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Mitigating the impact of organochlorine and pyrethroid residues in fresh and chemically washed spinach

Ali HUSSNAIN[1], Rai Muhammad AMIR[1*], Muhammad Azam Khan[2], Asif AHMAD[1], Shinawar Waseem ALI[3], Muhammad NADEEM[4], Kashif AMEER[1], Muhammad Asif KHAN[5], Shahid MAHMOOD[6], Imran Hayat[7]

Abstract

Spinach is highly contaminated with different pesticide residues. This study was designed to evaluate organochlorine and pyrethroid pesticide residues present in various washing treatments (T_0-T_{13}) of fresh and chemically washed spinach with respect to maximum residues limits. Efficiency of washing solutions (citric acid, acetic acid, garlic extract and ginger extract) of different concentrations (w/v %) along with tap water in spinach was investigated for residues dissolution. Results showed that the highest reductions in endosulfan, deltamethrin and cypermethrin residues were 0.008 ± 0.008 mg.kg^{-1} (53%), 0.023 ± 0.024 mg.kg^{-1} (76%) and 0.017 ± 0.014 mgkg^{-1} (83%) in treatment of T_5 (10% acetic acid) followed by 0.035 ± 0.029 mg.kg^{-1} (50%), 0.051 ± 0.029 mg.kg^{-1} (73%) and 0.037 ± 0.048 mg kg^{-1} (81%) in treatment T_{11} (10% ginger extract) while the lowest reductions in residues were 0.304 ± 0.004 mg.kg^{-1} (23%), 0.432 ± 0.030 mg.kg^{-1} (35%) and 0.468 ± 0.016 mg kg^{-1} (38%) in treatment T_2 (5% citric acid), respectively. In conclusion, 10% acetic acid, ginger extract and mixture of acetic and citric acids can effectively minimize pesticide residues in treated spinach.

Keywords: spinach; endosulfan; cypermethrin; deltamethrin; gas chromatography; washing treatments.

Practical Application: Household chemicals may be exploited to remove pesticide residues.

1 Introduction

Spinach (*Spinacia oleracea*) is a rich source of vitamin K, vitamin A, vitamin C, magnesium, manganese, iron, vitamins B, vitamin B6, vitamin E, dietary fiber, potassium and calcium. A lifelong continuous practice is that spinach contains more iron than other green leafy vegetables (Amir et al., 2015). In Pakistan, there is indiscriminate use of pesticide on crops to get high yield and spinach is one of the most vigorous pesticide-contaminated vegetable (Khan et al., 2020). Poor and insufficient exploitation of pesticides is a matter of great stress. The pesticide residues including organochlorine (OC) and pyrethroid (PYR) pesticides pose several health hazards to human including birth issues, reproductive disorder, circulatory problems, respiratory problems, endocrine and immune disorders and impaired central nervous system (Amir et al., 2019).

OC has long half-life even its very minute quantity poses a serious human neural problem by interfering with acetylcholinesterase. In food products, the presence of pesticides residues is a matter of real concern (Amir et al., 2019). At a time when these foods are consumed fresh, the problem is particularly serious (Solecki et al., 2005). Pyrethroids are insecticides with short mammalian poisonous quality that are utilized as a part of both rural and urban territories all inclusive (Abdullah et al., 2016). After reaching the natural environment, pyrethroids go between the three stages of solid, liquid and gas and originate into organisms via food chains, resulting in significant health hazards (Tang et al., 2018).

Traditional washing is the simplest form to mitigate the pesticide residues at both commercial and household level. Residues of various pesticides are removed by various washing treatments (Kaushik et al., 2009). Washing with 10% acetic acid mitigated deltamethrin (79.68%), cypermethrin (89.99%), chlorpyrifos (94.21%) and endosulfan (70.32%) in spinach (Amir et al., 2019). Residues of pesticide can be reduced by washing with tap water but high temp. water washing and blanching are considered to be more useful (Ahmed et al., 2011).

