A TOXICOLOGICAL ANALYSIS OF THE RESIDUES OF PESTICIDE ON LADY FINGER (ABELMOSCHUS ESCULENTUS) BEFORE AND AFTER DHAVAN BY HARIDRA-JAL

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ABSTRACT

The concentration of various pesticide were well below the established tolerances but continuous consumption of such vegetables even with moderate contamination level can accumulate in the receptor's body and may prove fatal for human population in the long-term. Along with the green revolution uses of pesticides including profenofos is increased and increasing day by day. The pesticide residue including profenofos in all edible things including okra is crossing the maximum permissible limit and arising a big long term hazards in human beings due to inorganic agriculture. Okra is sprayed with a number of pesticides for greater yield, ripening or storage. Commonly used methods for cleaning this vegetable are washing with tap water, warm water, or salt water. In Ayurveda some Vishaghna dravyas are mentioned. So, the study was planned to observe whether the toxic residues are nullified or reduced in the solution of the Vishaghna dravyas. The drug, Haridra selected for this analytical study of pesticide residue is one of important drug mention in Ayurveda. Aacharya Charak has included 10 herbs in Vishaghna Mahakashaya including Haridra. So it was taken as a Dhavan dravya.

KEYWORDS: Dhavan dravya (washing material), Haridra (turmeric), Profenofos, Vishaghna dravya (antitoxic material).

INTRODUCTION

Agad tantra is one of the branches of Astang Ayurveda, which deals with the study of all types of poisons. Nowadays, the utilization of pesticides, fungicides, food colours, preservatives and cosmetics etc. are continuously increasing day by day which causes physical and mental hazards effect on human being.

The concentration of various pesticide were well below the established tolerances but continuous consumption of such vegetables even with moderate contamination level can accumulate in the receptor's body and may prove fatal for human population in the long-term.

Along with the green revolutions the farmers are using the pesticides, preservatives abundantly due to lack of knowledge. They are unaware of the biological and health related hazards of these poisonous chemicals and pesticides. These vegetables absorb some of the pesticides in the epicarp and pulp.

Lady finger is a commercial vegetable crop with considerable area under cultivation in India. Lady finger is sprayed with a number of pesticides for greater yield, ripening or storage like endosulfon 35%EC, Fenvalerate 20%EC, lindane 6.5%EC, Malathion 50%EC, profenofos 50%EC etc. Profenofos is chosen for this study because it is used frequently by farmers now days. Profenofos, an organophosphate insecticide is acetylcholinesterase inhibitor which is responsible for hydrolysing acetylcholine to choline and acetic acid.

Here arises the need of solvent which may nullify or reduces toxic residues from the fruits and vegetables by dissolving them during washing.
been classified as a moderately hazardous (Toxicity class II) pesticide by the World Health Organization (WHO).

**Product name** – Profenofos 50% EC  
**Chemical name** - O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate  
**Type** – Insecticide  
**Molecular formula** – C₁₃H₁₃BrClO₃PS  
**Structure** – ![Chemical Structure of Profenofos](image)

**Physical properties**  
- **Appearance** - Light yellow to brown color liquid  
- **Odour** - Pungent, like garlic or cooked onions  
- **Flammability** - Flammable  
- **Specific gravity** - 1.455 (20°C)  
- **Solubility** - In water 20 mg/L (20°C), readily miscible with most organic solvents  
- **Acceptable daily intakes (ADI)** - 0.02mg/kg bw/day[^2]

**Acute clinical features of profenofos poisoning**  
- Excessive salivation, sweating, rhinorrhea and tearing.  
- Muscle twitching, weakness, tremor, in coordination, muscle spasms.  
- Headache, dizziness, nausea, vomiting, abdominal cramps, diarrhea.  
- Respiratory depression, tightness in chest, wheezing, productive cough, fluid in lungs.  
- Pin-point pupils, watering of eyes, sometimes with blurred or dark vision.  
- Severe cases: seizures, incontinence, respiratory depression, loss of consciousness coma.  
- Cholinesterase inhibition.[^3]

