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Decontamination methods for reduction of insecticide residues in Brinjal and Chilli

Afroza Begum*, **Md. Sultan Ahmed, Syed Nurul Alam**

Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh

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Abstract

The study was conducted in the experimental field and Pesticide Analytical Laboratory of Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during 2014-15 to evaluate the effect of different decontaminating solutions in the removal of organophosphorus insecticide residues in brinjal and chilli. Five insecticide viz. diazinon, malathion, fenitrothion, quinalphos and chloropyrifos formulation were sprayed in brinjal and chilli plant. Estimation of residues was done using Gas Chromatograph equipped with Flame Thermaionic Detector. The results indicated that dipping in 2 % salt water solution for 15 minutes followed by washing under tap water plus cooking was found to be more effective in reducing all pesticides tested when compared with other treatment solutions both in brinjal and chilli. This study facilitated to standardize simple cost effective approaches to eliminate harmful pesticide residues from brinjal and chilli which could be adept by home makers.

* **Corresponding Author:** Afroza Begum ✉ afrozalopa16@gmail.com

Introduction

Indiscriminate use of pesticides particularly at fruiting stage and non-adoption of safe waiting period leads to accumulation of pesticides residues in consumable vegetables. This leads to contamination of vegetables with pesticide residues which have been reported by several researchers (Kumari *et al.*, 2002, 2003 and 2008). Furthermore, brinjal is highly cultivated vegetable crop consumed with peel as boiled in Bangladesh, attacked by variety of insect and disease pests, of which fruit borer is economically significant. In addition, chilli is also a high valued crop in the Bangladeshi circumstances, attacked by sucking pest most. To keep safe brinjal and chilli fruit from pest attack, farmers use a variety of pesticides, of which organophosphates and synthetic pyrethroids are leading. However, farmers are not looking at the safety intervals while harvesting i.e pre harvest interval (PHI), mentioned on the label of the used pesticide formulation. Consequently, the pesticide residues in the brinjal and chilli fruit at both farm gate and marketplace are common concern in Bangladesh.

Most of the pesticide residues have been associated in chronic neurotoxicity, endocrine disruption, immune impacts, genotoxicity, mutagenicity and carcinogenesis through routes that include ingestion of dietary residues. Several studies conducted in Kerala had led to a conclusion that the direct health effects of pesticide residues entering the human system through contaminated food are much more serious than the indirect effects through food chain and environment (Mathew TB *et al.*, 2012). Hence, it is essential to look for economical and easy method which can be accepted easily by homemakers. It is essential to diminish the residue level of vegetables before consumption. It has been reported that commercial and household processing such as washing, peeling, cooking, blanching and concentrating can reduce residue levels in food, which further minimize the impact of hazards on human health (Soliman, 2001; Zohair, 2001; Byrne and Pinkerton, 2004; Pugliese *et al.,* 2004; Zhang *et al.,* 2007).

Extensive literature review demonstrates that in most cases processing leads to large reductions in residue levels in the prepared food, particularly through washing, peeling and cooking operations (Soliman, 2001; Zohair, 2001). Shashi BV *et al.* 2015 revealed that, if brinjal fruits were washed by 2% salt water dimethoate is reduced to 78%. 82% of reduction in methylparathion, quinolphos reduced to 91%. endosulfan has got reduction up to 89% and 88.20% of reduction shown in profenophos. In addition, by processing with 2% salt water plus cooking the entire residues from the brinjal sample were drained out up to 100%.

However, there are some studies have been conducted on decontamination of different organochlorine and organophosphorus pesticides in different vegetables by different researcher. But no studies were conducted on decontamination of diazinon, malathion, fenitrothion, chloropyrifos and quinalphos pesticides in brinjal and chilli in Bangladesh circumstance. In the light of the above facts this study effort to assess the outcome of different decontamination techniques as well as to make a suitable and effective decontamination procedure to remove the above mentioned pesticide residues from brinjal and chilli by following different household preparations viz., washing with running tap water and detergent powder solution, dipping in 2% salt water solution, 2% vinegar water solution, 1% turmeric powder solution and luke warm water (36-40^{o} C).

Materials and methods

Reagents

Certified Reference Materials (CRM) of five different pesticides viz. diazinon, quinalphos, malathion, fenitrothion and chloropyrifos were used in the present study having purity > 99.99 per cent were obtained from M/s Sigma Aldrich and stored in a freezer at low temperature, without light and moisture. Solvent, acetonitrile used in the study were analytical graded. Analytical graded Magnesium sulphide manufactured by Scharlau, Sodium chloride and activated powder, charcoal manufactured by UNI-CHEM were purchased from M/s Sigma Aldrich, keeping in anhydrous condition in the laboratory.

A weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with n-hexane: toluene (1:1) to obtain a stock solution of 1000 mg kg-1.

Recovery study

The intermediary standards and working standards of 500, 300, 200, 100, 50 µg kg-1 were prepared by aptly diluting the stock solution in acetonitrile and used as standard check in analysis, linearity and recovery studies. The analytical method for estimation of residues of pesticides in brinjal and chilli has been validated by conducting recovery studies using control samples. Control samples were fortified with the above mentioned five level of standard.

Decontamination study

Brinjal and chilli plants were raised in the experimental field of Entomology Division, BARI, Gazipur. Agrizion 60EC (Diazinon 60ec) and Corolux 25 EC (Quinalphos 25 EC) @2ml per liter water. Fifanon 57 EC (Malathion 57 EC), Sumithion 50 EC (Fenitrothion 50 EC), Agriban 20 EC (Chloropyrifos 20 EC) @ 1.5 ml per liter water was sprayed in five different plots of each vegetable. Brinjal and chilli fruits were collected after 2 hours of insecticide spraying were subjected to dipping in different decontaminating solutions. The different decontaminating solutions were used in this experiment was normal tap water, 2% common salt solution (20g common salt dissolved in one litre water), 2% vinegar solution (20ml vinegar diluted in one litre water), 1% turmeric powder solution (10g turmeric powder dissolved in one litre water), luke warm water (36-40° C) and detergent powder solution (5 g detergent powder in one litre water).

Samples (250 g brinjal and chilli fruits) were dipped individually in these treatment solutions for fifteen minutes followed by washing under tap water. On the other hand, washing without dipping was carried out in two ways, one was washing for one minute under running tap water (Unprocessed) and another one was washed with detergent powder.

Another treatment was carried out by dipping in 2% common salt solution plus cooking at 100°C until softens. Samples were then homogenized after chopping into small pieces and the representative sample (25 g) in three replicates was used for residue estimation. A standard procedure of sample extraction and separation was done.

Extraction and separation

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used to extract diazinon, quinalphos, malathion, fenitrothion and chloropyrifos from different vegetables matrices. The method was slightly modified. Field collected samples (250g) were homozinized thoroughly with the blender (Mixer M-122, Bamix, Switzerland). Then single phase extraction of multiple analytes with a small volume (10 mL) of acetonitrile followed by liquid-liquid partitioning with the addition of 7.5 g of anhydrous $MgSO_4$ (Magnesium sulphide) plus 1 g of NaCl (Sodium chloride). Vigorous shaking is required for 1 minute after addition of $MgSO_4$ and NaCl. A quick shaking followed by centrifugation at 5000 rpm for 5 minutes (Laboratory Centrifuges, Sigma-3K30, Germany) which removes several interferences. After centrifugation approximately 2 mL of the supernatant should transfer to an eppendorf containing 100 mg PSA, 150 mg MgsO4 and 100 mg charcoal. Removal of residual water and clean-up of polar residues are performed simultaneously using a dispersive solid-phase (d-SPE) clean-up. The d-SPE clean-up carried out by just adding a primary secondary amine sorbent (PSA) directly to the acetonitrile extract. It is required to shake again for 2 minutes before another centrifugation at 10000 rpm for 5 minutes. At the final step of extraction, filtration through a 0.45um filter is required for injection. The instrumental parameters for GC-FTD are on the table 1.

Prior to the injection of the sample extract, standard solutions of different concentrations of each insecticide were prepared and injected with selected instrument parameters. The samples were calibrated (retention time, peak area etc.) against three to four pointed calibration curve of standard solution of concerned pesticide.

Each peak was characterized by its retention time. Sample results were expressed in ppm automatically by the GC software which represented the concentration of the final volume injected. From this value, the actual amount of pesticide residue present in the sample was determined by using the following formula:

Levels of insecticides present in processed and unprocessed commodity was estimated using the formula

$$\frac{\text{Peak area of sample} \times \text{Concentration of standard injected} \times \text{Volume of sample injected x Dilution Factor}}{\text{Peak area of standard x Volume of standard injected}}$$

Table 1. Instrument parameters for GC-FTD.

Instrument	Description
Gas Chromatograph	SHIMADZU-2010
Detector	FTD
Column	ATM™-1, 30 meters, 0.25 mm ID
Injector Temperature	250° C
Split Ratio	30.0
Carrier Gas	Hydrogen/Air
Carrier Gas flow	1.5 ml/min.
Column Oven Temperature	Initial temp. 150° C, hold time 1 min, @ 10° C /min 220° C, 2 min. hold, Total time 10 min.
FTD	280° C
Makeup flow	30 ml/min.

