

# Methods of Removing Pesticide Residues in Tomato (*Lycopersicon Esculentum*) and Gotukola (*Centella Asiatica*)

G.M.S.A. Sarada<sup>1</sup>, C.P. Rupasinghe<sup>1</sup>, C. Magamage<sup>2</sup>

<sup>1</sup>Department of Agricultural Engineering, Faculty of Agriculture, University of Ruhuna, Mapalana, Sri Lanka  
[ama123sarada@gmail.com](mailto:ama123sarada@gmail.com)

<sup>2</sup>Horticultural Crop Research and Development Institute, Department of Agriculture, Peradeniya, Sri Lanka  
[champamgmg@gmail.com](mailto:champamgmg@gmail.com)

**Abstract:** Pesticides are widely used by the farmers in agriculture to increase the yield by controlling any undesirable plant or animal species. Pesticide residues can remain in fruits and vegetables as a result of neglecting recommended dosages of pesticides at the application and neglecting pre-harvest intervals. It is important to identify methods for removing pesticide residues in order to ensure food safety. This study was conducted to identify the methods for removal of pesticide residues in vegetables, to evaluate the effectiveness of those methods and to suggest the most appropriate method among them. Tomato (*Lycopersicon esculentum*) and Gotukola (*Centella asiatica*) were selected for the study as according to previous studies they contain higher amounts of pesticide residues and both are consumed mainly as fresh salads. Samples were collected from Central Market, Kandy. Analysis was carried out for the pesticides of Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine, which are recommended for Tomato and Gotukola. Two samples from each vegetable were taken. In the first sample, tomato was spiked with 1000 ppm Fipronil and gotukola was spiked with 1000 ppm Pymetrozine. In the second sample, both vegetables were spiked separately with a mixture of all the four pesticides. Each spiked sample was divided into 4 portions; one part was kept as the control for direct residue analysis and the other three parts were subjected to removal methods; washing twice with tap water for 1 minute, dipping in 3% salt solution for 10 minutes and dipping in 4% vinegar solution for 10 minutes. The samples were extracted for pesticide residue analysis using QuEChERS, AOAC 2007.01 method. Residual analysis was carried out using Liquid Chromatography-tandem Mass Spectrometry (LC-MS-MS). According to the results, vinegar treatment has given the highest pesticide residual reduction. Salt treatment also has given considerable reduction percentages. Washing with tap water has given the lowest reduction percentages for all the four pesticides in both tomato and gotukola. So people are advised to consume these vegetables after dipping in 4% vinegar solution for 10 minutes. However, further studies should be carried out using more samples and for more pesticides by changing the concentrations of solutions and changing the time of applying treatments.

**Keywords**— LC-MS-MS; Pesticide residues; QuEChERS; Removal methods

## 1. INTRODUCTION

To maintain the quality of fruit and vegetable harvests by reducing losses, pesticides are used during cultivation of crops along with other pest control techniques to destroy pests and prevent diseases [1]. A pesticide is a substance or a combination of substances applied in order to prevent, regulate, destroy or repel any undesirable plant or animal species [2]. Pesticide use is increasing because it acts quickly, reduces toxins produced by food-borne organisms, and is less labor intensive than other pest control methods. However, the use of pesticides during production often retains pesticide residuals on fruits and vegetables after harvest [1].

When sprayed, pesticides decompose very slowly. Each pesticide requires some period for its dissipation [3]. Due to the high demand for fresh fruits and vegetables, pre-harvest intervals and recommended doses when using pesticides have been ignored by farmers and this may lead to higher pesticide residue (PR) levels [4]. Pesticides are applied to fruits and vegetables at different stages of production. Therefore, they may retain on different parts of the same fruit or vegetable [5]. The residual effect of pesticides in various food ingredients depends on several factors such as the nature of pesticide molecules, type of food, and environmental

conditions [6]. Mostly, the traces of pesticides are remained on the peel surfaces in fruits and vegetables [7]. Only small amounts of systemic pesticides can be ingested into flesh [8]. PR concentrations near the epidermis and in fruit stalks are higher in fruits and fruit-type vegetables than in pulp or pericarp. PR levels in leafy vegetables are lower on inner leaves than on outer leaves [9].

