DIETARY RISK ASSESSMENT OF FARMER PRACTICE IN BITTER GOURD (*Momordica charantia* L.) DIPPED IN INSECTICIDE SOLUTION

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ABSTRACT

A field study was conducted to investigate the dipping and spraying of bitter gourd fruits with insecticide solution at the National Crop Protection Center, University of the Philippines Los Baňos in January 2019. Bitter gourd fruits were sprayed with recommended dose and double the recommended dose of commercial products containing chlorpyrifos, profenofos and cypermethrin. The field trial results revealed all pesticide residues in both applications, after the post-harvest interval of 10, 7, and 7 days, respectively did not exceed the CODEX or ASEAN Maximum Residue Limits (MRLs) of several fruiting vegetables. A pesticide residue survey of 34 bitter gourd market basket samples were analyzed for various insecticide residues. A variable range of detectable insecticide residues were detected with chlorpyrifos, cypermethrin, endosulfan and profenofos. The effects of home processing were evaluated and the results demonstrated that washing with mild detergent solution was consistently a good home preparation for reducing residues substantially on bitter gourd fruits. In addition, soaking in vinegar alone, soaking in vinegar and boiling, as well as grilling also contributed to reduction of residues. A combination of home processing steps should be able to address food safety concerns. The dietary risk offered by dipping bitter gourd in chlorpyrifos, cypermethrin and profenofos is very low and similar in magnitude with that of spraying. The following maximum residue levels are recommended in bitter gourd: 0.2 mg/kg for chlorpyrifos and cypermethrin and 0.5 mg/kg for profenofos. Additional pesticide residue trials in the field need to be conducted to support an ASEAN MRL recommendation.

Key words: risk assessment, food processing, dietary risk

INTRODUCTION

Food safety is a public health priority. Dietary risk assessment of pesticide residues is an important component of food safety programs to ascertain risk. Bitter gourd (*Momordica charantia* L.) is a major part of the Filipino diet. Consumption of bitter gourd is on an average of 5 g/day or 2 kg/year representing a consumption of 0.6% of total food intake (FNRI 2014). Insecticide use in bitter gourd is meant to manage fruit flies, aphids, cutworms, cucurbit beetles, and leaf folders. Due to the enormity of the pest complex, farmers increase their insecticide use. In an informal survey with bitter gourd farmers and municipal agricultural officers, it was learned that some farmers resort to dipping bitter gourd fruits in insecticide solution as dipping entails less insecticide use, and in instances when farmers did not have a knapsack sprayer, application could still be done.

The Maximum Residue Limit (MRL) is the maximum concentration of a pesticide residue (expressed as mg/kg), to be legally permitted on or in food commodities and animal feeds. MRLs are based on Good Agricultural Practice (GAP) data and foods derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable (FAO 2022). These limits for pesticide residues in food are established by Codex Alimentarius Commission based on a risk assessment and limits for safe intake set by an FAO/WHO international expert scientific group named Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

Agricultural products, particularly foods, are listed as one of the 13 priority integration sectors in the ASEAN Economic Community Blueprint. Different laws and regulations of individual countries with different MRLs would pose barriers to trade of food products within the region. Therefore, member states have been striving to harmonize MRLs of pesticides in agricultural produce traded in the region. ASEAN also started to establish its own MRLs, generated from residue trials that are aligned with international standards. To date, a total of 880 ASEAN MRLs has been established for vegetables and fruits, involving a total of 71 pesticides (ASEAN 2021). Current ASEAN and Codex MRLs are available for several fruiting vegetables such as cucumber, eggplant and tomato but none for bitter gourd (*Charantia momordica* L.) (CAC 2023).

Cypermethrin residues in bitter gourd were detected in Mauritius and four (4) samples contained residues (0.20 mg/kg) exceeding the FAO MRL (0.07 mg/kg) (Ministry of Agro Industry and Food Safety 2015). Bitter gourd sprayed with profenofos and bifenthrin contained residues exceeding the MRL at 24 hours after spraying (Mirani et al. 2012). In India, residues of dicofol, ∑- endosulfan were detected in bitter gourd (Srivastava et al. 2011). Bitter gourd samples from India were contaminated with organochlorine, and pyrethroid insecticides (Kumari et al 2002). Residues of carbofuran, chlorpyrifos, malathion, fenvalerate and endosulfan have likewise been detected in bitter gourd samples from Pakistan (Latif et al. 2011).

