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Research Article

COMPARATIVE IN VITRO EXPERIMENTAL STUDY ON APPLE (*Malus domestica* Borkh.) BEFORE AND AFTER WASHING WITH USHNA (LUKEWARM WATER), LAVANA (5% NaCl SOLUTION) AND HARIDRA JALA W.S.R. PESTICIDE RESIDUE

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Abstract

Pesticides are frequently sprayed over the apple cultivated area to control pest and to fulfill its growing demand. As a result, pesticide residue levels (PRL) are high in apple fruit, which is a matter of health concern. Acharya Charaka has grouped ten Vishaghna Dravyas and named them as Vishaghna Dashemani, one of them is Haridra (*Curcuma longa* Linn.) hence it was selected for the present study. The main aim and objectives of this study is to assess the reduction in PRL on Apple (*Malus domestica* Borkh.) before and after washing with Ushna Jala (lukewarm distilled water), Lavana Jala (5% NaCl solution) and Haridra Jala and to ascertain the best washing media for apple. Procurement of raw drug viz. apple was done form three different sources of Pune market followed by its authentication. Standardization of Haridra Jala and experiments were carried out at State Public Health Laboratory, Pune. Results showed organophosphates group of pesticides viz. Malathion was mainly found in apple. The mean value of PRL on Apple before washing was found to be 0.047 ppm and after its wash with Ushna Jala (distilled water) was 0.027 ppm whereas no pesticide residue was detected after Dhavana with Lavana Jala (NaCl solution) and Haridra Jala. The result of reduction in the pesticide residue level after washing with Lavana Jala (5% NaCl solution) and Haridra Jala were highly significant. Lavana Jala and Haridra Jala are more efficient washing media for reducing pesticide residue in apples.

Key words: Apple; Pesticide residue; Haridra; Visha; Agadtantra.

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INTRODUCTION

The world we dwell in today is a polluted one. The fruits and vegetables are also polluted by the liberal use of pesticides to live up to their growing demand of this age of green revolution. In India, farmers and workers use these poisonous chemicals blindly and abundantly. They are unaware of the biological and health related hazards of these poisonous chemicals and pesticides. These fruits and fruit-vegetables absorb some of the pesticides in the skin and pulp. Pesticides have been linked to a number of health problems, including neurologic and endocrine (hormone) system disorders, birth defects, cancer, and other diseases.[1][2] Children are especially susceptible to the harmful effects of pesticide residues due to their lower body mass, rapid development and higher rates of consumption of affected products.[3][4] In children, exposure to certain pesticides from residues in food can cause delayed development; disruptions to the reproductive, endocrine, and immune systems; certain types of cancer; and damage to other organs.[5] Prenatal exposure to certain pesticides can affect cognitive development and behavior.[6] The world-wide deaths and chronic diseases due to pesticide poisoning number about 1 million per year.[7] Certain environmental chemicals, including pesticides termed as endocrine disruptors, are known to elicit their adverse effects by mimicking or antagonizing natural hormones in the body and it has been postulated that their long-term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer.[8][9][10] Here arises the need of the solvents, which may nullify or reduce toxic residues from these fruits and fruit-vegetables by removing them during washing. Washing fruits and vegetables helps remove some pesticide residues – but only for certain pesticides up to some extent.[11]

Apple is among widely used and cultivated fruit in India. Apple is a common and nutritious fruit that is eaten in all forms like raw, ripen or cooked form. Jammu and Kashmir is the largest apple producing state followed by Himachal Pradesh. Many pesticides are frequently sprayed over the apple-cultivated area to control pest and to fulfill its growing demand. As a result, pesticide residue level is high in this fruit, which is a matter of health concern.

In Ayurved, some Vishaghna Dravyas (drugs which nullify poisonous effect) are mentioned which are easily available and used in common household practice. Acharya Charaka has grouped ten Vishaghna Dravyas and named them as Vishaghna Dashemani,[12] one of them is Haridra (*Curcuma longa* Linn.) which is commonly used in the daily household practice and due to its ample availability it was selected for the present study for the present study.

As on today majority of the studies carried on pesticide residue was analytical type and only few such studies on reduction of pesticide residue by using Vishaghna Dravya has been conducted in Ayurveda. As turmeric is commonly used in the daily household practice and being one among the Vishaghna Dashemani**,** as stated by Acharya Charaka, it was selected for the study. Turmeric can be mixed with water and directly used for washing procedure.

AIM AND OBJECTIVES

To assess the reduction in pesticide residue level on Apple (*Malus domestica* Borkh.) before and after washing with Ushna Jala (lukewarm distilled water), Lavana Jala (5% NaCl solution) and Haridra Jala (turmeric water).

