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Study of effect of different types of veg wash on the microbial flora as well as residual pesticide Lambda cyhalothrin on vegetables

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ABSTRACT: A vegetable wash is a cleaning product designed to aid in the removal of dirt, wax and pesticides from agricultural produce before they are consumed. Different types of vegwash are composed of NaCl, citric acid, glycerine, potassium sorbate, sodium benzoate and Neem - Tulsi extracts. Present study focused on the effectiveness of the vegwashes available in the Indian market. By selecting seven samples of veg washes viz Nim wash, Veggie Clean, Wiz Vegetable & Fruit Wash, Dr + Rhazes, veg fru wash and OrgaNature, effect of them on the total viable bacteria on the surfaces of vegetables was tested, where washing with water was served as a control. Action of the vegwash was tested on indicators of faecal pollution like *E. coli* and *Pseudomonas* and on pathogens such as *Salmonella typhi*, *Shigella*, *Staph aureus*, and *Klebsiella*. To check its effectiveness for removal of pesticides, the HPLC method was carried out. Wiz wash, Nim wash and healing touch have shown inhibitory action on the majority of pathogens. Nim wash and healing touch have shown decrease in the total viable microflora of the vegetables. HPLC results have shown that these veg washes were unable to remove traces of pesticide present on vegetables.

KEY WORDS: bacteriological analysis, lambda-cyhalothrin, pathogens, vegwash, vegetables

I. INTRODUCTION

Contamination of vegetables and fruits with human pathogens may occur at any point throughout the production, harvesting, packing, processing, distribution, or marketing i.e. from transportation from the farm to the store shelf, where exposure to human or animal faeces is possible.[1] Numerous commercial washing formulations for fresh produce are available, including surfactant solutions, combinations of surfactants with organic or mineral acids, and alkaline washes that are claiming the removal of microbes and pesticides on vegetables and fruits.[2] According to manufacturers of vegwash liquids, rinsing alone with tap water is not enough to remove the germs and pesticide residues. Generally, vegetables retain 10^3 to 10^5 microorganisms cm-3 or 10^4 to 10^7 microorganisms g-1 if they are not washed properly. Some of the most noticeable bacterial types are lactic acid bacteria, Corynebacterium, Enterobacter, Proteus, Micrococcus, Enterococcus, Pseudomonas and several spore-formers.[3] Unwashed vegetables may make it possible to grow different types of molds, such as Alternaria, Fusarium, and Aspergillus on their surfaces. Listeria monocytogenes, Salmonella spp., Shigella spp, Clostridium botulinum, Clostridium perfringens and Escherichia coli are important pathogens reported to cause food borne infections associated with different vegetables. [4,5]

The fruits and vegetables we eat are usually sprayed with pesticides, making it harmful to health. Lambda-cyhalothrin is the pesticide said to be the most commonly used by vegetable growers. It has a low water solubility, moderate acute oral, dermal, and inhalation toxicity in humans. The residual levels of Lambda-cyhalothrin, a pyrethroid insecticide, has been determined in fruits by high-performance liquid chromatography (HPLC). [6] Available vegwashes in the market are composed of sodium chloride, citric acid, glycerine, potassium sorbate, sodium benzoate and plant extracts (Neem, Tulsi). Since the brand claims that it is a naturally derived cleaner, it is safe to use and will not deplete the nutritional value of food. It does not leave any aftertaste, smell or residue and is free from harmful preservatives, chlorine, soap and alcohol.[7]During the outbreak of corona, contamination of vegetables and food were the subject of concern all over the world. Owing to the threat, a number of companies entered in the field of vegwash production. Consumers assume that washing and sanitizing vegetables and fruits will reduce the microbial load, but in reality, research data on the said topic indicates that these conventional methods are not capable of reducing the microbial population on the produce. The response of microorganisms to washing and sanitizing treatments will depend on the conditions of contamination that affect attachment and survival on produce surfaces. However, better results obtained after use of cleaning agents such as chlorine dioxide, ozone and peroxyacetic acid. The studies evaluating the effect of veg wash are mainly based on artificially contaminated vegetables and fruits, where the results are mainly depending on the selection of the kind of microorganism used, time interval between inoculation and treatment,

degree of agitation, and temperature like parameters. While in present study, the total viable count of the own microflora of five agro based produce such as cucumber, tomato, cabbage, cauliflower and fenugreek, which are consumed in raw state as part of salad was analyzed after washing the produce with plain water as well as after treatment of seven different vegwashes separately. Action of the vegwash was tested on indicators of faecal pollution like *E. coli* and *Pseudomonas* and on pathogens such as *Salmonella typhi*, *Shigella*, *Staph aureus*, and *Klebsiella*. To check its effectiveness regarding removal of pesticides, the HPLC method was carried out.