Food processing is a requirement of the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) for evaluation of pesticides for MRL recommendations. Freshly applied residues are easier to remove than aged residues. The type of wash solution also affects the extent of residue removal and detergent has been found to increase the amount removed (Holland et al. 1994). The magnitude of residues in bitter gourd needs to be assessed due to the corrugated nature of the fruit surface which could retain higher amounts of pesticide residues. Therefore, reducing the concentration of residues before consumption would help in protecting consumer health.

The effects of household processing on pesticide residues showed a reduction of pesticide residues in various vegetables (Ahmed et al. 2011; Calumpang 2014; Chung 2018; Đorđević and Đurović-Pejčev 2016; Kaushik et al. 2009). Pesticide residues can be lowered significantly by simple household practices like washing, peeling, and cooking operations (Soliman 2001; Zohair 2001). In India, bitter gourd was contaminated with parathion and permethrin. Washing and cooking minimized the residues of nine pesticides to a range of 1.74 to 64.78 and 38.40 to 90.15 percent, respectively (Joshi et al. 2015). Traditional Pakistani food processes such as washing, salting, blanching, sun-drying, dehydrating, and frying also reduced residues (Mirani et al. 2012).

There is virtually no formal information on pesticide application through dipping of bitter gourd. Therefore, there is a compelling need to generate data about this practice to determine the risks it may impose on consumers. Furthermore, food processing measures should be assessed to determine how practices at the household level can reduce pesticide residues. The information on the dietary risks of pesticide dipping practice can aid in the setting of ASEAN MRL for bitter gourd. The study sought to survey insecticide residues in market basket bitter gourd samples, determine the magnitude of insecticide residues in bitter gourd dipped with insecticide solution, identify food preparation methods to reduce insecticide residues and assess the dietary risk of insecticide residues detected.

MATERIALS AND METHODS

Method recovery test of bitter gourd samples. Bitter gourd fruits were quartered, and two opposite sides of the divided fruit were used as samples. The remaining two opposite sides were discarded. Samples were sliced thinly and blended until the consistency is a puree. Fifty-gram (50) samples were weighed, extracted and subjected to GC analysis. The method was optimised by recovery studies before the determination of pesticides in samples. Trials were conducted for bitter gourd using the Pesticide Monitoring Development Program (PMDP) multi residue method (Migano 1999) to determine acceptability of the method for analysis of six insecticides: chlorpyrifos, cypermethrin, endosulfan, fenitrothion, diazinon and profenofos, by gas chromatography. The PMDP method was modified slightly to extract bitter gourd with three replications spiked at 0.05 mg/kg. Average recoveries ranging from 80 to 120% were obtained. The same extraction procedure and gas chromatograph conditions as applied for recovery studies were used for sample analyses.

The gas chromatograph (Shimadzu 2010, Japan) was equipped with a Rtx-5ms capillary column and an electron capture detector (ECD). The injection volume for each sample was 1 µL with the injector and detector temperatures set at 280 and 320°C, respectively. The initial oven temperature was set at 120°C then increased to 250°C at the rate of 10°C/min and was held for 3 minutes, further increased to 270°C at 3°C/min and then raised to 310°C at 75°C/min and was held for 1.8 minutes. Confirmation of any residues detected was done using a gas chromatograph - mass spectrometer (GC-MS Shimadzu TQ8040) in the selective ion monitoring (SIM) mode.

Pesticide Residue Trials

Field preparation. Pesticide residues in bitter gourd fruit were evaluated in field trials by dipping and spraying fruit using the recommended rate of insecticide application. The study was conducted in January 2019. Proper irrigation and fertilization were maintained and pest management was observed.