Abdullah et al. (2016) conducted a research to measure pesticide residues in contaminated spinach. The efficiency of chemical solutions such as acetic acid, citric acid, hydrogen peroxide, sodium chloride and sodium carbonate of different quantities (w/v %) along with tap water for the suspension of pesticide residues in spinach was studied the most astounding reduction in imidacloprid and acetamaprid residues individually Taking in to account all the above, the current research was

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[1]*Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan*
[2]*Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi, Pakistan*
[3]*Institute of Agricultural Sciences, Punjab University, Lahore, Pakistan*
[4]*Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Vehari, Pakistan*
[5]*Department of Food Technology, University of Agriculture Sub Campus Burewala, Burewala, Pakistan*
[6]*Institute of Food Science & Nutrition, University of Sargodha, Sargodha, Pakistan*
[7]*Department of Food Science and Technology, University of Poonch Rawalakot, Azad Kashmir, Pakistan*
*Corresponding author: raiamir87@yahoo.com

designed to determine the residual levels of endosulfan, deltamethrin and cypermethrin in spinach and to find the potential of different washing treatments to minimize these pesticide residues. At present very limited data is available not in Pakistan but throughout the world.

2 Materials and methods

2.1 Collection of samples

The spinach was grown in a controlled area where known quantity of pesticides such as cypermethrin, deltamethrin and endosulfan were administrated at regular intervals. Spinach samples were collected after a one day (24 h) standby time from a controlled field trial with the foliar spraying. The spinach samples were taken in the optimum degree of maturity from the controlled field trial in triplicate (Figure 1a and 1b). They were kept in airtight polyethylene bags for 24 h at normal room temperature (25 °C) and later transferred to the laboratory of Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University Rawalpindi for further analysis (Amir et al., 2019).

2.2 Washing solutions to treat spinach

Spinach samples were washed in various solutions such as acetic acid and citric acid at 5, 10, 2.5+2.5 and 5+5% w/v (g. 100 mL^{-1}) and in solutions of garlic and ginger at 5, 10, 2.5+2.5 and 5+5% w/v (g. 100 mL^{-1}) (Table 1). Tap water was also used to wash spinach samples (Zohair, 2001), the samples were kept immersed in a chemical solution for about 10 min at a temperature of 30 ± 5 °C.

2.3 Extraction of residues

Extraction of pesticides residues was carried out by Amir et al. (2019) with some modifications. Vegetable samples were chopped by using the knife into small pieces. A consistent paste/slurry was obtained by blending one kilogram of sample in the blender. 50 g of homogenized spinach slurry was mixed with 20 g anhydrous sodium sulfate, 10 g sodium chloride and 70-75 mL ethyl acetate. The whole mixture was shaken using a mechanical shaker at 240 rpm for 1 h and 20 min. After separation of phase, the supernatant was collected in an inert plastic bottle. The sample extract was filtered using Whatman (No. 4) filter paper. The filtered extract was stored at -40 °C before further analysis.

2.4 Clean-up of extract

The filtrate was passed through a column containing anhydrous sodium sulphate, silica gel, activated charcoal and glass wool for purification. Extract cleaning was carried out according to

Table 1. Plan of treatment, solutions and their concentrations.

Treatment (T)	Type of Solution	Percentage (%)
T_0	-	-
T_1	Tap water	-
T_2	Citric acid	5
T_3	Citric acid	10
T_4	Acetic acid	5
T_5	Acetic acid	10
T_6	Acetic acid+ Citric acid	2.5+2.5
T_7	Acetic acid+ Citric acid	5+5
T_8	Garlic	5
T_9	Garlic	10
T_{10}	Ginger	5
T_{11}	Ginger	10
T_{12}	Garlic + Ginger	2.5+2.5
T_{13}	Garlic + Ginger	5+5