II. Materials and Methods

Sample collection : Seven samples of veg wash were collected from different local markets in Nashik city, named as Nim wash, Veggie Clean, Wiz Vegetable & Fruit Wash, Dr + Rhazes, Veg fru wash and OrgaNature between January 2021 and January 22. Similarly fresh vegetables like Fenugreek, Cauliflower, Cabbage, Tomatoes, Cucumber were collected randomly from various farmers in Nashik city who are applying lambda cyhalothrin as pesticide for their produce between January 2021 and January 22. Samples of vegetables were collected in sterile plastic bags and transported to the laboratory for processing. These samples of vegetables were processed following the standard method.

Processing of the Samples of produce Samples were cut in small portions, weighed 5 gms of each sample, and in duplicate, were placed in sterile screw capped tubes, and stored in the refrigerator.

Processing of the samples of veg washes Based on specific directions by the manufacturer on the labels of the veg wash containers, the samples of vegetable wash liquids were diluted aseptically using sterile water. (Table 1.)

Table 1. Dilution of samples

Dilution of Samples

Sample Name	Veg wash sample (ml)	Water (ml)	Washing Time
1. Dr + Rhazes	15 ml	1000 ml	10 min
2. Orga Nature	10 ml	1000 ml	10 min
3. Veggie Clean	15 ml	2000 ml	5 min
4. Nim wash	20 ml	1000 ml	15 min
5. Veg Fruit	15 ml	500 ml	1 min
6. Wiz wash	50 ml	1000 ml	10 min
7. Healing touch	10 ml	1000 ml	2 min

The diluted veg wash was used for washing of samples of vegetables as per the guidelines by the manufacturer by maintaining aseptic conditions. After washing with veg wash, the same product was subjected to wash with distilled water, and these after-wash samples (VW) were plated using pour plate technique to enumerate the total count of bacteria. Control was kept, using distilled water for washing of duplicate sets of produce. These control samples i.e., D/W treated product, washed again with distilled water (DW) and these after-wash samples of water were used for the plating purpose.

Bacteriological analysis

Enumeration of total bacteria on vegetables Plate count agar was used for the enumeration of bacteria on Fenugreek, Cauliflower, Cabbage, Tomatoes and Cucumber, following a pour plate method after serial dilution of the VW and DW up to 10^6, 10^5 and 10^6 dilutions were selected for the pour plate method, and the total viable count of all the vegetables individually treated with all seven vegwash and D/W was recorded.

Antimicrobial sensitivity testing by using disk diffusion method : 24-hour old culture of pathogens *E. coli*, *Pseudomonas*, *Klebsiella*, *Shigella*, *Proteus*, *Staphylococcus aureus and Salmonella typhi* were selected for the testing effect of vegwashes. As per the guidelines on the labels of veg wash containers given by the manufacturer, dilution was carried out. Kirby-Bauer method was used for susceptibility testing of veg washes. After spreading the individual bacterial suspension on Mueller Hinton agar plate, disks soaked with diluted

vegetable wash were placed appropriately on the surface of agar plates. After 30 minutes of refrigeration at 4^0 C, the plates were incubated at 37^0 C for 24 hours and results were recorded.

Determination of the residual pesticide by HPLC method

Test sample preparation

Sample 1. Cauliflower – D/W wash,
Sample 2 Cauliflower –Wash by using Healing touch vegetable wash liquid,
Sample 3 Cauliflower –Wash by using Nim wash vegetable wash liquid.

Standard stock solution preparation for HPLC : Standard Lambda Cyhalothrin (10000 ppm) was prepared using methanol as a solvent.

Parameters applied for HPLC : Wavelength: 277nm, Mobile Phase: Methanol: Water (70:30), pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid), Sample Volume: 20µl, Flow rate: 1.0 ml/min, Pressure: 9-10 MPa, Run time: 10.06min.

III. RESULT AND DISCUSSION

Bacteriological analysis

Enumeration of total bacteria on Fenugreek

Colony forming units (CFU) after washing with veg wash and with distilled water.

