

Analysis of Residue of Pesticide Sprayed on Lady's Finger Before and After Washing with “Vishaghna Dravyas” (Antitoxic Fluids)

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Abstract:

Due to the potential harmful & toxic effects of pesticide to other non-targeted organisms than pests and diseases the presence of pesticide residues in fruits and vegetables is a concern for consumers. Some of the pesticides are persistent and therefore remain in the body causing long term exposure. With reference to an earlier study Lady's finger is supposed to be the most contaminated vegetable with residues of 18 pesticides belonging to organophosphate class. The washing of fruits & vegetables with water or soaking in solutions of salt and some chemicals e.g. chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, hydroxyl peracetic acid, iprodione and detergents are reported to be highly effective in reducing the level of pesticides. So the objective of the present investigation, was to evaluate the efficacy of Rock Salt solution and Vinegar Water for removal of pesticide residue from lady finger, after soaking it for 2-4hrs rendering it safe for consumption. Reduction in residual content of malathion pesticide from lady finger after washing with distilled water, rock salt solution and vinegar solution was considered in present study. Qualitative analysis of malathion was done by HPLC method the retention time of malathion was found to be 2.825 ± 0.3 , and in given sample the retention time in water (2.072) and RS (2.559) was found similar to malathion (2.825) retention time, which confirm the presence of malathion in given water and RS (Rock Salt) sample. The outcomes of the present investigation encourages use of rock salt solution and vinegar solution for cleaning of fruits & vegetables in order to reduce the harmful chemical pesticide residues, where S Aaindhav lavanjal (Rock salt solution) has proved to be

the best medium as compared to distilled water and vinegar solution in present study.

Keywords: pesticide residue, HPLC, rock salt, vinegar, vegetable wash

Introduction:

For pest management, pesticides are used during cropping to destroy pests and prevent diseases. However, the use of pesticides during production often leads to the presence of pesticide residues in fruits and vegetables after harvest (Carriger *et al.*, 2006; Mebdoua, 2018). Due to the potential harmful & toxic effects of pesticide to other non-targeted organisms than pests and diseases the presence of pesticide residues in fruits and vegetables is a concern for consumers, interfering with the reproductive systems and fetal development as well as their capacity to cause cancer and asthma in particular (Echobinchon, 1996). Some of the pesticides are persistent and therefore remain in the body causing long term exposure.

Lady's finger or *Abelmoschus esculentus* is an economically important vegetable grown in the tropical and sub-tropical part of the world. Because of its robust nature, dietary fiber and distinct seed protein balance of both lysine and tryptophan amino acids it is a good source of high fiber, Vit-C, folate, Calcium, Potassium, polysaccharides and anti-oxidants (Bawa, and Badrie, 2016). However, cultivation of okra crop suffers a number of biotic and abiotic factors, including insect pests and diseases and thus prone to many kinds of depreciations right from the time of harvesting to yielding (A rain, *et al.*, 2012). With reference an earlier study Lady's finger is supposed to be the most contaminated vegetable with residues of 18 pesticides belonging to organophosphate class. All organophosphates have a common mechanism of toxicity and can cause similar symptoms in humans who have too much exposure (Sherman, 1995). Where consumers are now aware of the fact that, simply washing vegetables with tap water does not remove these harmful chemicals and remains in notion for safe consumption. The washing with water or soaking in solutions of salt and some chemicals e.g. chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, hydroxyl per acetic acid, iprodione and detergents are reported to be highly effective in reducing the level of pesticides (Bajwa, & Sandhu, 2014).

Ayurveda, being the oldest medical science known to mankind, mainly aims at healthy living and long life unlike other medical sciences, which simply focuses on the treatment of

ailments and disease. The science of Ayurveda is very elaborate and vast. Rock Salt “Saindhav lavan” in Hindi, is one among 5 salts described by Ayurveda (Khandelwal et al,2012). Its main composition is sodium chloride. In Ayurveda Rock salt has been considered to pacify all the three doshas (Vata, Pitta and Kapha) and is considered as the healthiest form of salt in Ayurveda. Ayurveda acharyas recommend using this salt in day to day life.