Results and discussion

Recovery study

The analytical method was validated in terms of recovery study. The fortification study was carried out by spiking the untreated control samples to determine the recovery levels. The average recoveries of the method for diazinon, quinalphos, malathion, fenitrothion and chloropyrifos was 80-120%. The recovery %s in this study was equal or higher than 80%, which indicated good and validated analytical procedure. The requirements regarding the process, for the quantitative analysis of pesticide residue are generally considered satisfactory if recovery is over 70% (Sanco, 2007).

Decontamination study

The effect of decontaminating solutions of different household products on removal of pesticide residues in brinjal and chilli fruits at 2 hours after spraying are summarized in table 2 and 3 respectively. To determine the % of pesticide reductions, each treated sample concentration was compared to the unprocessed sample concentration. In the unprocessed samples, the total concentrations of the five studied pesticides were 2.5, 2.7, 2.79, 2.56, and 2.88 mg/kg for diazinon, fenitrothion, malathion, chlorpyriphos and quinalphos respectively in brinjal. The residues of sprayed pesticides in brinjal sample have got considerable reduction by all house hold processing methods were used. After the washing process, the reduction of pesticides in malathion, fenitrothion, diazinon and quinalphos treated samples were shown to be superior (9-75% reduction) than the reduction (12-59%) in chlorpyriphos treated samples. But it could be reduced upto 39% by dipped in normal water while fenitrothion residue reduced up to 33%. This indicated that chlorpyriphos was easier to remove by the water than the other treatment solution. Subsequently, quinalphos was easily soluble in 2% salt water solution (82% reduction) while chlorpyrifos showed less solubility (45% reduction) in brinjal. On the other hand, malathion treated sample was shown the highest reduction (up to 75%) than other pesticides in all washing solutions except 2% salt water solution in brinjal. This may due to the physiochemical property of these pesticides. Almost 42% of malathion and 40% quinalphos were removed from the treated sample with the dipped in normal water for 15 minutes followed by washing under tap water, while dipped in luke warm water for 15 minutes followed by washing under tap water resulted in a reduction of 50% and 45% respectively. It was found that dipped in 1% turmeric powder solution followed by washing under tap water were reduced 53-61% pesticide residues. 100% diazinon and malathion residue and 96% fenitrothion and quinalphos residue were reduced through dipping in 2% common salt solution for 15 minutes followed by washing under tap water plus cooking.

Similar type of observation has been reported by Shashi BV *et al*, 2015, i.e. 98.02% quinalphos residue was reduced from brinjal through 2% salt water washing plus cooking.

Table 2. Effect of different household processing in reducing pesticide residue from Brinjal at 0 DAS.

Processing methods	Treated pesticides	Residue levels (mg kg^{-1})	Reduction %
Unprocessed sample	Diazinon	2.50	0
	Fenitrothion	2.70	0
	Malathion	2.79	0
	Chloropyrifos	2.56	0
	Quinalphos	2.88	0
T1= Washing with running Water	Diazinon	2.23	11
	Fenitrothion	2.43	10
	Malathion	2.43	13
	Chloropyrifos	2.25	12
	Quinalphos	2.62	09
T2= Dipping in normal water for 15 mins.	Diazinon	1.58	37
	Fenitrothion	1.81	33
	Malathion	1.62	42
	Chloropyrifos	1.56	39
	Quinalphos	1.73	40
T3= Dipping in 2% common salt water solution for 15 mins.	Diazinon	0.98	61
	Fenitrothion	0.99	63
	Malathion	0.69	75
	Chloropyrifos	1.41	45
	Quinalphos	0.52	82
T4= Dipping in 2% vinegar water solution for 15 mins.	Diazinon	1.85	26
	Fenitrothion	2.16	20
	Malathion	1.70	39
	Chloropyrifos	2.05	20
	Quinalphos	2.25	22
T5= Dipping in 1% turmeric powder solution for 15 mins.	Diazinon	1.10	56
	Fenitrothion	1.24	54
	Malathion	1.09	61
	Chloropyrifos	1.20	53
	Quinalphos	1.15	60
T6= Dipping in luke warm water for 15 mins.	Diazinon	1.38	45
	Fenitrothion	1.38	49
	Malathion	1.12	50
	Chloropyrifos	1.43	44
	Quinalphos	1.59	45
T7= Washing with detergent powder	Diazinon	1.00	60
	Fenitrothion	1.03	62
	Malathion	0.89	68
	Chloropyrifos	1.05	59
	Quinalphos	1.04	64
T8= Dipping in 2% common salt water solution for 15 mins.+cooking until soften	Diazinon	0.00	100
	Fenitrothion	0.11	96
	Malathion	0.00	100
	Chloropyrifos	0.72	72
	Quinalphos	0.12	96