Spraying field trials. Three rows of bitter gourd were sprayed, with a plastic sheet barrier around it to eliminate cross contamination. The insecticide was sprayed only once prior to sampling and fruits were sampled on selected days based on preharvest interval. Bitter gourd fruits were sprayed with chlorpyrifos, cypermethrin, and profenofos, using the recommended rate for either vegetables or watermelon. Priority insecticides were based on detections in market basket samples. About 1 kilogram of bitter gourd was collected at the same sampling schedule as that of the dipping trials.

Fig. 1. Dipping of bitter gourd fruit in insecticide solution in a plastic container.

Dipping trials. Bitter gourd fruits were dipped individually in the field. Each fruit was covered with a net bag and only fruits of marketable size were sampled. Four fruits (total of about 1 kg) were collected after dipping. Bitter gourd fruits were dipped in chlorpyrifos, cypermethrin, and profenofos, using both the recommended rate and double recommended rate.

Home preparation. Bitter gourd fruits were dipped in recommended rate and double the recommended rate solution. The fruits were harvested one day after spraying or dipping. All fruits were quartered longitudinally, and two opposite quarters were processed. Laboratory samples were sliced using a kitchen knife, blended (Osterizer 4172- 051, Oster, USA), extracted using acetonitrile, cleaned up using SPE C18 and Envicarb, and analyzed by GC-ECD. The following home processes were evaluated: unwashed, boiled in water for 15 minutes, unwashed, grilled for 20 minutes over electric stove top, washed with liquid detergent solution (0.05%), washed with liquid detergent and grilled or boiled, soaked with rubbing using a rubber foam and rinsed in tap water, soaked in mild vinegar solution (1%) for 5 minutes with mild rubbing using kitchen foam, soaked in vinegar and boiled for 15 minutes, and squeezed with salt, as in a salad and rinsed with water 3 times.

Dietary risk of insecticide residues detected. An estimated daily intake (EDI) was calculated (mg/kg body weight) for the insecticide evaluated (WHO 1997). The values were compared with the Acceptable Daily Intake (ADI) of the particular insecticide.

$$\text{EDI} = \frac{\text{Residue detected (mg/kg)} \ast 0.125 \text{ kg bitter gourd consumed per day}}{55 \text{ kg/body weight}}$$

The calculations used the following assumptions:

1. Daily intake of 0.125 kg of bitter gourd or ½ of medium sized fruit, representing a maximum daily intake.
2. Average weight of 55 kg body weight for Filipinos

RESULTS AND DISCUSSION

Field trials of bitter gourd fruits sprayed and dipped in insecticide solution.

Chlorpyrifos residues. Bitter gourd fruits were dipped or sprayed using the recommended rate. It appears that retention of chlorpyrifos residues in bitter gourd is not affected by the method of application, either spraying or dipping. Residues did not differ at 1 day after spraying or dipping and likewise at 10 days after application, which is the recommended pre-harvest interval (PHI) for chlorpyrifos in bitter gourd (Table 1). It is noted, that these residues, ranging from 0.001 to 0.005 do not exceed the CODEX or ASEAN MRLs of several fruiting vegetables. Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 1.2 x 10^{-5} mg/kg bw chlorpyrifos per day, that corresponds to 0.12% and 0.10% of the ADI for dipped and sprayed bitter gourd, respectively at 1 day after application.

Table 1. Insecticide residues (mg/kg) in bitter gourd using the recommended rate.

Insecticide	Dipped	Concentration	Sprayed	Concentration
Chlorpyrifos	1 DAS	0.005	1 DAS	0.004
Chlorpyrifos	10 DAS *	0.001	10 DAS	0.001
Cypermethrin	7 DAS *	0.01	7 DAS	0.02
Profenofos	7 DAS *	0.024	7 DAS	0.018

*PHI based on label information, field conditions

A maximum residue level of 0.5 mg/kg is recommended for chlorpyrifos in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which are lower than 0.2 mg/kg. This is the same for the existing ASEAN MRL for tomato (Table 2).