Table 2: TVC on vegetable sample of Fenugreek

Sample	Sample 1: First wash water of Fenugreek with Veg wash		Sample 2: Second wash water of the same vegetable i.e., Fenugreek with distilled water.
Veg wash / D/W	Dilution		Dilution
	10^5	10^6	10^6
1.Distilled water	25 CFU x 10^5	0.5CFU x 10^5	0.3 CFUx 10^5
2. veggie clean	15 CFU x 10^5	1.2 CFU x 10^5	0.9 CFU x 10^5
3. wiz wash	92 CFU x 10^5	7.2 CFU x 10^5	6.0 CFU x 10^5
4. Dr $^+$ Rhazes	72 CFU x 10^5	2.1 CFU x 10^5	1.9 CFU x 10^5
5. veg fru wash	66 CFU x 10^5	5.2 CFU x 10^5	2.4 CFU x 10^5
6. Orga nature	48 CFU x 10^5	29 CFU x 10^5	2.7 CFU x 10^5
7. Nim wash	70 CFU x 10^5	0.2 CFU x 10^5	0.1CFU x 10^5
8. Healing touch	uncountable	Uncountable	0.1CFU x 10^5

Enumeration of total bacteria on Tomato : Colony forming units (CFU) after washing with veg wash and with distilled water.

Table 3: TVC on sample of Tomato

Sample	Sample 1: First wash water of **Tomato** with Veg wash		Sample 2: Second wash water of the same vegetable i.e., **Tomato** with distilled water.
	Dilution		
	10^5	10^6	10^6
1.Distilled water	uncountable	uncountable	uncountable
2. veggie clean	4CFU x 10^5	0.2CFU x 10^5	0.01CFU x 10^5
3. wiz wash	4CFU x 10^5	0.2CFU x 10^5	0.01CFU x 10^5
4. Dr $^+$ Rhazes	uncountable	1.4CFU x 10^5	0.01CFU x 10^5
5. veg fru wash	uncountable	0.2CFU x 10^5	**No growth**
6. Orga nature	uncountable	uncountable	0.45CFU x 10^5
7. Nim wash	2x 10^5	**No growth**	**No growth**
8. Healing touch	uncountable	uncountable	0.04 x 10^5

Enumeration of total bacteria on Cabbage

Colony forming units (CFU) after washing with veg wash and with distilled water.

Table 4: TVC on vegetable sample of Cabbage

Sample	Sample 1: First wash water of **Cabbage** with Veg wash		Sample 2: Second wash water of the same vegetable i.e., **Cabbage** with distilled water.
	Dilution		
	10^5	10^6	10^6
1.Distilled water	3CFU x 10^5	0.2CFUx 10^5	0.01CFU x 10^5
2. veggie clean	5CFU x 10^5	0.1CFU x 10^5	0.01CFU x 10^5
3. wiz wash	1CFU x 10^5	0.1CFU x 10^5	0.02CFU x 10^5
4. Dr $^+$ Rhazes	21CFU x 10^5	1.8CFU x 10^5	1.2CFU x 10^5
5. veg fru wash	160CFU x 10^5	12.4CFU x 10^5	11.2CFU x 10^5
6. Orga nature	10CFU x 10^5	1.1CFU x 10^5	1.0CFU x 10^5

7. Nim wash	1CFU x 10^5	0.1CFU x 10^5	0.01CFU x 10^5
8. Healing touch	uncountable	uncountable	0.1CFU x 10^5

Enumeration of total bacteria on Cauliflower : Colony forming units (CFU) after washing with veg wash and with distilled water.

Table 5: TVC on vegetable sample of Cauliflower

Sample	Sample 1: First wash water of **Cauliflower** with Veg wash		Sample 2: Second wash water of the same vegetable i.e., **Cauliflower** with distilled water.
	Dilution		
	10^5	10^6	10^6
1.Distilled water	uncountable	26.5 CFUx 10^5	22.4 CFU x 10^5
2. veggie clean	140 CFU x 10^5	4.8 CFU x 10^5	3.2 CFU x 10^5
3. wiz wash	uncountable	32.4 CFU x 10^5	29.2 CFU x 10^5
4. Dr $^+$ Rhazes	860 CFU x 10^5	72.0 CFUx 10^5	62.0 CFU x 10^5
5. veg fru wash	640 CFU x 10^5	5.2 CFUx 10^5	4.2 CFU x 10^5
6. Orga nature	612 CFU x 10^5	50.8 CFUx 10^5	49.0 CFU x 10^5
7. Nim wash	70 CFU x 10^5	0.2CFUx 10^5	0.2 CFU x 10^5
8 . Healing touch	uncountable	uncountable	0.1 CFU x 10^5

Enumeration of total bacteria on Cucumber : Colony forming units (CFU) after washing with veg wash and with distilled water.