Fresh fruits and vegetables make up 30% of the human diet and therefore considered to be a major food source that makes people highly exposed to pesticides. Also they contain a lot of PR because mostly they are minimally processed or consumed in raw forms [10]. Tomato (*Lycopersicon esculentum*) and Gotukola (*Centella asiatica*) were selected for this study considering several reasons. Tomato is a widely cultivated vegetable in countries all over the world. It is also called as the apple of poor men due to its popularity among majority of people, especially the poor as it is available at lower prices. It is having high nutritive value and many economic benefits and mostly consumed as fresh salads, sauce, ketchups, soups and as many other products. Tomato is a vegetable which is highly susceptible for pest attacks like leaf minor and fruit borer in the field. So pesticides are heavily used in tomato cultivation [11]. Gotukola is consumed as a food and a beverage and it is a valuable medicinal plant having antioxidant and neuroprotective

properties. It belongs to the family apiaceae and is a creeping perennial herb. It is grown naturally and also cultivated as a crop and has a higher demand due to its medicinal properties. It is consumed as a vegetable in many Asian countries including Sri Lanka. It is consumed raw as salads and also it can be cooked. Beverages are also prepared using this plant [12]. Pesticides are heavily used in cultivation of both of these crops and both are consumed in fresh form. So there is a higher risk of ingestion of PR by the consumers.

Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine are the pesticides that were analyzed in this study. Fipronil is a phenylpyrazole broad spectrum insecticide. It is highly effective and is used widely for the control of various soil and foliar insect species of rice, fruits and vegetables [13]. Thiamethoxam is a neonicotinoid which can control many species of sucking and chewing insects in many agricultural crops [14]. Relatively higher water solubility of 4100 mg/l at 25°C [15]. It is recommended all over the world for foliar application, soil and seed treatments in large number of agricultural crops [16]. Imidacloprid is an effective broad spectrum neonicotinoid insecticide primarily acting against sucking insects and also against some Coleoptera, Diptera and Lepidoptera. Due to its excellent systemic activity and excellent residual properties, it is particularly suitable for soil application, seed treatment and foliar application and suitable for a variety of economically important crops due to its wide range of activity and excellent crop tolerance [17]. Pymetrozine is a highly active systemic insecticide which is specifically used to control sucking insect pests. Also used to control whiteflies and aphids in crops. It is a highly selective pesticide having a water solubility of 290 mg/l at 25° C [18].

Residue analysis provides an overview of the degree of pesticide contamination of fruits and vegetables and the polluting delay of chemical contamination in ecosystems. Analysis of PR is very important for assessing the safety of certain pesticides used [3]. In order to protect and improve human health and the environment, it is essential to identify and determine the level of harmful contaminants in our food and the environment. PR measurement helps to protect our community, and to improve better production practices [2]. Pesticides are present in fruits and vegetables at low concentrations, usually in parts per million (ppm). Currently, there are many analytical methods for detecting PR values, all of which involve certain basic steps such as sampling, sample preparation, extraction, clean-up, identification, etc. [19]. Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry (GC-MS, LC-MS) and Gas Chromatography, Liquid Chromatography tandem Mass Spectrometry (GC-MS-MS, LC-MS-MS), with triple quadrupole mass analyzers are the commonly used techniques in multi-residue target pesticide analysis in present days [3].

The presence of excess PR can cause blindness, cancer, liver and nervous system damage, and more. Long-term effects can reduce live sperm and fertility, increase

cholesterol levels, increase child mortality, and lead to metabolic and genetic disorders [20]. So it is very important to identify methods for removing PR from fruits and vegetables. Various studies have been carried out to identify such methods. Washing with water will reduce the PR of vegetables to some extent. Fresh fruits and vegetables are usually washed with tap water after harvesting, but most pesticides are hydrophobic, so this has a little effect on PR [21]. Washing with dilute salt (sodium chloride) is also an effective way to reduce food surface contamination, especially for fruits and vegetables. Washing with chemicals such as chlorinated water and ozone water, and diluents of other chemicals such as acetic acid and sodium bicarbonate have been very successful in removing PR from fruits and vegetables [6]. Washing vegetables with tap water and detergent solutions prior to cooking is important to decrease the residue concentration. Washing with Acetic acid solutions (at 10% concentration for 20 min, Washing with Sodium chloride solutions (at 10% concentration for 20 min), washing with tap water (for 20 min) and refrigeration reduces PR in vegetables like cabbage [22]. Washing with detergents is more effective than washing with just tap water. Washing and peeling removes about 10-85% of insecticides. Cooking and frying reduces about 25-100% PR in vegetables. Washing with tamarind water or vinegar gives a reduction of 95% of PR [23]. Cumulative effect of washing under tap water, soaking in 2% salt solution and cooking reduces PR in tomato by 95% [24].