The decrease in chlorpyrifos residues over a period of 10 days may be influenced by factors including volatilization and photodegradation. Higher temperature favors volatilization and photodegradation due to hydrolysis, which occurs when chlorpyrifos is exposed to sunlight (Akoto et al. 2016). Under UV light or sunlight, chlorpyrifos undergo hydrolysis which occurs readily in presence of water at about pH 6 and very readily above pH 8. This releases its metabolite, 3,5,6-trichloro-2-pyridinol, which undergo further decomposition (JMPR 1972). Hence, the reduction of chlorpyrifos residues in bitter gourd when exposed to field conditions.

Table 2. Maximum Residue Limits (mg/kg) of test insecticides set for various fruiting vegetables.

Insecticide	Commodity	ASEAN MRL*	Commodity	CODEX MRL**
Cypermethrin	Fruiting vegetables, cucurbits	0.07	Fruiting vegetables, cucurbits	0.07
	Eggplant	0.03	Eggplant	0.03
	Tomato	0.2	Tomato	0.2
			Pepper, sweet	0.1
Chlorpyrifos	Tomato	0.5	Tomato	0.5
	Pepper, sweet	2	Pepper, sweet	2
Profenofos	Tomato	10	Tomato	10

*ASEAN MRL 2021 (FAO 2022a) ** CODEX MRL (CAC 2023)

Cypermethrin residues. Levels detected in sprayed (0.01 mg/kg) and dipped (0.02 mg/kg) bitter gourd did not decline up to 7 days and are essentially the same for either sprayed (according to GAP) or dipped (non-GAP practice) (Table 1). When cypermethrin is applied, the parent compound is the major identified residue with very little absorbed or translocated. Metabolites result from ester hydrolysis and hydroxylation processes. Exposed residues are subject to isomerisation, presumably by a photolytic process (JMPR 1972). Cypermethrin residues of dipped and sprayed bitter gourd also did not exceed the ASEAN MRL of eggplant and cucumber (0.2 mg/kg), a cucurbit like bitter gourd. Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 3.0×10^{-5} mg/kg-bw cypermethrin per day, that corresponds to 0.06% and 0.08% of the ADI for dipped and sprayed bitter gourd, respectively at 1 day after application.

A maximum residue level of 0.2 mg/kg is recommended for cypermethrin in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which resulted in residues that were much lower than 0.2 mg/kg. This value is the same for the existing ASEAN MRL for cucumber (Table 2).

Profenofos residues. Profenofos residues in bitter gourd fruits that were dipped or sprayed using the recommended rate contained residues of 0.02 mg/kg at 7 days, which is the recommended PHI for profenofos in bitter gourd. The retention of profenofos residues was not affected by the method of application (Table 1). Profenofos residues in dipped (0.024 mg/kg) and sprayed (0.018 mg/kg) bitter gourd did not exceed the MRL for tomato (2 mg/kg) and sweet pepper (0.5 mg/kg), which are both fruiting vegetables (Table 2). Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 1.48×10^{-4} mg/kg-bw profenofos per day, that corresponds to 1.48% of the ADI. Profenofos is slowly absorbed and metabolized in plants. Profenofos was the major residue when harvested several weeks after the last application, and profenofos underwent hydrolysis of phosphate ester to form CGA 55960 4-bromo-2-chlorophenol and its sugar conjugate (JMPR 2008).

A maximum residue level of 0.5 mg/kg is recommended for profenofos in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which resulted in residues that were much lower than the ASEAN MRL for sweet pepper (Table 2). Both vegetables (bitter gourd and sweet pepper) belong to the same food group of fruiting vegetables.