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MATERIAL AND METHODS

A] Analytical study

1. Procurement of raw drugs required for the study i.e. apple and turmeric

Apples were procured from three different sources of Pune market while turmeric was from local source of Nigdi market, Pune. Apples from all these sources were taken in same quantity i.e. 50 g each. They were packed and labeled as sample 1, 2, 3 and were sent for authentication. Samples 1 & 2 were procured from local fruit vendor of Nigdi and Kothrud local market and sample 3 from More Supermarket, Kothrud.

2. Authentication of raw drugs i.e. apple and turmeric

The samples of Apple and turmeric were authenticated and identified by the Department of Botany, Dr. D. Y. Patil College of Arts, Commerce and Science Akurdi, Pune. (Image 1)

3. Analytical study of turmeric and Haridra Jala (Turmeric water)

Physicochemical analysis of turmeric and turmeric water were carried out at State Public Health Laboratory, Pulgate Pune.

B] Experimental study-

Materials

Instruments and apparatus used were Gas Liquid Chromatography apparatus, porcelain dish, knife, conical flask, measuring jar, funnel, filter paper, beaker, stand, extraction funnel and rotary vacuum evaporator. In the experiment reagents used were – Dichloromethane, Sodium hydroxide, Hexan and Activated Charcoal, which is an adsorbent.

Methods

Study type and Site

Study was in-vitro experimental study which was carried out at State Public Health Laboratory, Pulgate, Pune.

Pilot Study

Before going for actual experimental study a pilot study was conducted on single sample of apple collected from different sources of Pune markets. During the study, only Malathion belonging to organophosphates group of pesticides was found on sample no.3 which was collected from More supermarket, Kothrud, Pune. No pesticide residue was found on sample no.1 & 2. The procedure for the pilot study was similar to that of as actual study but single sample was used. (Image 3)

Grouping of Samples

The experimental study was divided into four groups as follows as shown in Table 1.

1. Control group (Unwashed apple group) - Group A

3 samples of apples in which Malathion was found in pilot study (viz. the apples collected from More Market, Kothrud, Pune) were taken for analysis and labelled as 1, 2 and 3. 50gm of each sample was taken and analyzed without washing for pesticide residue by Gas Liquid Chromatography and readings were noted.

2. **Experimental group-3 groups**

This group was divided into 3 groups Group B, Group C and Group D as shown in Table 1.

Under each group three samples were taken and analyzed for pesticide residues by G.L.C, observations were noted and compared with the control group. Results were noted accordingly.

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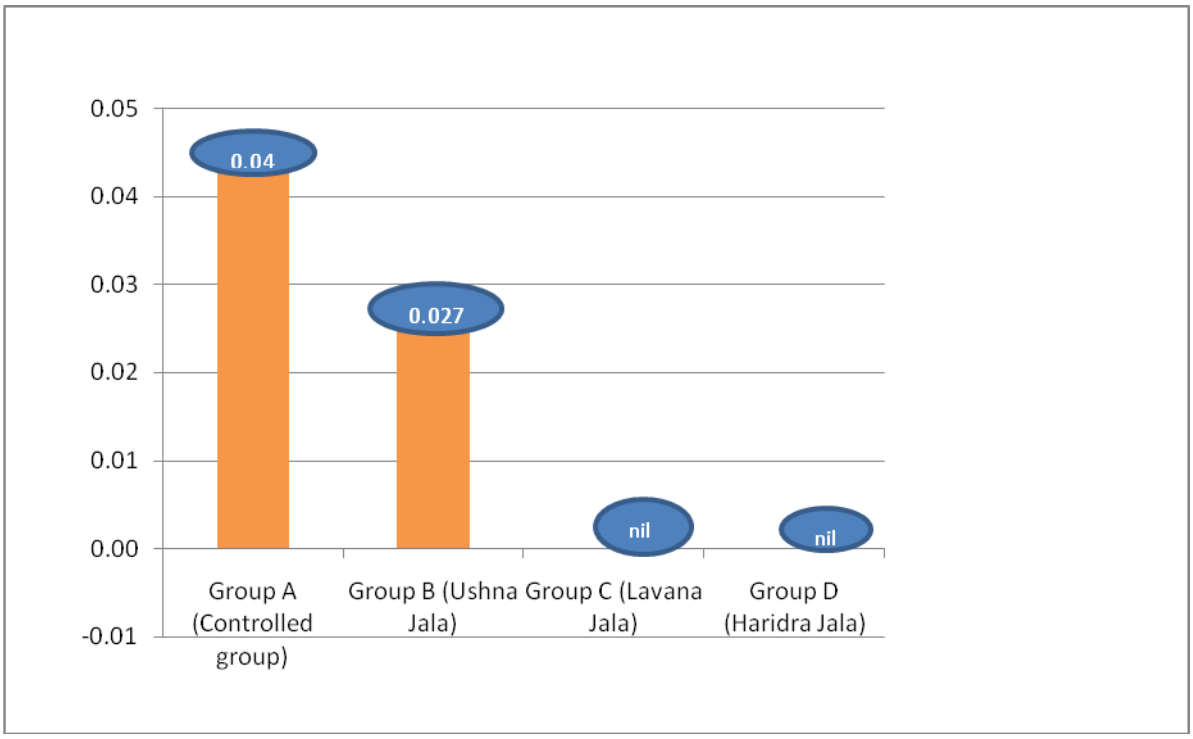
Table 1: Grouping of samples