## 2. MATERIALS AND METHODOLOGY

### 2.1 Sample Collection

Tomato (*Lycopersicon esculentum*) and Gotukola (*Centella asiatica*) samples were collected from Kandy market. They were brought to the laboratory in ice boxes and they were stored in the refrigerator.

### 2.2 Preparation of the Standard Stock Solutions of Pesticides

Fipronil, Thiamethoxam, Imidocloprid and Pymetrozine were selected for the study according to the pesticide recommendations for tomato and gotukola by the Department of Agriculture, Sri Lanka. The 1000 ppm solutions were prepared using Certified Reference Materials (CRM) of pesticides. Purity percentages of Certified Reference Materials were taken into consideration for deciding the weight of the CRM to be used.

Table 1: Purity percentages of pesticide standards

Pesticide	Purity %
Fipronil	98

Thiamethoxam	99.3
Imidacloprid	100
Pymetrozine	99.9

Source: Sigma-Aldrich

The standards of selected pesticides were taken out from the refrigerator. The calculated weights from each pesticide standard were measured using the electronic analytical balance. They were dissolved in LC grade Methanol in separate volumetric flasks. They were shaken well and vortexed. They were labelled and stored in the freezer at 4-6° C.

### 2.3 Preparation of the Pesticide Mixture and

The prepared standard stock solutions of Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine in 1000ppm concentrations were mixed in equal volumes in a 10ml volumetric flask. It was shaken well and vortexed.

### 2.4 Spiking of Pesticides

Two samples from each vegetable (tomato and gotukola) were taken. In the first sample; tomato was spiked with 1000 ppm Fipronil (recommended pesticide for tomato) and gotukola was spiked with 1000 ppm Pymetrozine (recommended pesticide for gotukola) at 2 ppm concentration in weight basis. In the second sample; both tomato and gotukola were spiked separately with the prepared pesticide mixture at 1 ppm concentration in weight basis. The spiked samples were mixed well from time to time for uniform distribution of pesticides and they were kept under room temperature for about 24 hours for better absorption of the pesticides.

### 2.5 Treatments

After 24 hours, each spiked sample was divided into 4 portions; one portion was kept as the control for direct residue analysis and the other three portions were subjected to removal methods. One part was washed twice with tap water for 1 minute each time. Other part was dipped in 3% salt solution for 10 minutes. The other part was dipped in 4% vinegar solution for 10 minutes. Three replicates were taken from each of the four portions of tomato and gotukola. The treated samples were cut into coarse pieces and they were stored in the freezer at -18° C in sealed polythene bags. The knife and the cutting board were washed well with water and acetone after cutting each treated sample to avoid cross contamination [25]. Followed experimental procedures for each sample are given in table 2.

Table 2: Summary of the methodology

Sample No.	Vegetable	Spiked/ Not spiked	Sample Type
	Tomato	Spiked with 1000	Control
			Water treated

Sample 1	Gotukola	Spiked with 1000 ppm Pymetrozine	Salt treated
			Vinegar treated
Sample 2	Tomato	Spiked with 1000 ppm pesticide mixture	Control
			Water treated
			Salt treated
	Gotukola	Spiked with 1000 ppm pesticide mixture	Vinegar treated
			Control
			Water treated
Salt treated			
Vinegar treated			

### 2.6 Sample Extraction Procedure

Sample extraction was done using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) AOAC 2007.01 method [26]. First, 100ml of acetonitrile acidified with 1% of acetic was prepared. Frozen samples were taken out from the refrigerator, one at a time. They were grinded separately using the grinder. The grinder was cleaned well with water and acetone after grinding each sample to avoid cross contamination. Three 50ml centrifuge tubes were taken and 10±0.1g of the grinded sample was added into each tube in order to take replicates. Each test tube was brought under the fume hood and 10ml of acidified acetonitrile was added to it. Then a 7.5g QuEChERS Extraction Pouch containing 6g Magnesium Sulphate and 1.5g Sodium Chloride was added to it. The tube was shaken well immediately after adding the Extraction Pouch to avoid formation of clots and then it was vortexed for 1minute.