Survey of market basket bitter gourd samples. In some market basket samples, bitter gourd fruits contained detectable insecticide residues of the 4 commonly used insecticides fenitrothion, profenofos, chlorpyrifos, and cypermethrin. More detections were noted in the later part of 2018, and this coincided with the rise in market price of bitter gourd. It can be surmised that farmers used insecticides during this time to protect their harvest, thus terminal residues could be detected. A total of 34 samples were analyzed for various insecticide residues. Low level insecticide residues (less than 1 ppm) were detected. A total of 10 samples (30%) did not contain detectable residues, and 24 samples (70%) had detectable insecticide residues of chlorpyrifos, profenofos, cypermethrin and endosulfan. Trace levels of diazinon and fenitrothion were detected in only 2 samples. The most common insecticide detected was chlorpyrifos, in 50% of samples. Low levels of endosulfan, cypermethrin, profenofos, diazinon and fenitrothion were also detected. Some samples contained 2 or 3 insecticide residues but usually 1 insecticide was quantifiable with other(s) present in only trace amounts. The highest level detected was endosulfan (1.23 mg/kg), which is unauthorized use as it is no longer registered with the Fertilizer and Pesticide Authority since 2015 (Table 3).

The residue levels detected are below the recommended maximum residue level, except for the highest level detected in cypermethrin (Table 2). This information can be used by agricultural technicians to conduct farmer information campaigns in areas where use of insecticides may exceed the recommended dosage and/or timing. Additional information on pesticide management as well as information on biological control, intercropping and other pest management strategies are necessary so that farmer reliance on synthetic pesticides are minimized.

Table 3. Monitoring of insecticide residues in market basket bitter gourd samples. (n =34)

	Number of samples	Residues detected (mg/kg)	Percent of samples analyzed
Chlorpyrifos	17	0.001 – 0.084	50
Cypermethrin	5	Trace – 0.49	15
Endosulfan	3	0.001 – 1.23	9
Profenofos	1	Trace – 0.002	3
Diazinon	1	Trace	3
Fenitrothion	1	Trace	3

Home preparation of bitter gourd dipped at recommended rate. Home preparation is a good means to reduce insecticide residues. Boiling or cooking of unwashed bitter gourd dipped at the recommended rate, resulted in 85 to 90% reduction of residues of the 3 insecticides evaluated. Washing with mild liquid detergent, squeezing in salt and soaking in vinegar (as in a salad) and soaking in vinegar prior to boiling reduces chlorpyrifos, profenofos and cypermethrin residues considerably (Table 4).

Table 4. Percent reduction of selected insecticide residues using various home preparation methods.

Insecticide	Washed, mild liquid detergent	Soaked in vinegar and boiled	Squeezed in salt and soaked in vinegar	Unwashed, Boiled
Chlorpyrifos	78.4	71.6	62.0	89.0
Profenofos	63.5	61.4	63.5	90.0
Cypermethrin	55.2	64.0	38.1	84.6

Washing the bitter gourd dipped in either chlorpyrifos, profenofos or cypermethrin with mild liquid detergent solution reduced residues by 78.4%, 63.5 % and 55.2%, respectively. This is within the range reported in earlier studies for sweet pepper (62.58%) and eggplant (100%) (Radwan 2005). A 50-60% reduction in cypermethrin residues was determined in eggplant dipped in cypermethrin and subsequently washed with liquid detergent solution (Holland et al. 1994).

Soaking in mild vinegar solution and cooking bitter gourd in water resulted in significant reduction of insecticide residues in bitter gourd fruits that had been dipped. Our results are consistent with the previous studies which showed considerable reduction of initial residues by 68.5%, and 100% on eggplant and sweet pepper (Chandra 2015; Kiwango 2018).

Squeezing in salt then soaked in vinegar was meant to simulate preparing bitter gourd salad. Both organophosphate insecticides, chlorpyrifos and profenofos, were reduced substantially (about 60%) by the process while more cypermethrin residues were retained in the waxy layer of the bitter gourd skin (38% reduction). Cypermethrin is a non-polar insecticide and is expected to adhere to the waxy surface. (JMPR 2008).

Direct boiling of unwashed bitter gourd can also lead to substantial reduction of insecticide residues (85-90%). A reduction of 50-100% of several organophospate insecticides, such as chlorpyrifos, was observed for boiling (Satpathy et al. 2012). Hydrolysis of chlorpyrifos is known to be affected significantly by increased temperature (Hui et al. 2010). Boiling of vegetables was found to be more effective than washing in dislodging the residues in various fruiting vegetables. However, some intact residues or more toxic metabolites may be found in the boiled water (Chung 2018).