Sample no.	Control group (A)	Experimental group		
		Wash with distilled water (B)	Wash with 5% NaCl soln (C)	Wash with *Haridra Jala* (D)
1	A1(50 gm)	B1(50gm)	C1(50gm)	D1(50gm)
2	A2(50gm)	B2(50gm)	C2(50gm)	D2(50gm)
3	A3(50gm)	B3(50gm)	C3(50gm)	D3(50gm)

Chart 1: Levels of Malathion (in ppm) in Sample 3



3. Preparation of solutions

Preparation of Lavana Jala (5% NaCl solution)

It was prepared by mixing 50 g of NaCl (sodium chloride) in 1000 ml of distilled water i.e.5% NaCl solution.

Preparation of Haridra Jala

Preparation of Haridra Jala was done at State Public Health Laboratory, Pulgate, Pune. As there is no direct reference regarding preparation of the Haridra Jala.

It was decided to prepare the same according to Sharangdhar's Kashaya method i.e. 1 part of Haridra and 4 parts of water was mixed thoroughly and used for Dhavan.[13] This preparation was termed as Haridra Jala.

4. Procedure of experimental study

A) Extraction of sample

50g sample was soaked in Acetonitrile: Water (65:35) solution for overnight then filtered and mixed along with the known quantity of the filtrate in a separator funnel. To this filtrate saturated NaCl solution was added (to break the emulsion).

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The sample was soaked in petroleum ether (50ml*3) for organophosphate compounds and in dichloromethane (50ml*3) for organochlorine compound for overnight and then it was filtered through filter paper having layers of sodium hydroxide and activated charcoal, which are adsorbent in nature so as to obtain clear transparent colorless filtrate by separating matrix and small particles of apple from it.

Petroleum ether phase

The petroleum ether phase was collected and treated with 5 ml concentrated sulphuric acid and was allowed to stand. Acid helps in removing impurities (only for OC pesticides, OP gets degraded in acid)**.** The acid layer was removed and the petroleum ether layer was washed till it got acid free which was checked by litmus paper. Then petroleum ether layer was passed through anhydrous sodium sulphate (to remove moisture) and evaporator using rotary vacuum evaporator.

Aqueous phase

Aqueous phase with dichloromethane was extracted for column clean up. Then the colorless clear filtrate was concentrated using rotatory vacuum evaporator to obtain the concentrated extract by evaporating filtrate.

B) Analysis of extract

The detection and estimation of the extraction was done by Gas liquid chromatography (G.L.C.) apparatus.

Principle of G.L.C Apparatus

G.L.C is a technique for separation of mixtures into components by a process which depends on the redistribution of the components between a stationary phase and gaseous phase.

Injection of the filtrate

The extract which was obtained after clean up was diluted with 1ml hexane of which 2ul was injected in G.L.C. apparatus by a micro syringe to obtain respective graphs which were compared with the standards of pesticides, provided by W.H.O. This procedure was done for the control group samples which were collected from the market and readings of pesticidal residues were obtained. Again this same procedure was repeated with the experimental group i.e. apple after washing in Ushna, Lavana and Haridra jala respectively to obtain respective graphs and readings of pesticidal residues. The three readings which were obtained by three experimental groups were compared with readings of the control group separately.

OBSERVATIONS AND RESULTS

Observations

During analysis of turmeric, loss on drying was found to be 08.09% w/w, Total Ash content was 07.48% w/w, Acid insoluble ash was found to be 0.68% w/w, Total starch content was 43.15% and Lead chromate test was found to be negative. (Image 1) During analysis of Haridra Jala, (5 % aqueous solution of turmeric powder) pH was found to be 6.25, specific gravity was 1.0043 and total solids were found to be 02.45%. (Image 2)

In pilot study, it was observed that Malathion belonging to organophosphates group of pesticide was found in sample no.3. The mean value of pesticide residue level on Apple before washing i.e. in control group (Group A) was found to be 0.047ppm. The mean value of pesticide residue level on Apple after washing with Ushna Jala (Group B) was 0.027 ppm. The mean value of pesticide residue level on Apple after washing with Lavana Jala (Group C) and Haridra Jala (Group D) was not detected as shown in Images 1,2,3,4,5.