Then the tubes were centrifuged at 3000 rpm for 3 minutes. Then the aliquot from the supernatant layer was transferred into dispersive SPE clean-up kits. For tomato, 15ml general clean-up kit was used as it is a lightly pigmented vegetable and for gotukola, 2ml pigmented clean-up kit was used as it is a highly pigmented vegetable. The 4ml General Kit contains 400mg Primary, Secondary Amine (PSA) and 1200mg Magnesium Sulphate. The 2ml Pigmented Kit contains 50mg PSA, 50mg Bulk Carbograph and 150mg Magnesium Sulphate. After transferring the aliquot, the tube was shaken well and was vortexed for 1 minute. After finishing this step, the vortexed Solid Phase Extraction (SPE) tubes were centrifuged at 3000rpm for 3minutes. The supernatant layer was taken into a plastic syringe and it was filtered into LC vials using 0.22µm Nylon

filters. Finally the filtered extract was subjected to LC-MS-MS analysis [26].

### 2.7 Analysis of PR

PR were analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS) technique. Thermo Scientific Endura Triple Quadrupole Liquid Chromatography Tandem-Mass Spectrometer of Ultimate 3000 series was used for the analysis. Ultra-High Performance Liquid Chromatography (UHPLC) is used in this. It consists of pump, auto sampler and column compartment. The stationary phase consists of column stored with methanol, having a length of 100mm, internal diameter of 2.1mm and a particle size of 2.6µm. Column temperature is maintained at 30<sup>0</sup> C. The injection volume is 5µl. The flow rate is 0.3mlmin<sup>-1</sup>. The mobile phase comprises of (A) Ammonium formate and Formic acid in water and (B) Ammonium formate and Acetic acid in Methanol. The LC vials were stored in the tray in definite positions and the samples were auto injected by the injector.

### 3. RESULTS AND DISCUSSION

Washing of vegetables is required in domestic level as well as in commercial level in order to remove biological contaminants and also the PR. Several procedures have been suggested to remove PR in vegetables without any clear scientific evidences [27]. Mostly the highest amounts of PR remain in the outer surface of the vegetables and they can be removed by washing. Tomato and Gotukola are consumed mainly as fresh salads so it is important to apply these treatment methods in order to remove PR before consumption [12, 23]. So the effectiveness of three types of washings in removal of PR were evaluated by this research and the obtained results are indicated below using tables and graphs. The reduction percentages of PR were calculated using the following equation 1.

$$\text{Average reduction percentage} = \frac{\text{Average PR in control} - \text{Average PR in treated sample}}{\text{Average residue level in control}} \times 100 \quad (1)$$

#### 3.1 The Effectiveness of the Treatments in Removing Pesticide Residues in Tomato Spiked with Fipronil

According to Table 3 and Fig.1, all the three treatments have reduced the initial Fipronil residue levels in tomato in different percentages. The water treatment has given the least reduction percentage of 24.3%. Vinegar treatment has given the highest reduction percentage of 85.69% and salt treatment also has given a higher reduction percentage of 78.13%. So according to the results, the vinegar treatment is the most suitable treatment to reduce the Fipronil residue level in tomato while salt treatment also can be used and water treatment is the least suitable method.