The antimicrobial properties of vinegar in the context of food preparation have been recently described by researchers, however, the use of undiluted vinegar is also mentioned for cleaning of dentures (Entani, et al., 1998; Shay, 2000; Sengun and Karapinar, 2005).

Other constituents of vinegar include vitamins, mineral salts, amino acids, poly phenolic compounds like, galic acid catech in, caffeic acid, ferulic acid; and non volatile organic acids like, tartaric, citric, malic, lactic (Johnston & Gaas, 2006). Coconut and cane vinegar, containing 4-7 % acetic acid is commonly used in India.

So the objective of the present investigation was to evaluate the efficacy of Rock Salt solution and Vinegar Water for removal of pesticide residue from lady finger, after soaking it for 2-4 hrs rendering it safe for consumption.

Materials & Methods:

Sample Collection

For present investigation, the lady finger samples were collected directly from the villages around Bhopal city, M.P. India, where use of pesticides on vegetable crops is a common practice. The fresh sample were brought to laboratory and kept at -20°C in a refrigerator till the start of experiment.

Experimental plan

In present investigation, malathion was considered to be analyte of interest that was supposed to be detected as residual pesticide from collected lady finger samples. There were three groups of lady finger were prepared; Group-1 would be washed with a in distilled water, Group- 2 would be washed with water containing rock salt at 10g/l concentration and Group-3 would be washed with water containing vinegar at 10 gm/l concentration. Each

group was subjected to washing with their respected solution for 2 hours. The washed and cleaned samples would be then subjected to homogenization followed by extraction of analyte for quantitative analysis.

Sample Extraction

The extraction of vegetable samples was done according to the method suggested by William, (2005); Salman, and Ahmed, (2016), with suitable modification as per the requirement of present investigation. For which 10gm of vegetable samples was cut into small pieces and blended first then put into 250 ml of conical flask containing 100 ml of 1:1 hexane and acetone mixture. Sodium sulphate was also added to remove the water from sample. The maceration was allowed overnight followed by filtration through a clean cotton pad. The filtrate so obtained was centrifuged at 4000 rpm for 20 minutes. The supernatant is then carefully transferred to 10ml volumetric flask and allowed to evaporate till dryness to obtain extract which was finally re-dissolved in 5 ml of acetonitrile. This liquid portion was filtered through a 0.45 μ m dissociable nylon syringe filter twice then subjected to HPLC analysis.

Preparation of Standard Stock

10 mg of malathion (Sigma Aldrich) was used to prepare 1000ppm stock solution in acetonitrile, 1 ml of which was then used to suitably dilute to prepare a dilution series of standard concentrations of analyte which is depicted in table.

HPLC Analysis for Pesticide Residues

The prepared samples were analyzed on Isocratic HPLC system (Shimadzu LC-10) as per the method suggested by Ohlin (1986) and Dekok and Himestra in (1992) with suitable modification. The chromatographic conditions for analysis includes use of 250mm x 4.60mm, 5 μ m particle size reverse phase C-18 column (from Thermo Fisher); the mobile phase used was 7:3 mixture of Acetonitrile and distilled water at a flow rate of 1ml/min maintained at ambient temperature. 20 μ l of prepared samples extracts were injected in the system which were detected by UV/visible detector at 230nm wave length that was showing peak of detection at retention time of 2.825 ± 0.3 min approx. The residual concentration of malathion pesticide in each sample was evaluated by comparing the reading of sample analytes in the form of area under peak as chromatogram with standard plot malathion prepared under same chromatographic conditions.

Results:

The residual content of malathion pesticide in the lady finger samples were analyzed in their extract by comparing the peak areas of their chromatogram at retention time of 2.825 ± 0.3 min approx. with the calibration curve plot of standard malathion as depicted in table 1 and figure 1

Table 1: Standard concentration vs peak area generated of standard analyte malathion

S.N	Conc.($\mu\text{g/ml}$)	Area under peak in chromatogram
1	0	0
2	4	12859.9
3	6	16752.9
4	8	21354.6
5	10	23921.4