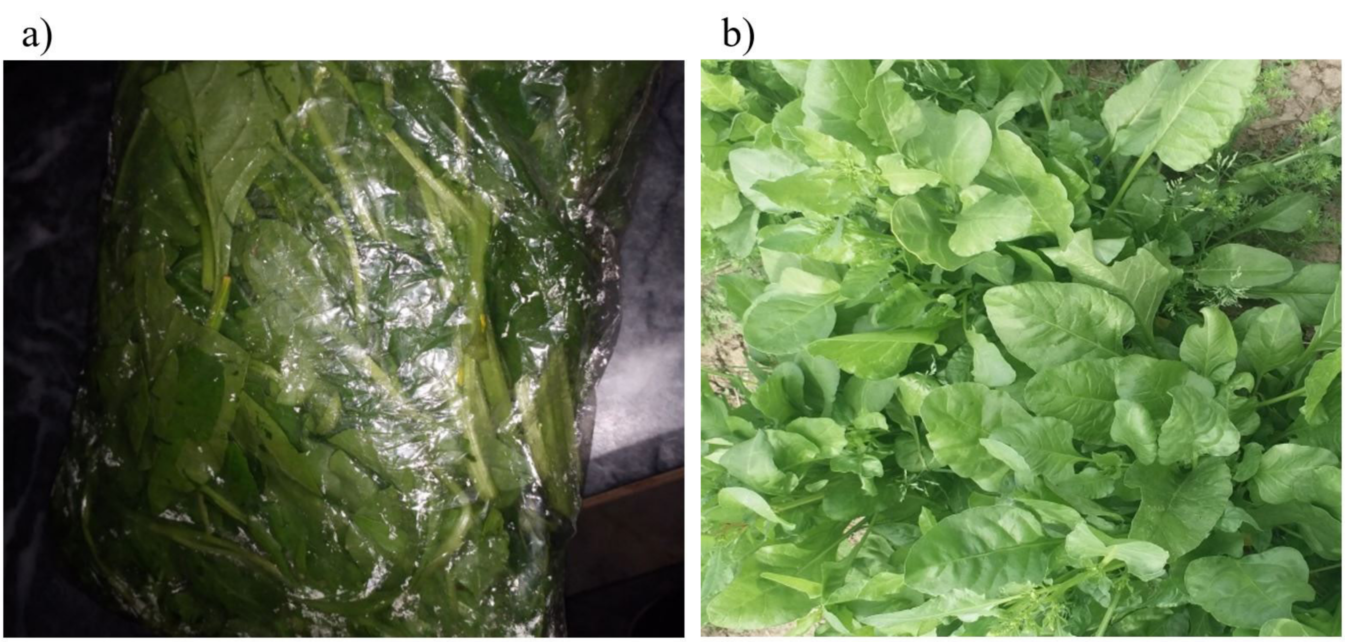

Figure 1. Sample collection of spinach samples (a); field trial of spinach sample (b).

the modified method of Baig et al. (2009) and stored at -40 °C until further GC-ECD analysis as shown in Figure 2.

2.5 Analysis of organochlorine and pyrethroid residues

The re-dissolved samples were subjected to a GC-ECD analysis according to the modified method of Amir et al. (2019) by an auto sampler into injector with the split closed for 2 min. The injector and detector temperature were 250 °C and 300 °C respectively. The temperature column was programmed from 130 °C (hold time: 1 min) to 200 °C at 10 °C min 1 (hold 10 min) and then from 200 °C to 232 °C for 1 min. The carrier gas was adjusted at 0.85 $mL.min^{-1}$ and the make-up at a flow rate of 60 $mL.min^{-1}$ at a temperature of 150 °C in an oven to measure organochlorine and pyrethroid residues. The gas chromatograph was equipped with a 63 Ni-ECD, a split/ split less injector operated in the split less mode, a fused-silica capillary chromatographic column (Agilent Technologies, Santa Clara, CA, USA) 60 m × 0.25 mm id. × 0.25 µm film thickness and an auto sampler. ChemStation software was used for instrument control and data treatment. Nitrogen was the carrier and make up gas (purity 99.9%).

Figure 2. Clean up of extracts.

