Effect of Household Processing Methods on the Removal of Pesticide Residues in Mustard Greens *Brassica juncea* (L.) Czern

Khin Mar Kyi
Pathein University
khinmark4@gmail.com

Nang Sanda Myint
Yangon University

Kay Lwin Tun
Yangon University

Abstract

Pesticides and other poisonous chemicals used in growing fruit and vegetables can pose arisk of developing cancer in people. In the present study, the effects of household processing on removal of pesticide (profenofos) residues in Brassica juncea (L.) Czern. mustard greens were determined. Study period lasted from February 2017 to July 2017. Mustard greens (405 plants), which had been grown for 45 days, were sprayed with 0.2% of the insecticide profenofos for 3 consecutive days. After 24 hours, mustard greens were harvested and divided between three groups and subjected to different household processing methods, i.e., washing with water, washing with water and dipping in a 1 % salt solution, and control (no processing). The pesticide residue amounts after processing were measured by Gas chromatography - Mass Spectrometry(GC/MS) method. High amount of residue were found in the control group, with residue levels of 93.45 mg/kg (milligram per kilogram). Much lower amounts of profenofos were found after washing of the mustard greens with tap water with (30 mg/kg), or after washing and subsequent dipping in a 1% salt solution (25.26 mg/kg) of sample had pesticide residues below maximum residue levels(MRL) then it is save for human consumption. Subjecting mustard greens to dipping in salt water was found to be more effective than only washing of mustard greens with tap water.

Keywords: *Pesticide, residues, washing, household preparation, profenofos.*

1. Introduction

Nowadays, people consume more fresh fruits and vegetables in the diet to improve their health. However, the consumption of fruits and vegetables that have been sprayed with pesticides (insecticides, herbicides, and fungicides) can pose hazards to human health. This is especially the case for vegetables, as they are often freshly consumed soon after field harvest without allowing much time for inactivation of pesticide residues [10].

Pesticides are extensively used all over the globe to control different pests [6]. In Myanmar, pesticides are used not only for protecting crops, but also for vector control. The consequences of their use and the realization that some foods contain residues of these compounds are of obvious importance to the consumers. The problem of contamination of food commodities, especially fruits and vegetables, with pesticide residues constitutes one of the most serious challenges to public health [7].

In Myanmar, people are eating fresh vegetables as salad, appetizer and fermented product in their daily meal. Among the vegetables in Myanmar, mustard leaves are eaten nation-wide. Mustard is best grown as summer or winter crop in Myanmar. It is eaten raw in salads or cooked as an essential ingredient with meat. In addition, fermented mustard has been used as a famous traditional Shan and Myanmar food. For its preparation, raw mustard leaves are washed with water and are then fermented in bottles or pots with salt. Processing of fresh vegetables before eating varies between households. Annual consumption of mustard leaves in Myanmar is increasing day by day. Farmers who are growing mustard green are using pesticides because it is mainly attacked by aphids, cabbage worms and loopers, slugs and flea beetles.

Therefore, testing for pesticide residues in mustard green that is used as part of the daily diet in Myanmar is urgently needed for food safety. The present study, is investigated the effects of different household processing methods on the removal of profenofos, a commonly used insecticide to understand the fate of its residues on mustard leaves.

2. Material and Methods

2.1. Plantation of mustard green, *Brassica juncea* (L.) Czern.

Fifteen-day old mustard green *Brassica juncea* (L.) Czern, which had not been treated with pesticide, was purchased from Pyi Thar Yar Agricultural Farm, Yankin Township. It was grown on a plot of land beside the Aquatic Bioscience Laboratory, Department of Zoology, Yangon University in February 2017 to July 2017. Mustard green was grown in three rows, each 9.75 m long and 0.61 m wide. In each row, 135 *Brassica juncea* plants were cultivated at 5 cm intervals. Mustards green were watered three times a day, at 6 am, 11am and 5 pm. They were grown for 45 days without being treated with pesticides or other compounds.

A total of 4 ml of profenofos (50% EC Myanmar Awba Group Co. Ltd) was diluted with 1 liter of tap water as outlined in the user instructions. Then, the solution was sprayed onto all mustard green plantation area using a one-liter pressurized handheld sprayer (Figure 1).The same amount of profenofos was applied for three consecutive days. Two days after the last application, mustard green was harvested to test for pesticide residue analysis.

Figure 1. *Brassica juncea* planted in aquatic bioscience laboratory, Department of zoology, University of Yangon.