**Chronic health hazards of profenofos poisoning**  
It is capable of interfering with the proper functioning of estrogen, androgen and thyroid hormones in humans and animals. These substances are called endocrine disruptors. Exposures can cause sterility or decreased fertility, impaired development, birth defects of the reproductive tract, and metabolic disorders. These chemicals have been shown to alter levels of male and female hormones, as well as certain thyroid hormones. Changes in these hormone levels affect development of organisms more than adults and can result in abnormalities in reproduction, growth, and development, as well as cancer and immune system disorders, even at very low levels of exposure.[^4]

**Scientific classification:**  
- **Kingdom** : Plantae  
- **Division** : Magnoliophyta  
- **Class** : Magnoliopsida  
- **Order** : Malvales  
- **Family** : Malvaceae  
- **Genus** : Abelmoschus  
- **Species** : A.Esculentus  

**Binomial name:** Abelmoschus esculentus[^6]  

The following *dravyas* were selected as *Dhavan dravya*  

1. Tap water Chemical formula - H₂O  
2. *Haridra-jal* preparation of *haridra-jal* -  
   - 25gm of *Haridra* and 1000 ml water were mixed thoroughly and used for *dhavan*. This preparation was termed as *Haridra-jal*.

**About haridra**  
- **Latin name** - *Curcuma longa*  
- **Family** - Zingiberaceae  

**Karma:** *Krimighna, Kushthghna, Varnya, Vishaghnna, Kaphapittranut, Pramehanashaka*  

**Therapeutic uses** - *Pandu, Prameha, Vrana, Visha vikara, Kusthha, Tyagroga, shtapitta, Pinas*.[^7]

**Pharmacological Actions:**  
It is antiinflammatory, cholagogue, appetite, haematonic, hepatoprotective, bloodpurifier, antioxidant, detoxifier and regenerator of liver tissue, antiasthmatic, antitumour, anticutaneous, antibacterial, antifungal, antiprotozoal, stomachic and carminative.[^6][^8][^9]

**CHEMICALS AND REAGENTS**  
1) 1% acetic acid in Acetonitrile 2) Magnesium sulphate (MgSO₄)  
3) Sodium Acetate (Anhydrous) 4) PSA (Primary secondary amine)  
5) Hexane 6) Acetone

**METHOD**  
*(a) Procurement of drug*  
Taken 400 okra plants from the green house of Rajasthan Agricultural Research Institute, Jaipur and cultivated them in 200 square meter area. The first spraying of profenofos on okra plants was applied during flowerings after cultivation. The 2nd and 3rd spraying of profenofos was applied in the interval of 10 days after 1st spraying. After that next day okra was collected.

**Okra**  
Okra an oligo purpose crop, but it is usually consumed for its green tender fruits as a vegetable in a variety of ways. These fruits are rich in vitamins, calcium, potassium and other mineral matters. The mature okra seed is a good source of oil and protein has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition. Its mature fruit and stems contain crude fibre, which is used in the paper industry.[^3]
(b) Procurement of Haridra

The Haridra used for the study was collected from Rasashastra Pharmacy, National Institute of Ayurveda, Jaipur.

(c) Procurement of Profenofos

- Dose of profenofos – Profenofos dissolved in water in dose of 10ml in 5 lit. (Profenofos 50%EC = 500gms ai./hac. and 500 lit. water is essential of 1 hac.)

Therefore the dose of profenofos is about 20ml for 1 lit.

Study design

Type of study : Experimental In-vitro study

Place of Study : Experiment was conducted at Dept. Of Entomology, Rajasthan Agricultural Research Institute, Jaipur.

Selection of samples

The sample for the experiment was randomly selected and was mainly divided into 2 groups.

1) Control group (without washed)
2) Experimental group (washed with tap water and Haridra-jal)

The experimental group was again divided into 2 sub groups each sub group having approximately 200gms of Okra.

Study design

Table 1 – The quantity of sample taken in control and experimental group

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control group</th>
<th>Experimental (trial) group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without washed (200gms)</td>
<td>Washed with Tap water (200gms)</td>
</tr>
<tr>
<td>Sample 1</td>
<td>15gms</td>
<td>15gms</td>
</tr>
<tr>
<td>Sample 2</td>
<td>15gms</td>
<td>15gms</td>
</tr>
<tr>
<td>Sample 3</td>
<td>15gms</td>
<td>15gms</td>
</tr>
</tbody>
</table>

METHOD OF EXPERIMENTAL STUDY

The experiment was done by "QuEChERS" method.