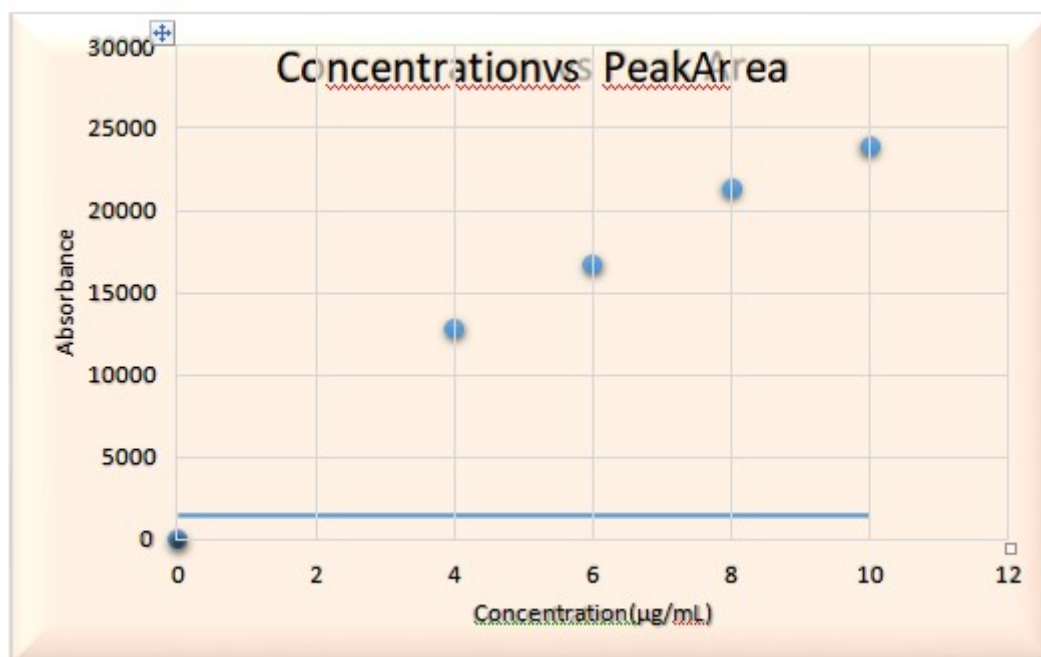


Figure 1: Concentration vs area under chromatographic peak standard curve plot of standard analyte malathion generated by linear regression function in MS Word 2013.

The retention time of malathion in sample extracts prepared from lady finger washed with plain distilled water and rock salt solution are 2.072 min and 2.559 min approx. respectively, which are comparable with retention time of standard analyte malathion *i.e.*, 2.825 ± 0.3 min approx. which confirm the presence of residual malathion in samples washed with water and rock salt solution. The amount of malathion removed by 3 types of solutions used in study are from per 10 mg of samples is depicted in table 2.

Table 2: Amount of malathion removed upon washing with distilled water, vinegar solution and rock salt solution

S.N.	Samples	Estimation of Malathion (ng per 10mg)
1.	D.W	0.0291
2.	Vinegar	0.0142
3.	R.S	0.0377

A small amount of the pesticide residue would be considered as removed by distilled water as compared to Saindhav Jal *i.e.*, 2.285% at retention time of 2.072. Vinegar water solution when used for washing of vegetables did extract residues of pesticides from the vegetables *i.e.*, 39.399% in retention time of 1.714. In HPLC, maximum peak was observed in Saindhav Jal (Rock Salt Solution) where, the residue extracted was 0.0377 ng/10 mg, which was greater than distilled water (0.0291 ng/10 mg) and Vinegar solution (0.0142 ng/10 mg). Figures 2, 3 and 4 depict the chromatogram of experiments G-1, G-2 and G-3 groups respectively for malathion residue from treated ladyfinger samples.