Table 6: TVC on vegetable sample of Cucumber

Sample	Sample 1: First wash water of **Cucumber** with Veg wash		Sample 2: Second wash water of the same vegetable i.e., **Cucumber** with distilled water.
	Dilution		
	10^5	10^6	10^6
1.Distilled water	264 CFU x 10^5	0.28CFU x 10^5	0.15 CFU x 10^5
2. veggie clean	11CFU x 10^5	1.7 CFU x 10^5	0.30 CFU x 10^5
3. wiz wash	uncountable	0.3 CFU x 10^5	0.2 CFU x 10^5

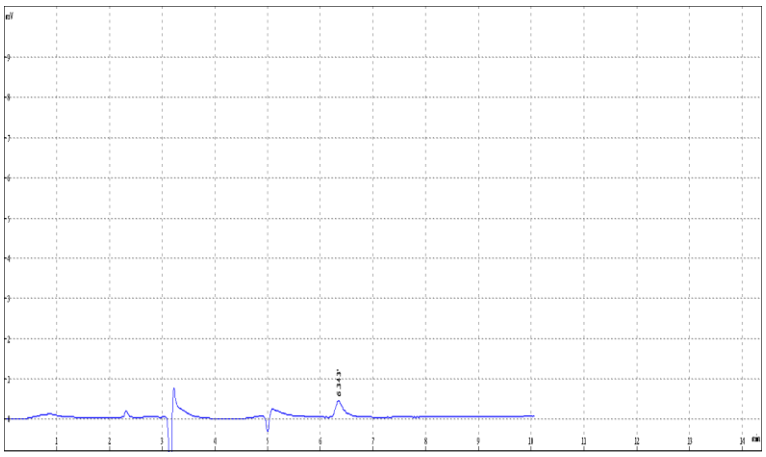
4. Dr + Rhazes	5 CFU x 10^5	2 CFU x 10^5	0.1 CFU x 10^5
5. veg fru wash	15 CFU x 10^5	0.8 CFU x 10^5	0.6 CFU x 10^5
6. Orga nature	uncountable	uncountable	3.2 CFUx 10^5
7. Nim wash	12CFU x 10^5	0.8 CFU x 10^5	0.6 CFU x 10^5
8 . Healing touch	uncountable	12.8 CFU x 10^5	0.31CFU x 10^5

Results of antimicrobial sensitivity

Table:7- Antimicrobial effect of veg washes on various pathogens

	E.coli	Pseudomonas	S.typhi	Shigella	Proteus	Klebsiella	S. aureus
1. Dr + Rhazes	1 cm	R	1.9 cm	1.7 cm	1.9 cm	R	1 cm
2. Wiz fruit wash	2.1 cm	0.7 cm	1.1 cm	1.5 cm	2 cm	0.9 cm	0.9 cm
3. Veg fruit wash	1.1 cm	R	R	R	1 cm	1 cm	1 cm
4. Nim wash	2.5 cm	1.5 cm	2.5 cm	2 cm	2.7 cm	1 cm	0.9 cm
5. Veggie clean	R	R	1.6 cm	1.5 cm	R	R	R
6. Orga Nature	0.8 cm	R	0.8 cm	1.1 cm	0.5 cm	R	R
7. Healing touch	R	0.9 cm	0.8 cm	0.5 cm	1.5 cm	1.5 cm	0.7 cm

Results of HPLC
Sample A is 10000 ppm solution of Standard Lambda Cyhalothrin
Wavelength: 277nm
Mobile Phase: Methanol: Water (70:30)
pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)
Sample Volume: 20µl
Flow rate: 1.0ml/min
Pressure: 9-10MPa
Run time: 10.06min

