography with Florisil (60-100 mesh, Supelco, analytical grade) to remove any residual components that may interfere with the high performance liquid chromatography (HPLC) analysis.

The concentrated extract was transferred to a glass column (1.5 cm i.d. X 40 cm length) containing a piece of glass wool, anhydrous sodium sulfate (5.0 g) and Florisil (10.0 g). The analytes were eluted using 20 ml of a mixture of ethyl acetate:diethyl ether (7:1) followed by 20 ml of hexane:acetone (3:1) and finally 20 ml of acetonitrile:toluene (3:1). The combined extracts were concentrated to 1 ml using a rotary evaporator at 40°C before evaporation to dryness under a stream of nitrogen gas. Finally, the extract was reconstituted to 1 ml with acetonitrile. The samples were filtered through a 0.45 μm nylon membrane into a vial before injection into the HPLC system.

Quality control and quality assurance were incorporated into the analysis. The analytical method was standardized by processing spiked samples. The accuracy and precision were also validated in accordance with the European Commission (EC) guidelines (SANCO/2007/3131). The precision was expressed as the relative standard deviation (RSD). Accuracy can be measured by analyzing samples with known concentrations and comparing the measured values with the actual spiked values. For the recovery experiments, pesticide-free tomato samples (20 g) from control plot were spiked, in three replicates, after homogenization by the addition of the appropriate volumes of standards of chlorpyrifos, cypermethrin and diazinon) at three different levels (0.05, 0.50 and 5.0 μg/ml). Control samples were processed along with spiked ones. The mixture was left standing for 1 h to allow equilibration. The processes of extraction, cleanup of pesticide residues were same as described above. Average per cent recoveries were ranged from 86 to 92 for chlorpyrifos, 80 to 85 for cypermethrin and 89 to 97 for diazinon with precisions ranging from 2.25 to 12.12% (TABLE 2).

Analysis was done by high performance liquid chromatography (Shimadzu, Japan) LC-10 ADvp, equipped with an SPD-M 10 Avp attached to a photodiode array detector (Shimadzu SPD-M 10 Avp, 200–800 nm). C18 Reverse Phase Alltech (250 × 4.6 mm, 5 μm) was used as analytical column and column temperature was maintained at 30°C. 70% Acetonitrile in distilled water was used as mobile phase at a flow rate of 1.0 ml/min. Prior to HPLC analysis, the samples and mobile phase were filtered through 0.45 μm of nylon (Alltech Associates, IL, USA) syringe filters. The chromatographs were obtained by a manual injection of 20 μl samples or standards. Suspected pesticide was identified by comparison with the retention time of the pure analytical standard. For preparation of calibration curve, equal volumes of differently concentrated standard solutions of chlorpyrifos, cypermethrin and diazinon were injected into HPLC. The variation of retention featured is within acceptable range of ±0.05%. Quantification was based on the calibration technique[8]. To determine the residual levels of pesticides, the following calculating equation was used:

$$\mathbf{R = (H_A \times V_{END} \times W_{ST}) / (H_{ST} \times V_I \times G)}$$

Here, R = mg/Kg for vegetable samples; G = Sample weight (Kg); V_{END} = Terminal volume of the sample solution (ml); V_I = Portion of volume V_{END} injected into HPLC (μl) column; W_{ST} = Amount of standard pesticides injected with standard solvent (μg); H_A = Peak

TABLE 2 : Performance characteristics of the analytical method employed for pesticide residues analysis in tomato at three spiking levels

Analyte	Method	% Recovery (n=3) (Repeatability / %RSD)		
		Spiking at 0.05 mg /kg	Spiking at 0.5 mg /kg	Spiking at 5.0 mg /kg
Chlorpyrifos	HPLC-PDA	86 (2.25)	88 (10.7)	92 (7.42)
Cypermethrin		80 (5.33)	85 (7.45)	82 (11.34)
Diazinon		97 (12.12)	93 (5.68)	89 (9.6)

n= number of replicates, Values are average of triplicate analysis

area obtained from V_I (mm^2); H_{ST} = Peak area obtained from W_{ST} (mm^2).

Health risk indices of the residues were computed using the obtained and food consumption. Health risk index was calculated using following equation[10].

Health risk index (HRI) = (Estimated daily intake / Acceptable daily intake)

Estimated Daily Intake was found by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (kg/day) and dividing by a body weight of 60 kg for adult population.