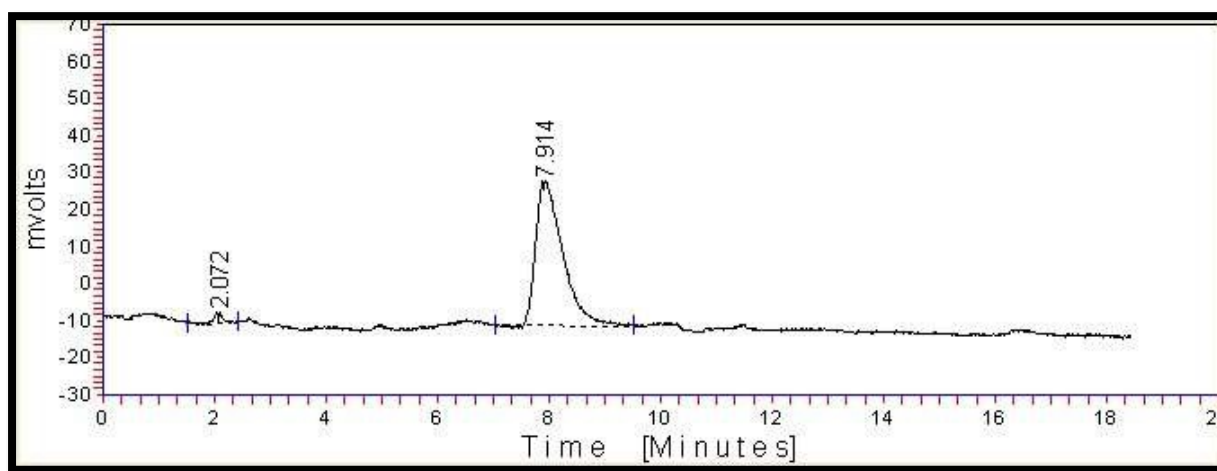


Figure 2: Chromatogram of sample extract G-1 for quantitative estimation of malathion in lady finger samples washed with plain distilled water at 230 nm.

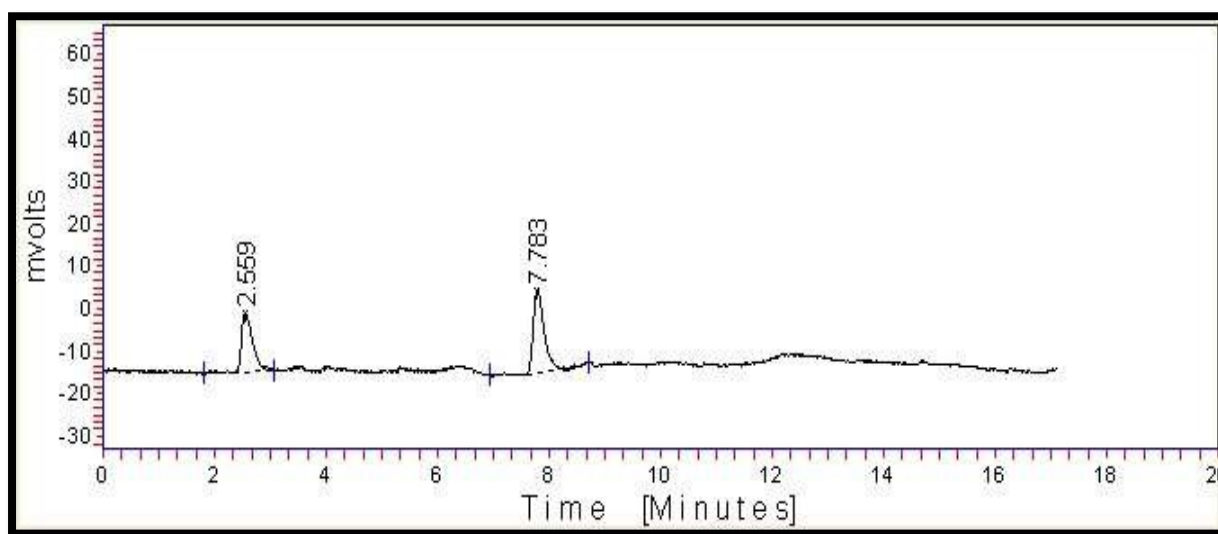


Figure 3: Chromatogram of sample extract G-2 for quantitative estimation of malathion in lady finger samples washed with rock salt solution at 230 nm.