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Image 1: Analysis of turmeric

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STATE PUBLIC HEALTH LABORATORY,PUNE-411001.
Email : statehealthlab@gmail.com Phone & Fax : 020-26330223/24/25 (Extn.208)

TEST REPORT

Report on sample of :- Ramdev Turmeric Powder (Lab Code No. P-895)
Sent by : Dr. Roopali Patel, P.D.E.A. College of Ayuveda & Research Centre, Nigdi, Pune
With letter No. -- Nil Date : 31/10/2017
Date of Receipt :- 31/10.2017 Date of Examination : 09/11/2017

RESULT OF EXAMINATION

Physical Appearance : Turmeric Powder : Yellow colour finely ground powder received in sealed loose polypack,

Microscopic Examination- Structure of Turmeric detected. Foreign body absent.

Sr. No	Parameters	P-895 Turmeric Powder (Ramdev) Results	Prescribed Standards as per 2.9.18(2)of Chapter 2 of Food Safety & Standards (Food Products & Food Additive) Regulations 2011
1	Moisture	8.09 %	Not more than 10 % by wt.
2	Total Ash on dry basis	7.48 %	Not more than 9.0 % by wt.
3	Ash Insoluble in dil. HCl on dry basis	0.68 %	Not more than 1.5 % by wt.
4	Colouring Powder expressed as curcuminoid content on dry basis	Test Not Performed	Shall not be less than 2.0 % by wt.
5	Test for added colouring matter/ lead Chromate/ morphologically extraneous matter	Negative/ Absent	Shall be Negative/ Absent
6	Total starch	43.15 %	Not more than 60 % by wt.

Opinion: (A) It is presumed that the representative sample from the stock is sent for analysis.
(B) This report is restricted to the sample submitter in the laboratory.
Remarks : (1) The above mentioned sample conforms to regulation no. 2.9.18(2) as per Food Safety & Standard (Food products Standards & Food Additives) Regulation 2011; with respect to the tests performed.

End of Report

Health Services Report No.SPHL/D-78/P-895/ 1413 /2017
Dated : 10 /11/2017

Forwarded with compliments to : Dr. Roopali Patel
P.D.E.A's College of Ayurveda & Reserch Centre
Nigdi, Pune.

Rs. 13015/-
Receipt No. 2853746
Date 31.10.2017

for Deputy Director of Health Sevices
State Public Health Laboratory
Pune - 411 001

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Image 2: Analysis of turmeric water

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STATE PUBLIC HEALTH LABORATORY,PUNE-411001.
Email : statehealthlab@gmail.com Phone & Fax : 020-26330223/24/25 (Extn.208)

TEST REPORT

Report on sample of :- Turmeric Powder- water (5%) (Lab Code No. P-899)
Sent by : Dr. Roopali Patel, P.D.E.A. College of Ayuveda & Research Centre, Nigdi, Pune
With letter No. – Nil Date : 31/10/2017
Date of Receipt :- 31/10.2017 Date of Examination : 09/11/2017

RESULT OF EXAMINATION

Physical Appearance : Turmeric Powder-Water (5% aquous solution) :

Sr. No	Parameters	P-899 Turmeric Powder-Water (5% solution)
1	pH	6.25
2	Specific Gravity	1.0043
3	Total Solids	2.45 %
4	Total Dissolved Solids	2.41 %

Opinion: (A) It is presumed that the representative sample from the stock is sent for analysis.
(B) This report is restricted to the sample submitter in the laboratory.
Remarks : Results are as above.

End of Report

Health Services
Report No.SPHL/D-78/P-899/ 1415 /2017
Dated : 10 /11/2017

Forwarded with compliments to : Dr. Roopali Patel
P.D.E.A's College of Ayurveda & Reserch Centre
Nigdi, Pune.