Alpha-cypermethrin and cypermethrin residues were found to be stable during hydrolysis conditions simulating pasteurization and boiling (JMPR 2011). The application of heat in cooking/boiling reduces residue levels and can enhance volatilization and hydrolysis (Holland et al. 1994). Cooking/boiling is the most effective treatment for reducing the residues of synthetic pyrethroids in different vegetables (Chauhan et al. 2014). Cypermethrin residues were reduced in bottle gourd and ridge gourd after cooking in water by 15–33% (Kadian et al. 2001). The age of the residues is an important factor in the retention of residues as these can move into the cuticular layer which is difficult to extract. This could explain the lower percent reduction of cypermethrin (15-33%) than this present study which used bitter gourd fruits that were collected one day after application.

Dipped at double recommended rate. These studies were conducted to assess a worst-case scenario when a double the recommended rate is used due to intense pest pressure in the field. Residues generated from a double recommended rate can likewise be reduced by washing with liquid detergent and subsequent boiling or grilling which resulted in substantial reduction of residues (44 to 90% reduction). These home processes are commonly practiced in Filipino households, like bitter gourd salad or sautéed mixed vegetables (*pinakbet*). Washing with mild detergent solution gave the highest reduction (69 to 80% reduction) that was consistent for all three insecticides tested. Soaking in vinegar as a preparatory step prior to grilling resulted in substantial reduction of initial residues (69 to 81%). Grilling by itself did not reduce much of the organophosphate insecticides, profenofos and chlorpyrifos, while 89% reduction was obtained by the pyrethroid, cypermethrin (Table 5).

Previous studies showed that the grilling process was most effective in reducing cypermethrin residues in eggplant (50%) while only 41% reduction was obtained by boiling in water (Walia et al. 2010). Roasting or grilling was found more effective than cooking in reducing insecticide residues in eggplant (Thanki 2012). In general, washing and soaking can only lead to a certain degree of reduction in residue level, while other processes such as peeling, soaking in chemical baths and blanching can reduce pesticide residues more effectively (Chung 2018).

Table 5. Percent reduction with home processes practice of the insecticide residues in bitter gourd dipped in double dose above the recommended rate.

Insecticide	Percent Reduction			
	Washed with liquid detergent, boiled	Washed with liquid detergent, grilled	Grilled	Soaked in vinegar and grilled
Chlorpyrifos	69.5	Not done	32.46	68.9
Profenofos	80.0	65.2	43.8	81.1
Cypermethrin	70.1	44.1	89.8	Not done

CONCLUSION

Seventy percent (70%) of the market basket bitter gourd samples bought from Metro Manila and Region 4A, Philippines wet markets and supermarkets had low level insecticide residues. Low levels of chlorpyrifos, endosulfan, cypermethrin and profenofos were detected in some samples. Home preparation methods can further protect consumers from exposure to insecticides residue. The greatest reduction in residues for the three commonly used insecticides were obtained by: boiling, washing with mild liquid detergent solution, soaking in vinegar and boiling, and soaking in vinegar and grilling. A combination of home processing steps should be able to address food safety concerns.

The dietary risk offered by dipping bitter gourd in chlorpyrifos, cypermethrin and profenofos is very low and similar in magnitude with that of spraying. All estimated daily intake values are below 1% of the ADI. The following maximum residue levels are recommended in bitter gourd: 0.2 mg/kg for chlorpyrifos and cypermethrin, and 0.5 mg/kg for profenofos. Additional pesticide residue trials in the field need to be conducted to support an ASEAN MRL recommendation. These may be conducted in the various countries in the ASEAN region.

The information gap on reduction of pesticide residues in specific food processes peculiar to traditional or modern Asian cuisine needs to be addressed especially in commodities that receive more pesticide applications due to the presence of a pest complex in the field and the increasing temperature under field conditions which hastens pest development. Food processing is the final step to safeguard consumer safety from dietary risk.

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