The experimental results were expressed as mean. All parameters for inter-group differences were analyzed by repeated student's *t*-test. The statistical program SPSS 16.0 was used for these analyses. A level of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The residual level of chlorpyrifos and associated human risk index (HRI) as well as the percent (%) of reduction of residual pesticide and human risk due to household processing is summarized in TABLE 3. On zero day chlorpyrifos level in tomato sample was determined 3.26 mg/kg with a HRI value of 0.22. Washing and boiling/cooking causes a time dependant reduction of residual pesticide. Human risk index value was also reduced significantly (p<0.05) by washing and boiling/cooking treatment in a time dependant manner with highest % of HRI reduction at 30 second washing (20.91%) and 15 minutes of boiling/cooking (69.05%). On 3rd day of pesticide treatment, washing and boiling/cooking treatment causes a gradual time dependent reduction of residual pesticide. Human risk index value was also reduced significantly (p<0.05) by washing and boiling/cooking treatment in a time dependant manner with highest % of HRI reduction at 30 second washing (63.16%) and 15 minutes of cooking (84.21%). Chlorpyrifos levels were reduced to 0.39 mg/kg which is below MRL (0.5 mg/kg) by washing and boiling treatment on 3rd day of post-hervestive storage and thereby reached at safe level for consumers.

The residue levels of cyeprmethrin and associated human risk with the % of reduction of residual pesticide and human risk by household processing has been summarized in TABLE 4.

On zero day, chlorpyrifos level was determined 2.1 mg/kg with a HRI value of 2.33. A time dependant reduction of residual pesticide due to washing and boiling/cooking was observed like chlorpyrifos. A significant (P<0.05) time dependant reduction of HRI value

TABLE 3 : Effect of household processing (washing and boiling) on residual level of chlorpyrifos in tomato at zero (0) and three (3) day

Treatment	Residual chlorpyrifos levels on zero (0) day				Residual chlorpyrifos levels on 3rd day			
	chlorpyrifos concentration (mg/kg)	% of Reduction	HRI	HRI R`eduction (%)	chlorpyrifos concentration (mg/kg)	% of Reduction	HRI	HRI Reduction (%)
Control	ND	-	-	-	ND	-	-	-
Unprocessed	3.26	0	0.22	0	2.81	0	0.19	0
10 second washing	2.37	12.91^a	0.16	12.91	2.15	23.49	0.14	26.32^a
20 second washing	2.19	19.45^b	0.15	19.45	2.14	23.84	0.14	26.32^a
30 second washing	2.15	20.91^c	0.14	20.91	1.03	63.35	0.07	63.16^b
5 minutes boiling	1.89	30.18^d	0.13	30.18	1.03	63.35	0.07	63.16^b
10 minutes boiling	1.20	55.45^e	0.08	55.45	0.64	77.22	0.04	78.95^c
15 minutes boiling	0.83	69.05^f	0.06	69.05	0.39	86.12	0.03	84.21^d

HRI= Human risk index that was calculated considering daily tomato consumption rate as 0.2 kg and acceptable daily intake as 0.05[4]; ND = Not detected. Data are mean of three observations (n=3). Inter-group differences were analyzed by repeated t-test. Values in the same column with different alphabets ($^{a\text{-}f}$) are significantly different at P<0.05.

TABLE 4 : Effect of household processing (washing and boiling) on residual level of cypermethrin in tomato at zero (0) and three (3) day

Treatment	Residual cypermethrin levels on zero (0) day				Residual cypermethrin levels on 3rd day			
	Cypermethrin concentration (mg/kg)	% of Reduction	HRI	HRI Reduction (%)	Cypermethrin concentration (mg/kg)	% of Reduction	HRI	HRI Reduction (%)
Control	ND	-	-	-	ND	-	-	-
Unprocessed	2.1	0	2.33	0	1.7	0	1.89	0
10 second washing	1.9	9.52	2.11	9.39^{a}	1.5	11.76	1.67	11.35^{a}
20 second washing	1.85	11.90	2.06	11.78^{a}	1.38	18.82	1.53	18.44^{b}
30 second washing	1.51	28.10	1.68	27.99^{b}	1.28	24.71	1.42	24.35^{c}
5 minutes boiling	1.42	32.38	1.59	32.28^{b}	1.05	38.24	1.17	37.94^{d}
10 minutes boiling	1.23	41.43	1.37	41.34^{c}	0.9	47.06	1.00	46.81^{e}
15 minutes boiling	1.19	43.33	1.32	43.25^{c}	0.79	53.53	0.88	53.31^{f}

HRI= Human risk index that was calculated considering daily tomato consumption rate as 0.2 kg and acceptable daily intake as 0.003[4]; ND = Not detected. Data are mean of three observations (n=3). Inter-group differences were analyzed by repeated t-test. Values in the same column with different alphabets ($^{a-f}$) are significantly different at P<0.05.