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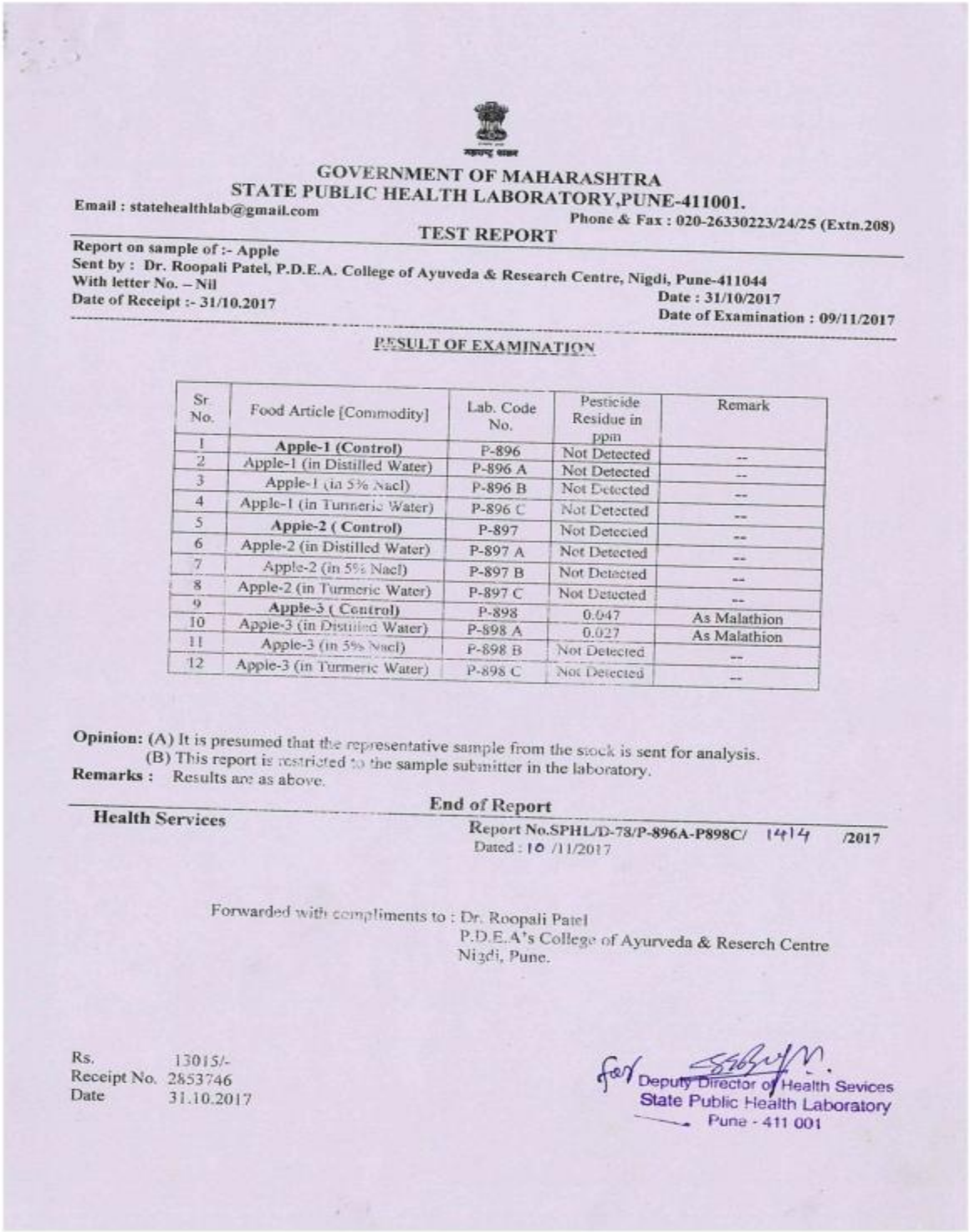
Image 3: Analysis of Pilot Study



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Email : statehealthlab@gmail.com
Phone & Fax : 020-26330223/24/25 (Extn.208)

TEST REPORT

Report on sample of :- Apple
Sent by : Dr. Roopali Patel, P.D.E.A. College of Ayuveda & Research Centre, Nigdi, Pune-411044
With letter No. – Nil
Date of Receipt :- 31/10.2017
Date : 31/10/2017
Date of Examination : 09/11/2017

RESULT OF EXAMINATION

Sr. No.	Food Article [Commodity]	Lab. Code No.	Pesticide Residue in ppm	Remark
1	**Apple-1 (Control)**	P-896	Not Detected	--
2	Apple-1 (in Distilled Water)	P-896 A	Not Detected	--
3	Apple-1 (in 5% Nacl)	P-896 B	Not Detected	--
4	Apple-1 (in Turmeric Water)	P-896 C	Not Detected	--
5	**Apple-2 (Control)**	P-897	Not Detected	--
6	Apple-2 (in Distilled Water)	P-897 A	Not Detected	--
7	Apple-2 (in 5% Nacl)	P-897 B	Not Detected	--
8	Apple-2 (in Turmeric Water)	P-897 C	Not Detected	--
9	**Apple-3 (Control)**	P-898	0.047	As Malathion
10	Apple-3 (in Distilled Water)	P-898 A	0.027	As Malathion
11	Apple-3 (in 5% Nacl)	P-898 B	Not Detected	--
12	Apple-3 (in Turmeric Water)	P-898 C	Not Detected	--

Opinion: (A) It is presumed that the representative sample from the stock is sent for analysis.
(B) This report is restricted to the sample submitter in the laboratory.
Remarks : Results are as above.

End of Report

Health Services
Report No.SPHL/D-78/P-896A-P898C/ 1414 /2017
Dated : 10 /11/2017

Forwarded with compliments to : Dr. Roopali Patel
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Nigdi, Pune.