Liang and coworkers (2012) reported that 63.40, 60.00, 50.00, 31.10 and 66.70 per cent reduction in the residues of trichlorfon, dimethoate, dichlorvos, fenitrothion and chlorpyriphos respectively, were observed in cucumber when dipped in 2 % sodium chloride solution for 20 min. These results agree with those obtained by Zohair (2001) who reported that soaking of contaminated potatoes in neutral (NaCl) solution (5 and 10 %) for 10 min resulted in 100 percent removal of pirimiphos methyl residues while dipped in 2% common salt solution for 15 minutes followed by washing under tap water plus cooking resulted in cent percent reduction of malathion and diazinon residues from brinjal fruit in this study. In normal water, 2% vinegar water solution, 1% turmeric powder solution and luke warm water, the efficiency of pesticide reduction were not as effective as 2% common salt solution. The cause and effect of the salt solution is still not known and needs further investigation.

However, in unprocessed chilli, the total concentrations of the five studied pesticides were 3.6, 3.05, 3.29, 3.56, and 2.86 mg/kg for diazinon, fenitrothion, malathion, chlorpyriphos and quinalphos respectively. Washing with detergent powder and dipped in 2% common salt solution for 15 minutes followed by washing under tap water plus cooking could remove 10-29% and 32-61% of residue respectively. But, the other six (6) treatments could not remove pesticide residue from chilli. Though dipped in 2% common salt solution for 15 minutes followed by washing under tap water could not remove any residue from chilli, cooking after dipped in 2% salt solution could it up to 61%. Even though 2% common salt solution, 1% turmeric powder solution, luke warm water and 2% vinegar water solution could remove pesticides residue from brinjal, no residues were removed by these household solutions from chilli.

The effects of different household solution in different crops depend on the interaction between the combination of the properties of pesticides and vegetables with environmental factors that determines the extent of pesticide absorbance, penetration and degradation (Amvrazi EG., 2011).

Table 3. Effects of different household processing in reducing pesticide residue from Chilli at 0 DAS.

Processing methods	Treated pesticides	Residue levels (mg kg-1)	Reduction %
Unprocessed sample	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T1= Washing with running Water	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T2= Dipping in normal water for 15 mins.	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T3= Dipping in 2% common salt water solution for 15 mins.	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T4= Dipping in 2% vinegar water solution for 15 mins.	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T5= Dipping in 1% turmeric powder solution for 15 mins.	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T6= Dipping in luke warm water for 15 mins.	Diazinon	3.60	0
	Fenitrothion	3.05	0
	Malathion	3.29	0
	Chloropyrifos	3.56	0
	Quinalphos	2.86	0
T7= Washing with detergent powder	Diazinon	2.81	22
	Fenitrothion	2.75	10
	Malathion	2.37	28
	Chloropyrifos	2.53	29
	Quinalphos	2.49	13
T8= Dipping in 2% common salt water solution for 15 mins.+cooking until soften	Diazinon	1.70	53
	Fenitrothion	1.22	60
	Malathion	1.28	61
	Chloropyrifos	2.42	32
	Quinalphos	1.92	33

The physiochemical properties of the pesticides, such as water solubility, volatility etc. as well as the composition and properties of plant surface (waxy cuticle and roots), pore size of fruit surface etc. depends on the rate of pesticide movement and dissipation (Amvrazi EG., 2011). In addition, the temperature of the washing water and the type of washing has an influence on the residue level.

Pesticides that are absorbed by the plant surface (waxy cuticle and roots) enter the plant transport system are termed as systemic pesticides. Contact pesticides remain on the surface of the plant. The concentration of pesticides was found to be higher in fruit stalk on exocarp and food receptacle in fruits and vegetables. (S. Yoshida, H. Murata and M. Imaida, 1992).

Conclusion

The present results indicated that the reduction of pesticide residues varies from pesticide to pesticide and crop to crop. It is recommended that commonly used pesticides may be tested for different type of crops with different type of pesticide. The study evidently revealed that dipping in 2% common salt water solution for 15 minutes followed by washing under tap water plus cooking until soften was the best treatment to eliminate residues of majority of insecticides from both brinjal and chilli under this experiment. The results of earlier workers (Aktar MW. *et al.*, 2010 and Dhiman N. *et al.*, 2006) have been reported similar results reducing the pesticide residues from brinjals and other vegetables. This result could be followed by the household makers in reducing pesticides from brinjal and chilli as a cost and time effective method.

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