Table 3: The average pesticide residue levels and average reduction percentages given by each treatment in the Tomato sample spiked with Fipronil

Sample	Average Residue (ppb)	Average Reduction (%)
Control	500.93	-
Water treated	379.1925	24.3%
Salt treated	109.555	78.13%
Vinegar treated	71.665	85.69%

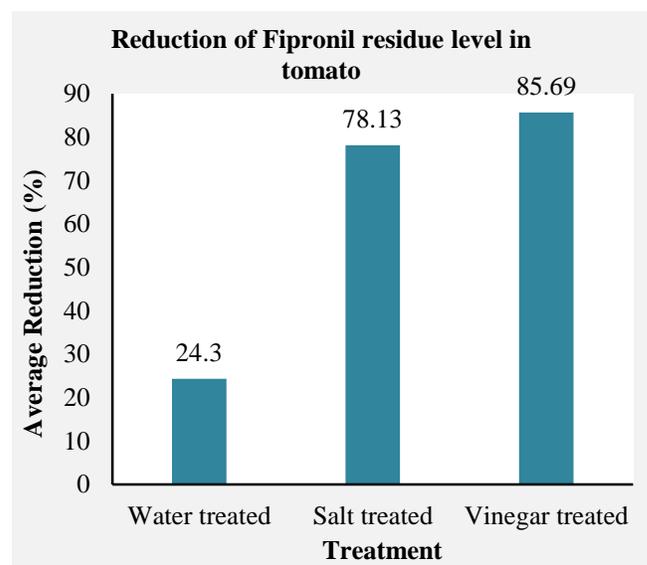


Figure 1: Average reduction percentages given by each treatment in Tomato spiked with Fipronil

#### 3.2 The Effectiveness of the Treatments in Removing PR in Gotukola Spiked with Pymetrozine

According to the data obtained (Table 4 and Fig. 2), vinegar treatment has almost completely removed the Pymetrozine residues in gotukola, as a percentage of 93%. Salt treatment has also given a higher reduction percentage of 84.66%. Water treatment has given comparatively lower percentage of reduction. So washing with 4% vinegar solution is the most suitable treatment to remove the Pymetrozine residues in Gotukola and the 3% salt solution is also suitable. Washing with water is the least successful treatment in removing Pymetrozine residues in Gotukola.

Table 4: The average PR levels and average reduction percentages given by each treatment in the Gotukola sample spiked with Pymetrozine

Sample	Average (ppb)	Reduction (%)
Control	532.145	-
Water treated	261.225	50.91%
Salt treated	81.635	84.66%
Vinegar treated	37.29	93%

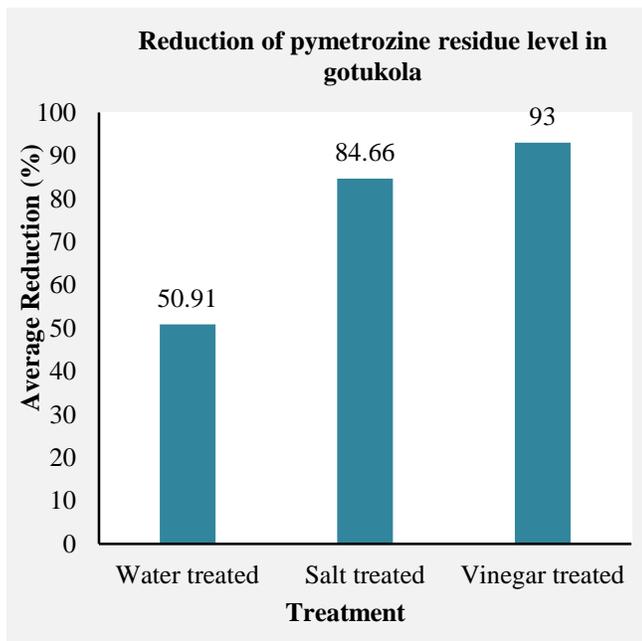


Figure 2: Average reduction percentages given by each treatment in Gotukola spiked with Pymetrozine

Table 5: Average PR levels (ppb) in tomato spiked with pesticide mixture before and after applying the treatments and the average reduction percentages given by the treatments

Sample		Fipronil	Thiamethoxam	Imidacloprid	Pymetrozine
Control	Residue (ppb)	524.87	333.38	430.34	459.03
Water treated	Residue (ppb)	370.01	307.51	401.79	383.50
	Reduction %	29.50%	7.76%	6.63%	16.45%
Salt treated	Residue (ppb)	153.52	172.80	314.99	279.41
	Reduction %	70.75%	48.16%	26.80%	39.13%
Vinegar treated	Residue (ppb)	65.2	136.90	297.15	147.96
	Reduction %	87.58%	58.93%	30.95%	67.76%