Rs. 13015/-
Receipt No. 2853746
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Graph 1: Standards of Organophosphate pesticides in Samples

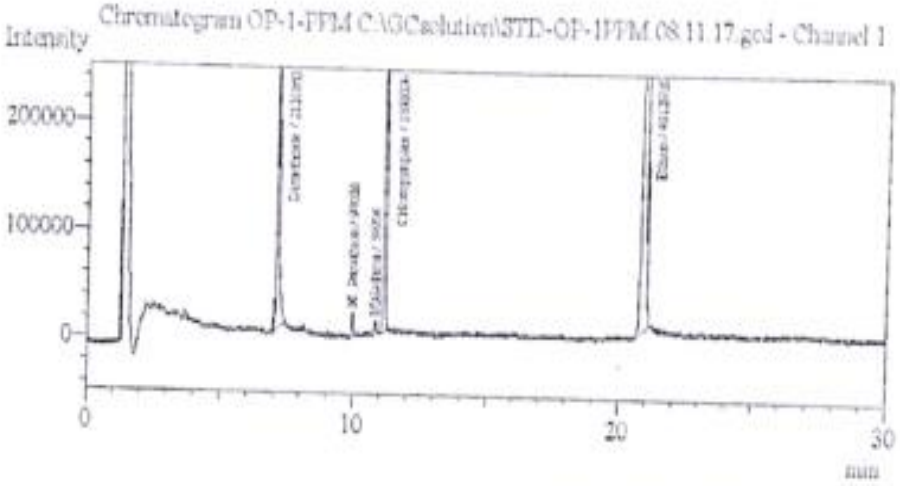
System Configuration

[Instrument]
Instrument Name : Instrument 1
Instrument Type : GC-2010
Communication Type : RS-232C COM1

OP. std - 1 ppm

Sample Information

OP-1-PPM
OP
01/10/2018
C:\GCsolution\STD-OP-1PPM 08.11.17.gcd
C:\GCsolution\tcol.vin\op_08112017.gcm



Peak Table - Channel 1

Peak#	Ret.Time	Area%	Name
1	7.093	23.4355	Dimethoate
2	9.566	1.0849	M. Parathion
3	10.825	0.6585	Malathion
4	11.144	20.8134	Chlorpyriphos
5	20.904	54.0076	Ethion
Total		100.0000	

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Graph 2: Graph of OP in Control group A of Sample 3

③ - control - op

System Configuration

[Instrument]
Instrument Name : Instrument1
Instrument Type : GC-2010
Communication Type : RS-232C COM1

Sample Information

Apple op
OP
01/10/2017
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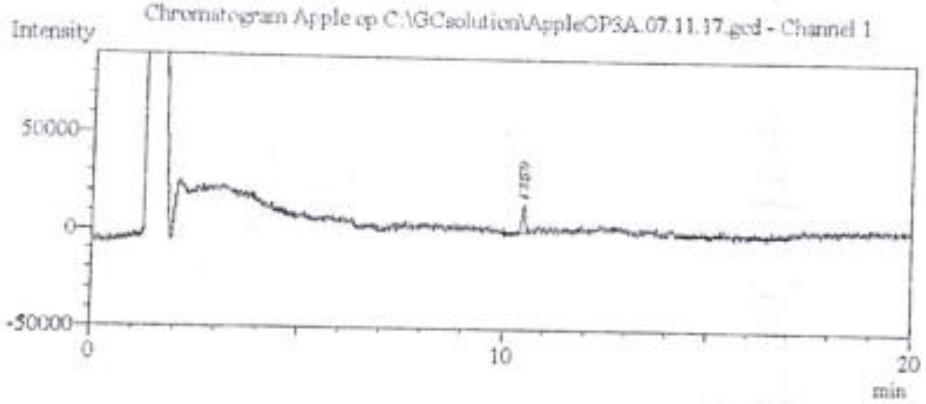
C:\GCsolution\OP_1PPM.06.11.17.gcd



Peak Table - Channel 1

Peak#	Ret.Time	Area%	Name
1	10.496	100.0000	malathion
Total		100.0000	

50 mg sample – 1000 ml dil – 2 ml inj

Area of Std Malathion 59904 = 0.004 mg
70578 = 0.0047 mg
2 ml = 0.0047 mg
1000 ml = 2.35 mg
50 ml = 2.35 mg
1 ml = 0.047 ppm