A total of 45 housewives were interviewed to determine the mostly commonly used method of vegetable processing in the home. Interviewed were three groups; first group were university teacher, second group were university office-staff and third group were restaurant, university campus. Thereafter, the three most commonly used methods control (without washing), dipping in 1 % salt solution for 5 min., washing with tap water were selected for evaluation in the present study.

2.2. Different processing of *Brassica juncea* (L.) Czern. to remove profenofos

A sample of mustard plants was taken in a "Z shape fashion" from different rows. Roots from the harvested mustard green plant was removed. Samples were weighed (not include roots) and divided between three groups (1kg/group). Each group was subjected to a household processing method, as follows:

2.2.1. Control (Without washing)

A total of 1 kg mustard green sample was chopped into 4 cm pieces and kept in a Zip-lock bag (18 x 12) until further analysis.

2.2.2. Dipping in 1 % salt solution for 5 min.

A total of 65 grams of table salt was added to 6.5 L of water. It was stirred for 5 min. until completely dissolved to know pesticide residue.Then, mustard green was chopped into pieces and dipped in the saline for 5 min before being washed twice with water (approx. 6.5 L). Washed mustard green was placed into a plastic basket (14 x 9 x 9), dried with absorbent tissue and kept in a Zip-lock bag until analysis.

2.2.3. Washing with tap water

A total of 1kg mustard green sample was washed in 6.5 liters of water for 1 minute. Then water was changed and the washing repeated for a second and third time before drying and storing of mustard green as explained above. All samples were brought to at Plant Protection Division, Department of Agriculture (Yangon) to check the pesticide residues. The pesticide residue was measured using GC/MS (Gas chromatography-Mass Spectrometry) method.

2.2.4. GC-MS analysis:

Sample preparation: The extraction method employed (15) is as follows: A representative 10-g portion of previously homogenized sample was weighed in a 200-mL polytetrafluoroethylene (PTFE) centrifuge tube. Then 10 mL of ethyl acetate was added, and the tube was shaken vigorously for 3s by hand. Next, 1.5 g of sodium chloride and 8 g of magnesium sulfate were added, and the tube was shaken in an automatic axial extractor for 15 min. The extract was 3700 rpm for 5 min centrifuged. Then the extract containing the equivalent of 1 g of sample per mL in 100 ethylacetate was injected directly into the Gas chromatography.

2.2.5. GC-MS conditions:

The separation of the pesticides from the whole vegetable extract was carried out using an Agilent 7890 GC system with two 15 m X 0.25 mm, 0.25-µm d_f Agilent HP–5MS UI Ultra Inert GC columns connected through an auxiliary programmable control module (PCM). The vegetable samples were put in splitless mode through an ultra-inert liner those a glass wool frit (Agilent). The injection volume was 2 µL. The injector temperature was held at 280 °C during the whole running time. Helium (99.999% purity) was used as the carrier gas. The oven temperature program was as follows: 60 °C for 1 min, then 60–120 °C at 40 °C/min, and 120–310°C at 5 °C/min. The analytic separation was achieve below retention time locking conditions, using clorpyrifos methyl as the locking compound at a retention time of 18.11 min. The flow rates in column 1 and column 2 were 1.225 mL/min and 1.425 mL/min, respectively. The total run time was 40.5 min with 3 additional minutes for back flushing. Back flushing was carried out to eliminate unwanted heavy materials from column 1, thereby shortening the analysis time, reducing system maintenance, and prolonging column life. The back flush conditions were set as follows: the oven temperature was set at 310 °C, the pneumatics control module (PCM) pressure was 50 psi, and the inlet pressure was 1 psi. These conditions allow a negative flow on the column 1.

2.2.6. Mass Spectrometry

The GC system was connected to an Agilent Technologies model 7200 TOF-MS instrument equipped with an electron ionization source. The ion source and quadrupole analyzer temperatures were set at 280 °C and 150 °C, respectively. The TOF analyzer was operated at two different acquisition rates, 2 GHz and 4 GHz, acquiring data in the *m/z* 45550 mass range. Perfluorotributylamine was used for daily MS calibration. The accurateness of the generated ions was controlled through an inner mass calibration performed

before each injection. Reduction percentage was calculated according to the following formula.

$$\textbf{Reduction percentage} = \frac{\textbf{Residue levels}}{\textbf{Control value}} \times \textbf{100}\%$$

Table1. Process methods for vegetable washing used by housewives in Myanmar

Sr. No	Method	Water Liters	No. of housewives
1	wash with little salt	6.5 L	1
2	wash with one tea spoon of salt (10 g)	6.5 L	16
3	wash with two tea spoon of salt (20 g)	6.5 L	2
4	wash with three tea spoon salt (30 g)	6.5 L	10
5	wash with tap water for 3 times	6.5 L	16
	Total household		45

Table 2. Profenofos residue in mustard green following different household processing methods.