### 3.3 The Effectiveness of the Treatments in Removing PR in Tomato Spiked with Pesticide Mixture

According to Table 5 and Fig. 3, it is clear that vinegar has given the highest reduction percentages in all the four pesticides when they are applied as a mixture. Salt treatment also has given higher reduction percentages but less effective than vinegar. Water treatment has given the lowest reduction percentages in all the four pesticides. So it is evident that vinegar is the most suitable treatment to remove the residues of given four pesticides in tomato, even when they are available as a mixture.

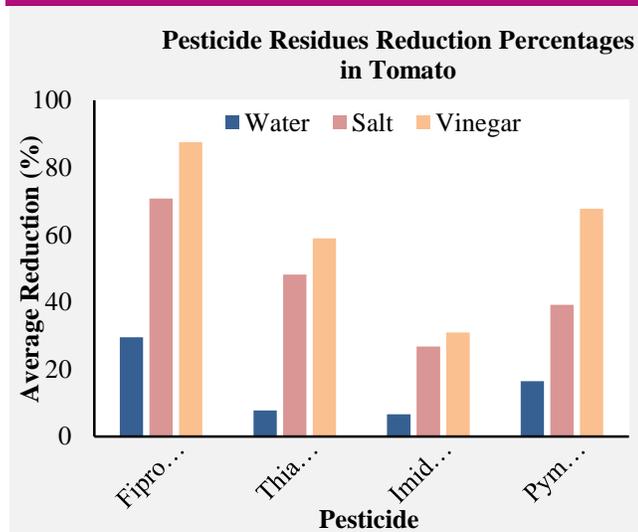


Figure 3: Average reduction percentages given by each treatment in Tomato spiked with pesticide mixture

Table 6: Average PR levels (ppb) in Gotukola spiked with pesticide mixture before and after applying the treatments and the average reduction percentages given by the treatments

Sample		Fipronil	Thiamethoxam	Imidacloprid	Pymetrozine
Control	Residue (ppb)	639.14	706.61	594.82	557.56
	Reduction %				
Water treated	Residue (ppb)	431.815	599.57	507.43	230.42
	Reduction %	32.44%	15.15%	14.69%	58.67%
Salt treated	Residue (ppb)	308.97	459.71	358.90	24.89
	Reduction %	51.66%	34.94%	39.66%	95.53%
Vinegar treated	Residue (ppb)	209.27	316.10	229.13	14.22
	Reduction %	67.26%	55.26%	61.48%	97.45%

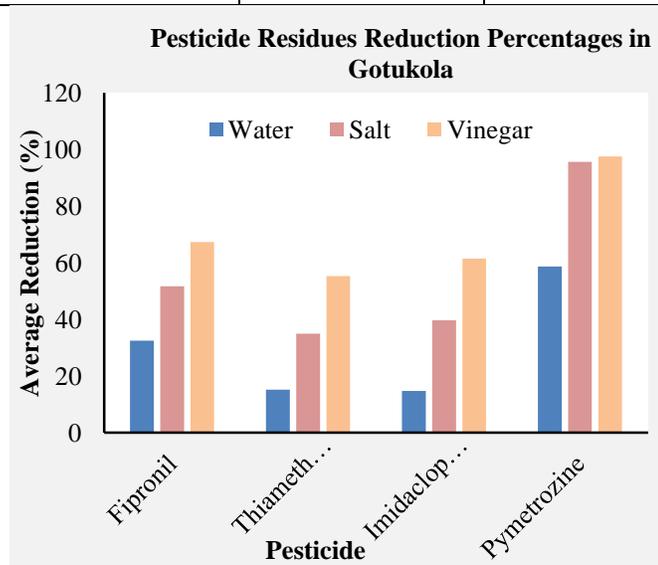


Figure 4: Average reduction percentages given by each treatment in Gotukola spiked with pesticide mixture

Processes such as photolysis, hydrolysis, oxidation and reduction, metabolism, temperature, pH, etc.