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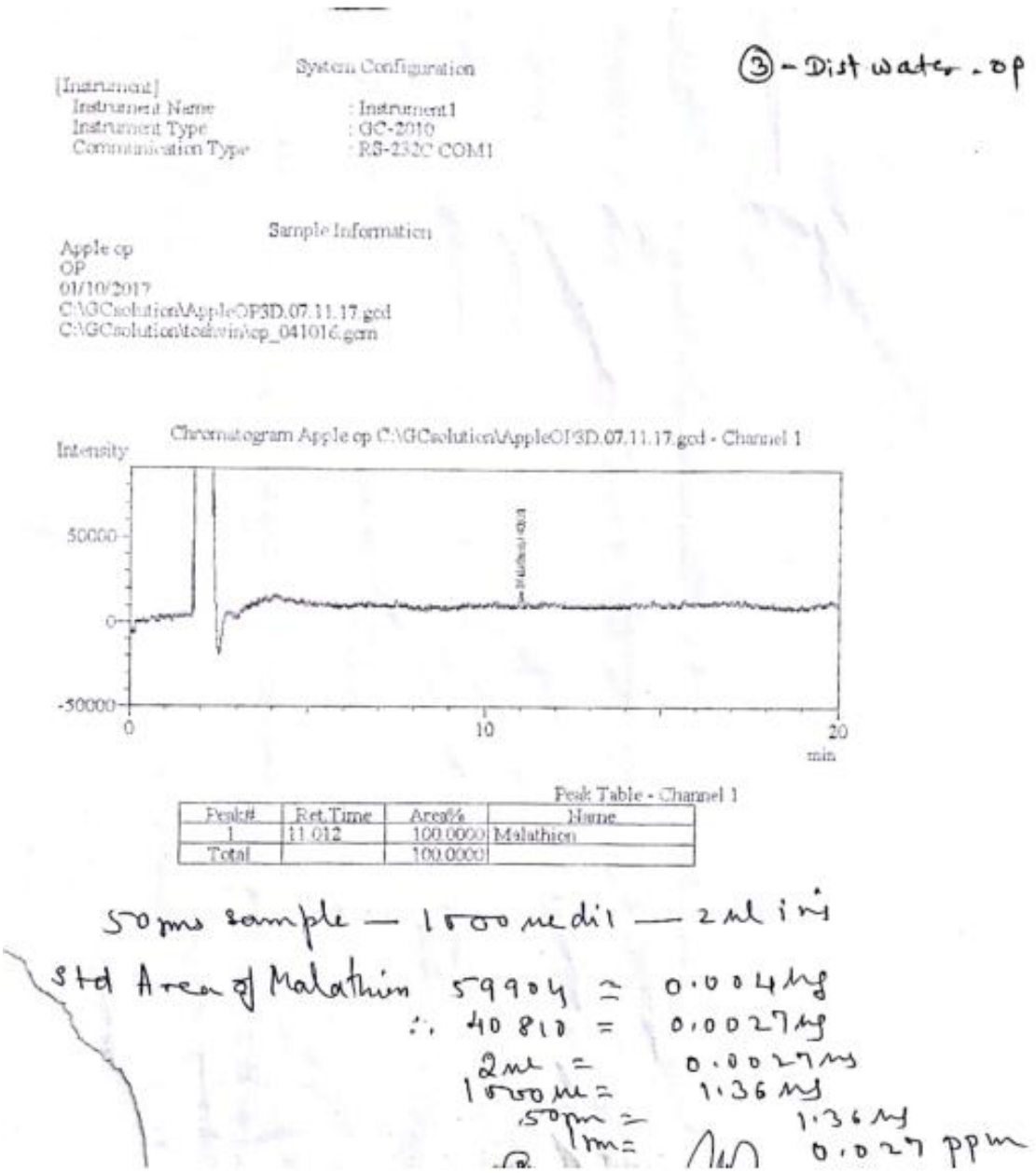
Graph 3: Graph of OP in Group B of Sample 3