Processing method	Reduced residue Levels(mg/kg)	Reduction (%)
Control(without washing)	93.45	0(control value)
Tap water washing	30	32%
1% salt solution washing	25.26	27%

3. Results

The amount of profenofos residue in mustard green sample was substantial reduction by different household processing methods. To understand the different household method used for vegetable processing, 45 households were interviewed. Triple-washing of mustard leaves with tap water was the most commonly used method among these households (16 of 45 interviews), followed by washing with one tea spoon (10g) of salt (16/45), washing with three tea spoons (30 g) of salt (10/45), washing with two tea spoons (20 g) of salt (2/45) and washing with little salt (1/45) (Table 1).

The different household preparations such as washing, cooking, washing plus cooking, and salt-water washing, contribute to reducing pesticide residues [11]. While tap-water washing did not remove profenofos to below the *"Maximum Residue Level"* (MRL), washing with 1% salt water was very effective in the removal of profenofos to values below MRL. The maximum residue levels of pesticide residue in food are stipulated by regulatory bodies in many countries. According to [2], the maximum pesticide residue for *Brassica* spp. ranged from 30 to 50 mg/kg. The level of residue in the present study is 30 mg/kg in samples washed with tap water and 25.26 mg/kg in samples dipped in 1% salt solution. Both methods thus can reduce the level of profenofos to an acceptable level.

In Myanmar, fermented mustard green has been used widely in traditional foods. Fermented mustard greens are produced commercially. For the preparation of fermented mustard greens, we would like to recommend that washing with tap water and dipping in 1% salt solution should be done to remove a substantial proportion of the profenofos.

Control processing (without washing) was found to yield the highest residue levels, at 93.45 mg/kg. In samples washed with tap water, the residue level was 30 mg/kg and the samples washed with tap water and dipped in 1 % salt solution, the lowest residue levels of 25.26 mg/kg, or a 27%, reduction was found in table 2.

Control sample (without washing) mustard green contained maximum residues. 1% salt solution wash and washing with tap water removed residues adsorbed on mustard green surface. Dipping in 1% salt solution decreased the water soluble pesticides profenofos. Therefore 1% salt solution washing was more effective in eliminating pesticide residues as compare to tap water washing. These operations such as salt washing and tap washing play a role in the reduction of residues in mustard green through household processing may help in reducting pesticide residues for human consumption.

Washing as a process is common in most households since it can be done with simply available plain water and also with solutions formulary from chemicals readily available in a household kitchen [4].

4. Discussion

Pesticides are used indiscriminately and excessively across the globe, and their residues remain in the food, water, fruits, vegetables and thus, in the diet [1] [5]. As a result, these pesticide residues can enter into the human body which can lead to chronic disorders. Thus, the removal of these residues from food commodities is very essential [9].

The present findings are very similar to those reported by [8]. She studied the effect of chlorpyrifos residue on the mustard green washed with water after 24 hr of harvesting. She detected 35% of residue in the samples and suggested that plants should be harvested with a minimum time period of 2 to 5 days after being treated with pesticide. The present study 'with watering' retained 30% residue; pesticide residual amounts are likely to differ between the different types of pesticide.

[3] have removed profenofos residues by washing with tap water for 10 min, reducing amounts to 49.4%, or by washing with a 2% salt solution for 10 min, reducing profenofos amounts by 55.9%. In the present work, profenofos on mustard greens was reduced to 25.26 mg/kg in response to a 1% salt solution and to 30mg/kg after washing in tap water. Profenofos control samples contained 93.45mg/kg. Variation in outcomes may reflect a different concentration of salt, different duration of dipping, or the use of different vegetables or different method of washing.

Thus, based on the results obtained in this study it can be concluded that by processing the mustard greens with the traditional processing methods if it helps in the removal of pesticide residues below Maximum Residue Levels, then it is safe for human consumption.

5. Conclusion

Myanmar, being an agrarian economy, is dependent on agriculture for its development. Use of pesticides on vegetables is an inevitable part of agriculture but their unscientific usage can cause significative health adversities. There is a need to regulate pesticide intake to lead a healthy life. According to the present study, profenofos can successfully be removed from *Brassica juncea* (L.) by washing the mustard greens and then dipping them in a 1% salt solution. Therefore, vegetable consumers are advised to not eat these vegetables until after proper dipping treatment for at least 5 minutes in salt before consumption.

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