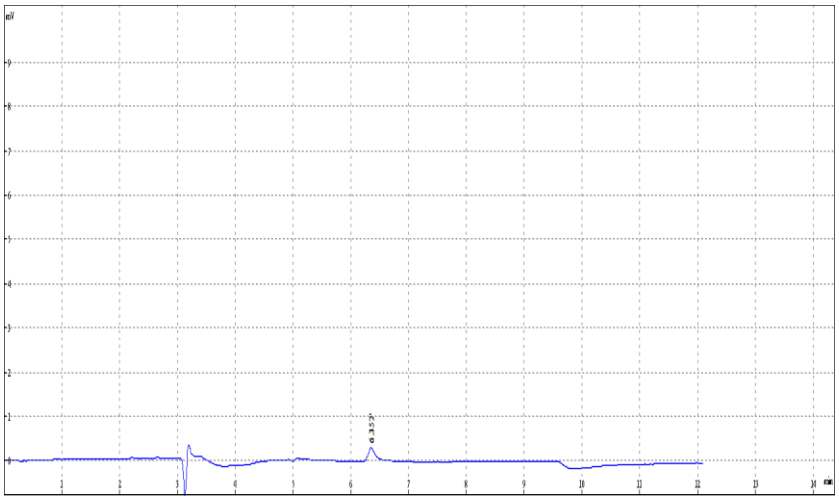


Time	Area	Resolut.	T. Plate Num	Asymmetry
6.343	8375	0.00	5619	1.31

FIG.1. UV scan of standard using concentration 10000 ppm.

Sample 1 is Standard Lambda Cyhalothrin 2000ppm

Wavelength: 277nm
Mobile Phase: Methanol: Water (70:30)
pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)
Sample Volume: 20µl
Flow rate: 1.0ml/min
Pressure: 9-10MPa
Run time: 12.09min



Time	Area	Resolut.	T. PlateNum	Asymmetry
6.352	3781	0.00	7301	1.36

FIG.2. UV scan of standard using concentration 2000 ppm

Sample 01 is D/W wash of vegetables.

Wavelength: 277nm
Mobile Phase: Methanol: Water (70:30)
pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)
Sample Volume: 20µl
Flow rate: 1.0ml/min

Pressure: 9-10MPa
Run time: 8.23min

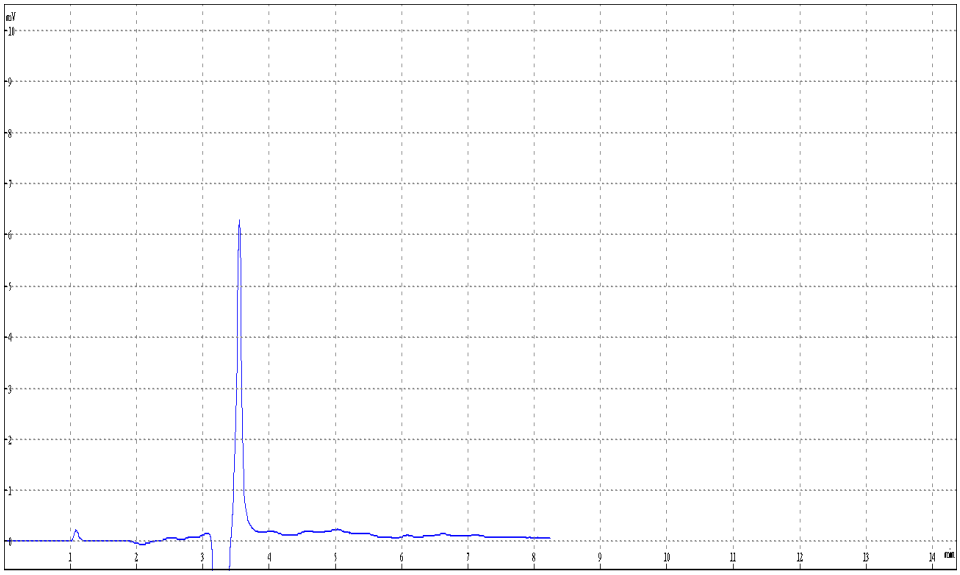


FIG.3. Graph of Sample 1.