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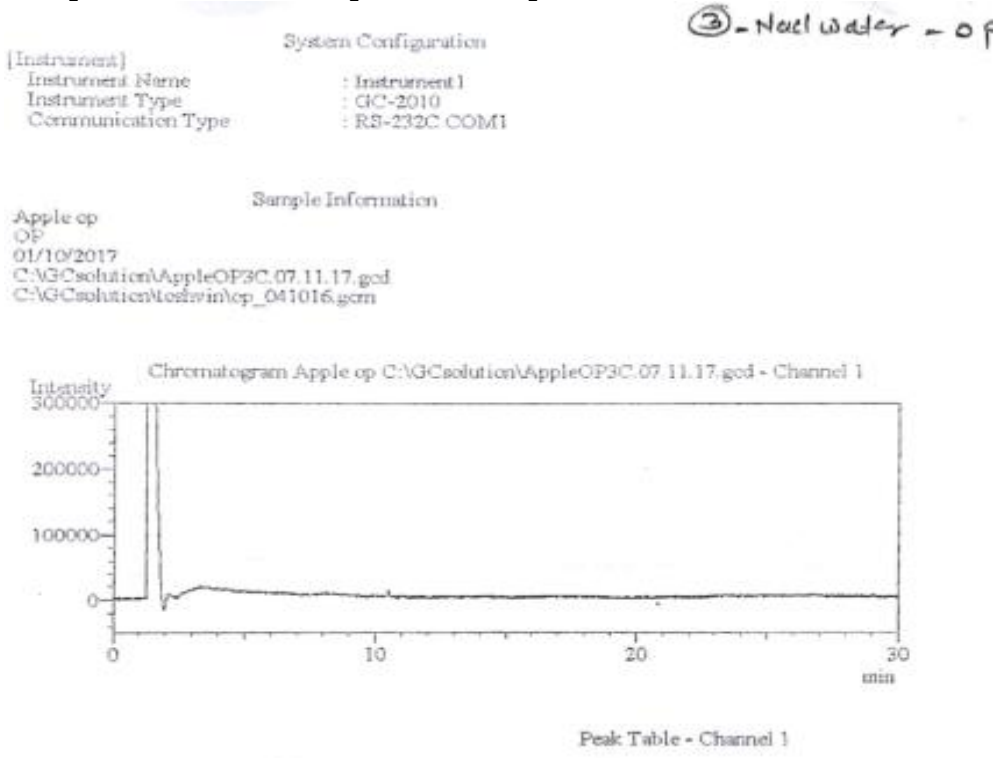
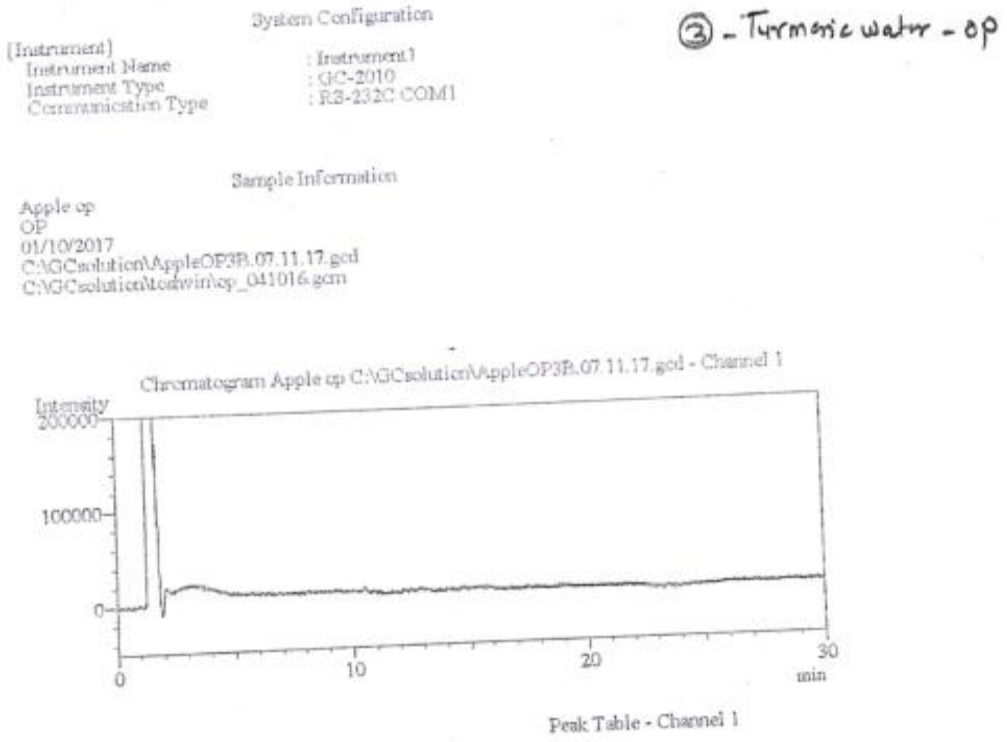
Graph 4: Graph of OP in Group C of Sample 3



Graph 5: Graph of OP in Group D of Sample 3



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Results

The result of reduction in the pesticide residue level after washing with Lavana Jala (Group C) and Haridra Jala (Group D) were significant. The reduction in the pesticide residue level after washing with Ushna Jala (Group B) was proved to be insignificant.

DISCUSSION

It was found that Malathion belonging to organophosphates group of pesticides was mainly used as pesticides on Apples. This may be due to its easy availability, cost-effectiveness and its efficacy to control the pest in India. The result of reduction in the PRL after washing with Haridra Jala (Group D) and Lavana Jala (Group C) were highly significant. This may be due to Vishaghna properties of Haridra as mentioned by Acharya Charaka[14] and due to pesticide soluble affinity of pesticide residue in Lavana Jala (5% NaCl solution). Organophosphates are soluble in water, esters, alcohols and fatty hydrocarbons[15] and Haridra Jala contains water, alcohol and essential oils.[16] Curcumin, the most active polyphenolic constituent, is the active ingredient in the traditional herbal remedy and dietary spice turmeric.[17] May be the action of these constituents help in reduction of pesticide residues. And as mentioned that organophosphtes are soluble in water, esters, alcohols and fatty hydrocarbons, the significant results observed and in case of Lavana Jala (5% NaCl solution) may be due to its ability to destabilize the chemical bonds which makes cleaning off pesticides easier and reduces potency because of the breakdown of the packaged molecules.

CONCLUSION

The present experimental study confirms that Haridra Jala and Lavana Jala (5% NaCl solution) reduce Malathion (pesticide residues) which is sprayed on apples. Haridra Jala and Lavana Jala are more efficient washing media as compared to Ushna Jala (distilled water). Further a study can be framed with all drugs of the Vishaghna Dashemani.

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