2.6 Statistical analysis

All experiments were conducted in triplicate, and data was analyzed using Origin 8.6 (Microcal Software Inc., Northampton, MA, USA), Microsoft Excel (version 2013) and SAS (version 9.4, SAS Institute, Cary, NC, USA) software using one-way analysis of variance (ANOVA). All measurements were recorded as mean ± S.D. in triplicate manner (n = 3). The difference between the means was compared by applying Duncan's multiple range test at significance level of $p < 0.05$ (Johnson & Bhattacharyya, 2019).

3 Results and discussion

Sample was collected from the supervised field trail. Raw/ fresh spinach was taken as a control group symbolized as T_0. There are thirteen groups symbolized as T_1-T_{13} as shown in Table 1.

3.1 Endosulfan residues in fresh and chemically washed spinach

The results regarding endosulfan depicted significant variation among different washing treatments presented in (Figure 3). Results shown tap water T_1 reduces residues of endosulfan 11%. Washing solutions of citric acid and acetic acid 5% and 10% mitigate 23%, 38%, 36% and 53% endosulfan residues in treatments T_2, T_3, T_4 and T_5 respectively. Similarly, combination of citric acid and acetic acid solutions 2.5+2.5% and 5+5% eliminated 33% and 48% endosulfan residues in treatments T_6 and T_7 respectively. Likewise, garlic and ginger solutions of 5% and 10% concentration resulted in 28%, 45%, 35% and 50% endosulfan residues reductions in treatments T_8, T_9, T_{10} and T_{11} respectively. Furthermore, garlic and ginger solutions of 2.5% and 5% concentration each mix reduced the level of endosulfan residues up to 31% and 45% in Treatment T_{12} and T_{13}, respectively. Conclusively, 10% acetic acid solution showed better reduction potential than other washing solutions. while

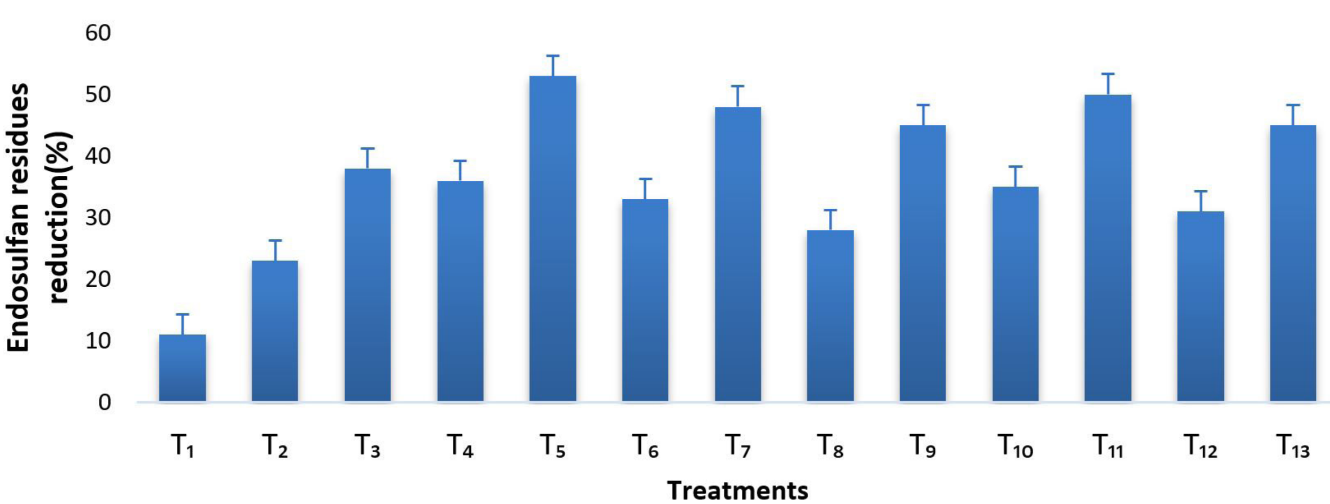

Figure 3. Mitigation percentages of endosulfan residues in treatments from T_1 to T_{13}.