### 3.4 The Effectiveness of the Treatments in Removing PR in Gotukola Spiked with Pesticide Mixture

According to the results obtained (Table 6 and Fig. 4), it was found that vinegar has given the highest reduction percentages in all the four pesticides, when available as a mixture. Salt treatment also has given higher reduction percentages in all four pesticides. Water treatment has given the lowest reduction percentages in all the four pesticides. So it is clear from the results that dipping in 4% vinegar solution is the most suitable treatment to reduce the above mentioned PR in Gotukola, even when available as a mixture of pesticides.

can affect vegetable PR (28). The major factor affecting the effectiveness of the treatments in removing PR is the solubility of the pesticides in tap water, salt solution and vinegar solution. Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine are water soluble pesticides. Their solubility may have affected for the effective removal of the PR. Also the physicochemical properties of the pesticide, the time of contact with the treatment solution, the concentrations of the solutions, the nature of the vegetable, pH of the solutions and the temperature affect for the effectiveness of the removal [29; 30]. The effect from temperature can be neglected as all the treatments were carried out under room temperature of 28°C and same water was used for direct washing and also for preparing the solutions. The main component of salt is Sodium chloride, and in vinegar it is Acetic acid and tap water contains chloride ions. The pH of tap water used in this research was around 6.8, pH of the salt solution was 6.67 and pH of the vinegar solution was 3.7. At the same time Acetic acid, the main component of vinegar acts as a buffer. According to the results obtained during the research, the effectiveness of the removal of PR in *Lycopersicon esculentum* and *Centella asiatica* has increased with the decrease of pH values.

According to the results obtained from this research all the three treatments; washing with tap water, 3% salt solution and 4% vinegar solution have reduced the PR levels that were present in the control samples. Washing just with tap water for one minute has removed all the four pesticides in tomato and gotukola in lowest percentages compared to other two methods. Dipping in 3% salt solution for 10 minutes has removed all the four pesticides in tomato and gotukola in higher percentages. Dipping in 4% vinegar solution has given the highest percentages of reduction in all the four pesticides Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine.

Many researches have been carried out previously using different treatments to find an appropriate method for removal of pesticide residues in fruits and vegetables. Washing with tamarind water or vinegar has given a reduction of 95% of PR [23]. Acetic acid of 5% concentration has removed over 40% of PR present in tomato [27]. Washing tomato under tap water has reduced the residue levels by 48-53%. Washing with 2% salt solution has reduced the residue levels in tomato by 78%-90% [24]. Washing with tap water has given the least reduction percentages for PR in cabbage, reducing the residue levels by 15.2%-17.6%. Washing with 10% sodium chloride solution has given reduction percentages of 65%-74.1%. Washing with 10% acetic acid solution has given the highest reduction percentages of 74%-79.8% [22].

So it is clear that the results obtained from this study agree with the results of previous researchers [6], [22], [23], [24], [27]. But the results obtained in this kind of a research may vary according to the type of pesticide and type of vegetable used. So it is better to carry on further researches by changing the pesticides, increasing the number of samples, changing the concentrations of the treatments and changing the time of treatment.

#### **4. CONCLUSION AND RECOMMENDATIONS**

This research was conducted to identify the most suitable method that could be practiced specially in domestic level to remove the PR present in tomato and gotukola. And the effectiveness of three treatments were evaluated in this research to find out the most suitable treatment among them. From the results obtained from the research, it is concluded that the PR of Fipronil, Thiamethoxam, Imidacloprid and Pymetrozine present in Tomato and Gotukola could be effectively removed by all the three treatments; washing twice with tap water for one minute each time, washing with 3% salt solution for 10 minutes and washing with 4% vinegar solution for 10 minutes. Washing with vinegar solution is the most effective treatment in removing all the four pesticides. Washing with salt solution is also effective, but less effective than the vinegar treatment. Washing with tap water is the least effective method in removing the above mentioned four pesticides present in tomato and gotukola. So the consumers of tomato and gotukola are advised to use them after being washed properly with 4% vinegar solution for at least 10 minutes. Suggestions for future research are to carry out the

research by changing the concentrations of the solutions, changing the time of treatment, increasing the sample amount, applying treatments for more pesticides and for other vegetables. It is better if the changes in sensory properties of the vegetables are evaluated after each treatment.

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