TABLE 5 : Effect of household processing (washing and boiling) on residual level of diazinon in tomato at zero (0) and three (3) day

Treatment	Residual diazinon levels on zero (0) day				Residual diazinon levels on 3rd day			
	Diazinon concentration (mg/kg)	% of Reduction	HRI	HRI Reduction (%)	Diazinon concentration (mg/kg)	% of Reduction	HRI	HRI Reduction (%)
Control	ND	-	-	-	ND	-	-	-
Unprocessed	0.61	0	2.03	0	0.43	0	1.43	0
10 second washing	0.32	47.54	1.07	47.45^{a}	0.23	46.51	0.77	46.39^{a}
20 second washing	0.2	67.21	0.67	67.16^{b}	0.13	69.77	0.43	69.70^{b}
30 second washing	0.14	77.05	0.47	77.01^{c}	0.1	76.74	0.33	76.69^{c}
5 minutes boiling	0.09	85.25	0.30	85.22^{d}	0.07	83.72	0.23	83.68^{d}
10 minutes boiling	0.08	86.89	0.27	86.86^{d}	0.05	88.37	0.17	88.34^{e}
15 minutes boiling	0.06	90.16	0.2	90.15^{d}	0.02	95.35	0.07	95.33^{f}

HRI= Human risk index that was calculated considering daily tomato consumption rate as 0.2 kg and acceptable daily intake as 0.001[4]; ND = Not detected. Data are mean of three observations (n=3). Inter-group differences were analyzed by repeated t-test. Values in the same column with different alphabets ($^{a-f}$) are significantly different at P<0.05.

by washing and boiling/cooking was observed with highest % of HRI reduction, 27.99% by 30 second washing and 43.25% by 15 minutes of cooking. On 3rd day of pesticide treatment, washing and boiling/cooking causes a time dependant gradual reduction of residual pesticide. Washing and cooking treatment also significantly (p<0.05) reduced % of HRI value in a time dependant manner with highest % of HIR reduction at 30 second washing (24.35%) and 15 minutes of cooking (53.31%).

The residual pesticide level of diazinon and associated human risk with residual pesticide and HRI value reduction rate (%) due to household processing has been summarized in TABLE 5. On zero day diazinon level was found 0.61 mg/kg with a HRI value of 2.03 while on 3rd day of storage it was found 0.43 mg/kg with a HRI value of 1.43. Both residual pesticide and HRI value was reduced in parallel to the washing and cooking treatment time. Washing and cooking causes a significant time dependant reduction of HRI value with highest HRI value reduction at 30 second washing (77.01%) and 15 minutes of cooking (90.15%). Washing and cooking treatment causes a time dependant reduction of residual pesticide in case of 3rd day of pesticide treatment. HRI value was also reduced significantly ($p<0.05$) in a time dependant manner by washing and cooking treatment with height HRI reduction at 30 second washing (76.69%) and 15 minutes of cooking (95.33%). Hence, levels of diazinon were reduced to 0.02 mg/kg which is still above MRL (0.01 mg/kg) by washing and boiling treatment on 3rd day of post-harvest storage.

The results of the present study was consistent with several other studies those showed that washing reduced the pesticide residual level with time[16,17,23]. The efficacy of washing treatment depend on the physiochemical properties of the pesticides like water solubility, hydrolytic rate constant, volatility and octanol-water partition coefficient, in conjunction with the actual physical location of the residues[17]. Washing processes lead to reduction of hydrophilic residues which are located on the surface of the crops. Hence, decontamination of surface residues is amenable to simple washing procedure. Boiling leads to a gradual decrease of residual pesticide for chlorpyrifos, cypermethrin and diazinon in a time dependant manner. On the basis of post-harvest time frame boiling was found to decrease the HRI value from 30- 86%, 32-53% and 85-95% for chlorpyrifos, cypermethrin and diazinon respectively. Degradation and volatilization rates of residues are increased by the cooking temperature generally. Besides these, low volatility and relative stability toward heat mediated hydrolysis of pesticide may also contribute to the residual level of pesticide during cooking. Previous study also showed a gradual time dependant decrease in residual pesticide level in food with heat treatment[1,13,24]. The results of the present study showed that post harvest storage and household processing can effectively reduce the residue level even under MRL level that is safe for consumers, which could be used as generalized and/or partial pesticides associated risk management practice.

CONCLUSION

The results of the present study showed that post harvest storage and household processing can effectively reduce the residue level even under MRL level that is safe for consumers. Therefore, longer post-harvest storage time as well as household processing could be generalized and/or partial pesticides associated risk management practice.

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