10% ginger solution removed more residues than garlic solutions with different concentration. The residual level of endosulfan in number of samples in raw and chemical washed spinach were detected above the maximum residue limits (MRLs). But there was a high reduction in residues limit after applying different treatments like acetic acid solution and ginger solution treatments leads the residual level below to MRLs. The results of recent study are similar with the previous findings of Randhawa et al. (2014) who determined the level of endosulfan residues in spinach, cauliflower, potato, brinjal, tomato, and okra.

These results have also conformity with the finding of Abdullah et al. (2016) who investigated that residues of imidacloprid and acetamaprid were removed up to 69%-71% by using 10% acetic acid solution. The data obtained during this study are parallel with the previous findings of Amir et al. (2019) who found washing with 10% acetic acid mitigate deltamethrin (79.68%), cypermethrin (89.99%), chlorpyrifos (94.21%) and endosulfan (70.32%) in spinach. Randhawa et al. (2014) studied that washing demonstrated a huge impact on the removal of endosulfan in various vegetables. The way toward washing minimizes endosulfan up to 27% in spinach and 28% in cauliflower. It was seen that washing removes 15 to 28% in endosulfan residues.

3.2 Deltamethrin residues in fresh and chemically washed spinach

The results pertaining to deltamethrin shown significant variation among different washing treatments depicted in (Figure 4). In the recent study found, tap water T_1, removes 17% deltamethrin residues and washing solutions of citric acid and acetic acid 5% and 10% decreases 35%, 59%, 55% and 76% deltamethrin residues in treatments T_2, T_3, T_4 and T_5, respectively. Similarly, citric acid and acetic acid solutions collectively of 2.5+2.5% and 5+5% mitigated 47% and 70% in treatments T_6 and T_7 respectively. Likewise, garlic and ginger solutions of 5% and 10% resulted in 42%, 64%, 52% and 73% deltamethrin residues reductions in treatments T_8, T_9, T_{10} and T_{11}, respectively. Furthermore, garlic and ginger solutions of 2.5% and 5% together reduced the level of pesticide residues up to 45% and 69% in treatment T_{12} and T_{13} respectively. In all treatments, 10% acetic acid solution proved best for mitigation of deltamethrin residues than other washing solutions while in biological solutions 10% ginger solution reduced more deltamethrin residues than garlic solutions with different concentration.

Washing with chemical was more effective to lower the pesticides residue than tap water washing. The study showed that washing with water or chemical treatments were important to reduce the level of pesticide residues (Keikotlhaile et al., 2010). The recent data have similarity with the previous findings of Amir et al. (2019) who found washing with 10% acetic acid mitigate deltamethrin (79.68%), cypermethrin (89.99%), chlorpyrifos (94.21%) and endosulfan (70.32%) in spinach. These findings are also justified with the previous findings (Randhawa et al., 2014; Abdullah et al., 2016) who applied different household washing techniques for mitigation of pesticide residues in different vegetables. The recent work has also conformity with the findings of Zhang et al. (2007) who examined different concentration of sodium chloride and acetic acid, refrigeration, frying and tap water washing was successful reduced the level of 4 pesticides in cabbage. Tap water washing for 20 min minimize 17%, 17%, 19% and 15% and 10% acetic acid solutions for 20 min decreased 79%, 65.8%, 74% and 75.0%. Besides, 10% NaCl solution for 20 min reduced 67%, 65.0%, 73% and 74% and the decreases because of broiling (for 5 min) were 86%, 67%, 84% and 84% however because of refrigeration were 3%, 2%, 3% and 3%, respectively. Similarly, Bonnechère et al. (2012) quantified the effect of processing on the pesticide residues present in spinach. Spinach was sprayed with four fungicides and one insecticide deltamethrin. The impacts of processing were observed.10-50% residues decreased by washing with tap water.