3.3.4 Sample 02 is Healing touch liquid veg wash treated vegetable water

Wavelength: 277nm
Mobile Phase: Methanol: Water (70:30)
pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)
Sample Volume: 20µl
Flow rate: 1.0ml/min
Pressure: 9-10MPa
Run time: 9.72min

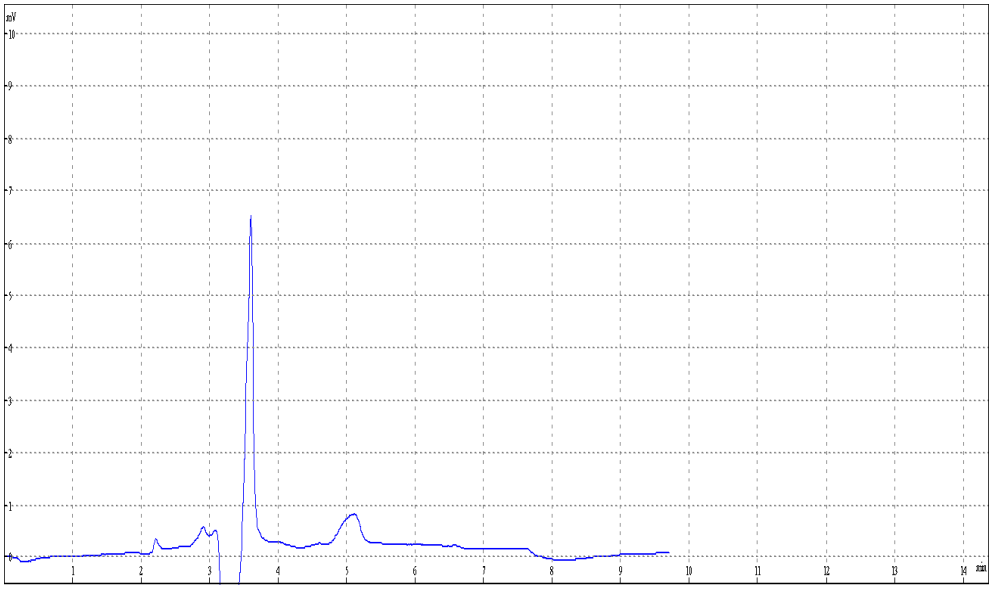


FIG. 4. Graph of Sample 2

3.3.5 Sample 03 is Nim wash liquid veg wash treated vegetable water

Wavelength: 277nm

Mobile Phase: Methanol: Water (70:30)

pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)

Sample Volume: 20µl

Flow rate: 1.0ml/min

Pressure: 9-10MPa

Run time: 8.19min

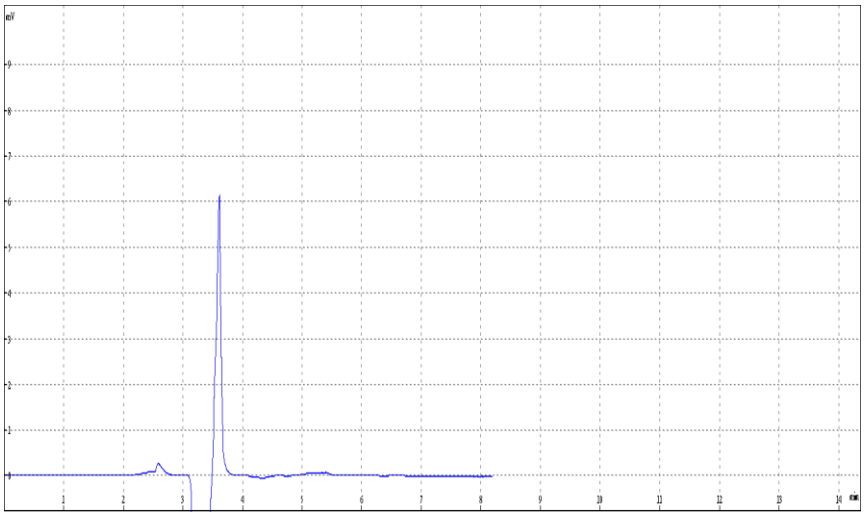


FIG. 5. Graph of sample 03

IV. DISCUSSION:

The veg washes named as Wiz wash, Nim wash and Healing touch have shown inhibitory action on majority of pathogens, while negligible effect of veg wash Veggie Clean, Dr + Rhazes, veg fru wash and OrgaNature is observed on the pathogens under the study, (Table number 2 to 6) When total viable count after treatment of vegwash is analyzed compared to other vegetable wash liquids, the Nim wash and Healing touch have shown drastic decrease in the microflora of the vegetables, (Table 7) By analyzing samples with HPLC for lambda cyhalothrin it is concluded that they are ineffective in removal of Lambda Cyhalothrin residues as tests are performed on the Lambda Cyhalothrin pesticide sprayed vegetables under the study, (figure 1-5)

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