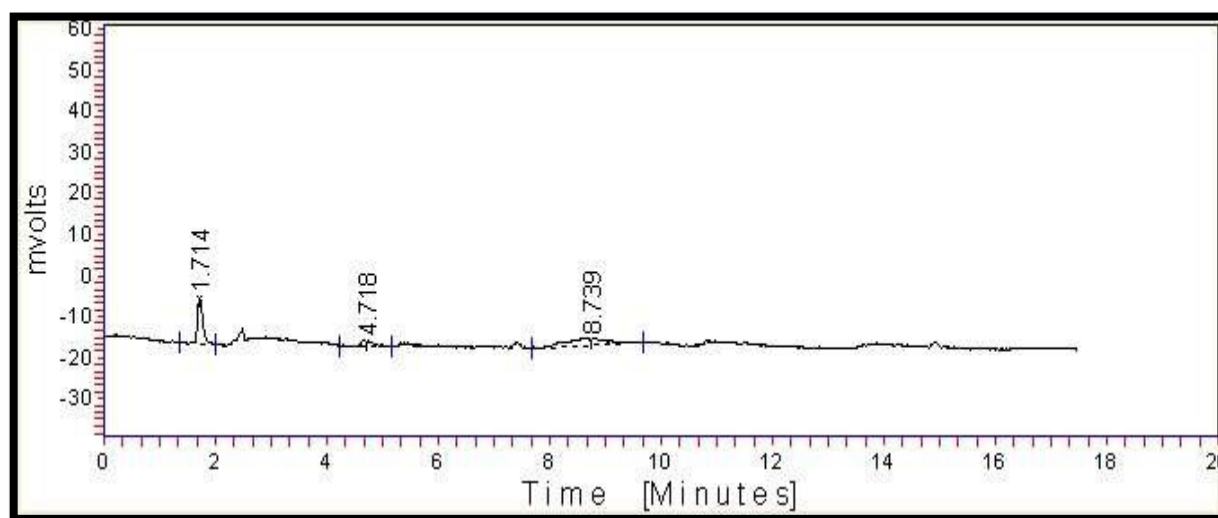


Figure 4: Chromatogram of sample extract G-2 for quantitative estimation of malathion in ladyfinger samples washed with vinegar solution at 230nm

Discussion:

Washing of vegetables is the most traditional and preliminary unit operation applied to remove debris and dirt from vegetables prior to consumption. It is the most prevalent practice in every household as it is the cheapest and easiest method of cleaning. Vegetables can be washed with plain water and also with the use of various chemical formulations in commercial and household processing. The extent of residue reduction by washing depends upon the physico-chemical properties of the pesticide, such as water solubility, hydrolytic constant, volatility, and octanol-water partition co-efficient in conjugation with the

actual physical location of the residue (Echobinchon, 1996).

Washing process leads to reduction of hydrophilic residues which are located on the surface of the crops. It is mention in a recently conducted research from Telangana Agriculture University stated that tap water reduced residues of pesticides only 17-39% (Pnumaka *et al.*,2017).

More over the composition of water varies from place to place and sometimes the heavy metals present in it render it unsafe for use. Therefore Distilled water was opted for research and analytical purpose. But it was noticed that only small amount of the pesticide residue was removed by Distilled water as compared to Saindhavjal *i.e.*, 2.285% at retention time of 2.072. Vinegar water solution when used for washing of vegetables did extract residues of pesticides from the vegetables *i.e.*, 39.399% in retention time of 1.714, but it affected the taste of the vegetables. Saindhavlan, (Rock Salt), inherits many medicinal properties and is easily available in every house (Khandelwal, *etal.*, 2012). Probable mode of action may be due to the hydrophilic nature of the pesticide. It was able to extract the residue of pesticide up to 42.033% against a retention time of 2.559.

Conclusions:

India, being an agrarian economy is dependent upon agriculture for its development. Use of pesticides on vegetables is an inevitable part of agriculture but their unscientific usage can cause significant health adversities. There is a need to regulate pesticide in take for leading a healthy life. In a developing country like India, where quite a large proportion of population live below poverty line, burden of deadly diseases can be a great curse. From the extensive review collected on the dissipation of pesticide residues and mechanism involved during house hold processing techniques it can be concluded that washing of vegetables with saindhavlan (Rock salt) has proved to be the best media as compared to distilled water and vinegar solution, to reduce the amount of pesticide residue on various vegetables, to its maximum, rendering it safe for consumption. While the present study used only one pesticide (Malathion), the Agricultural Industry, has access to many more chemicals that can penetrate into human body through various routes.

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