3.3 Cypermethrin residues in fresh and chemically washed spinach

The overall results of pyrethroid pesticide residues (cypermethrin) including control and treated group present in spinach with three replicates are presented in (Figure 5). All these findings are significant with respect to different washing techniques. Results shown that tap water T_1 removing 17%

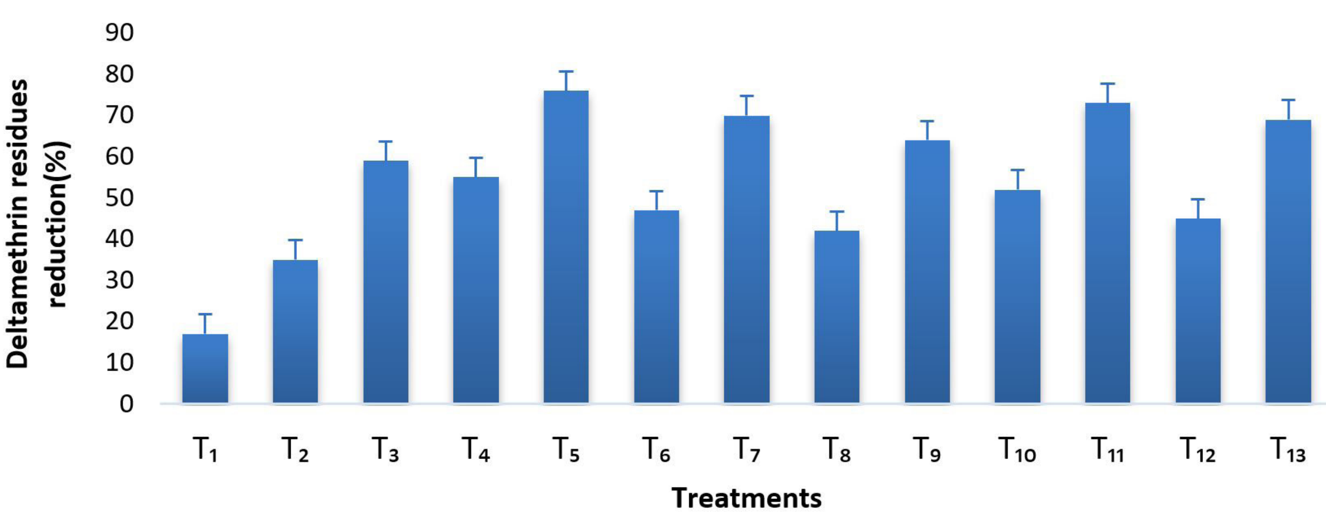

Figure 4. Mitigation percentages of deltamethrin residues in treatments from T_1 to T_{13}.

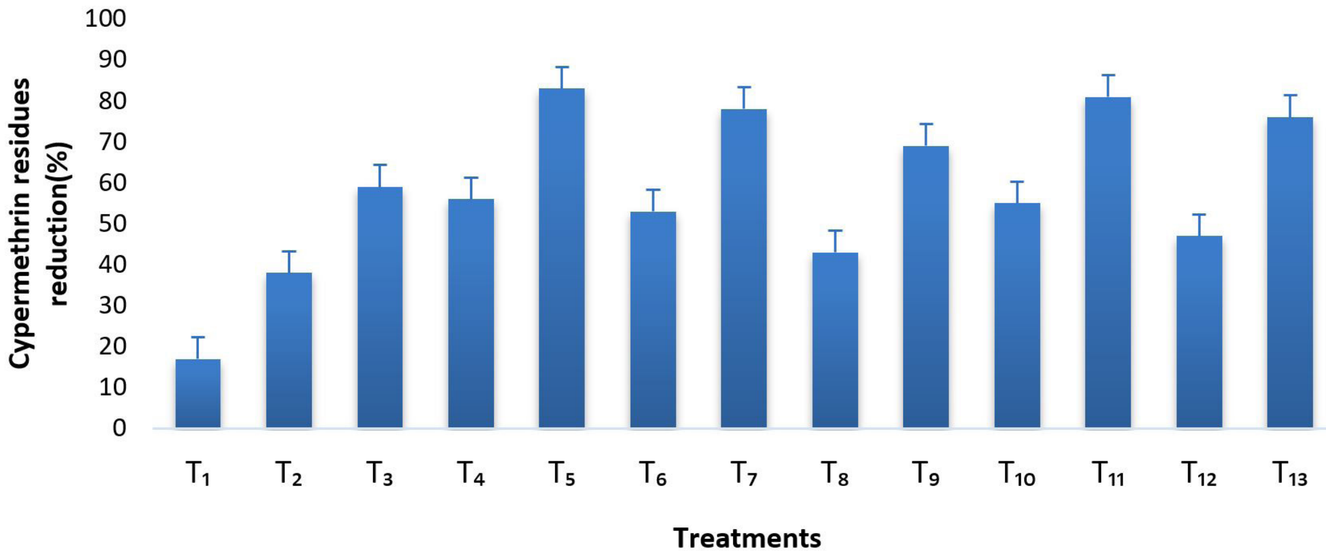

Figure 5. Mitigation percentages of cypermethrin residues in treatments from T_1 to T_{13}.

cypermethrin residue in spinach. Washing with acidic solutions like citric acid and acetic acid 5% and 10% mitigate 38%, 59%, 56% and 83% cypermethrin residues reductions in treatments T_2, T_3, T_4 and T_5, respectively. Furthermore, citric acid and acetic acid solutions collectively 2.5+2.5% and 5+5% concentration elucidate 53% and 78% in treatments T_6 and T_7 respectively. Moreover, garlic and ginger solutions of 5% and 10% resulted in 43%, 69%, 55% and 81% cypermethrin residues reductions in treatments T_8, T_9, T_{10} and T_{11}, respectively. Furthermore, garlic and ginger solutions of 2.5% and 5% concentration each mix together reduced the level of pesticide residues up to 47% and 76% in treatment T_{12} and T_{13}, respectively. In conclusion, all the washing solutions have the tendency to mitigate cypermethrin residues effectively, but 10% acetic acid solution showed better reduction potential than other washing solutions while 10% ginger solution reduced high level of residues than that of garlic and ginger solutions with different concentration.

The most widely recognized type of treatment is washing in which high percentage of pesticide residues reduced at household and commercial level. The result of the present study has conformity with the previous findings of Kumari (2008) who investigated residues of various pesticides which were removed by using of different washing treatments. Different chemical solutions were utilized to apply washing treatments that caused 20-77% reduction of pesticide residues in vegetables. While acetic acid and ginger solution showed high reduction of pesticide residues in spinach. It depends on washing adequacy relies upon site of the pesticides in vegetable, temperature, water solubility of the pesticide and type of wash. Polar water dissolvable pesticides are more effortlessly reduced as compare to the low polarity or less soluble pesticides like organochlorine pesticides. These results have relation with previous findings of Zohair (2001) who determined the mitigating impact of washing techniques to remove pesticide residues in various vegetables along with tap water washing.

From our results only tap water was used or in combination with different acidic, ginger or garlic solutions to mitigate pesticide residues. The acetic acid solution was more effective in reducing residues of endosulfan, deltamethrin and cypermethrin than other solutions. Delayed harvesting of vegetables after spraying with pesticide can reduce the adverse effects on peoples. Removal of pesticide residues is significant because they can carry harmful effects on human health, especially endocrine and immune disorders, and central nervous system problems. Chemical washing solutions of different concentrations were effective in reducing pesticide levels in spinach. The results further showed that removal of pesticide can be accomplished by washing with acetic acid, citric acid, ginger and garlic solutions. It has been discovered that the acetic acid solution was found to be most effective in removing pesticide residues. The results indicate a significant reduction in pesticide residues with increasing concentration of chemical washing solutions. This reduction indicates the ability to dissolve, higher concentrations of solutions can cause more dissolution and these results are consistent with those obtained through washing of fruits and vegetables.

4 Conclusion

This study demonstrated that the use of chemical reagents in washing treatments substantially reduced pesticide residues in spinach compared with tap water soaking alone. Among the applied solutions, acetic acid was found to be the most effective in reducing endosulfan, deltamethrin and cypermethrin residues in spinach followed by ginger extract and the mixture of acetic acid and citric acid, respectively.

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