Process for QuEChERS method

**Step 1 – Extraction Process**

Add 15 ml of 1% of Acetic acid in Acetonitrile to 15 ml homogenized sample in a 50 ml tube

Add 6.0 g MgSO₄ anhydrous & 1.5 g Sodium Acetate anhydrous shake vigorously for 1 min then centrifuge at > 1500 rpm for 1 min.

**Step 2 - Dispersive SPE Clean-up Processes**

Transfer 6 ml supernatant to the tube containing 300mg PSA + 900mg MgSO₄, shake vigorously for 30 sec then centrifuge at >1500 rpm for 1 min.

Withdraw the 2.0 ml supernatant & evaporate to dryness & make up to 2ml with Hexane: Acetone (3:1) for GC analysis.

**Step 3- Inject to GC for analysis**

OBSERVATIONS & RESULTS

It was observed in the study that the mean pesticide residue level in okra washed in Haridra-jal was reduced than that of the okra washed in tap water.

Table 2 – Mean Pesticide Residue Level of without washed, water washed and Haridra-jal washed groups

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Group</th>
<th>Result (in ppm)</th>
<th>Mean value (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>1.</td>
<td>Without washed (grp 1)</td>
<td>0.5426</td>
<td>0.5497</td>
</tr>
<tr>
<td>2.</td>
<td>Water washed (grp 2)</td>
<td>0.3251</td>
<td>0.3498</td>
</tr>
<tr>
<td>3.</td>
<td>Haridra-jal washed (grp 3)</td>
<td>0.1243</td>
<td>0.1623</td>
</tr>
</tbody>
</table>
Table 3 - Comparison of Mean pesticide residue level in between without washed and water washed groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>without washed</td>
<td>3</td>
<td>0.562</td>
<td>38.26%</td>
</tr>
<tr>
<td>washed with water</td>
<td>3</td>
<td>0.347</td>
<td></td>
</tr>
</tbody>
</table>

The mean pesticide residue level in without washed samples to be 0.562ppm and at the same time the mean pesticide residue level after washed with tap water was found to be 0.347ppm. The mean pesticide level was found less with tap water.

Table 4 - Comparison of Mean Pesticide Residue Level in between without washed and Haridra-jal washed groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>without washed</td>
<td>3</td>
<td>0.562</td>
<td></td>
</tr>
<tr>
<td>washed with Haridra-jal</td>
<td>3</td>
<td>0.142</td>
<td>74.74%</td>
</tr>
</tbody>
</table>

The mean pesticide residue level in without washed samples was found to be 0.562ppm and at the same time the mean pesticide residue level after washed with Haridra-jal was found to be 0.142ppm. The mean pesticide residue level was found less with Haridra-jal.

Table 5 - Comparison of Mean Pesticide Residue Level in between Water washed and Haridra-jal washed groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>washed with water</td>
<td>3</td>
<td>0.347</td>
<td></td>
</tr>
<tr>
<td>washed with Haridra-jal</td>
<td>3</td>
<td>0.142</td>
<td>58.23%</td>
</tr>
</tbody>
</table>

The mean pesticide residue level in okra washed in tap water was found to be 0.347ppm and at the same time the mean pesticide residue level after washed with Haridra-jal was found to be 0.142ppm. The mean pesticide residue level was found less with Haridra-jal.

DISCUSSION

Now days, the utilization of pesticides, fungicides, food colours, preservatives and cosmetics etc, are continuously increasing day by day which causes physical and mental hazards effect on human being. Fruits and vegetables are an important part of a balance diet. But due to contamination of pesticides they are not safe for us. Only water wash is not sufficient for washing the vegetables and fruits, so there is a need of a dhavan dravya which can be used as most efficient washing agent than water.

Okra is a common and nutritious vegetable. It is one of the favorite vegetables of Indians and also one of the earliest vegetables cultivated by man. It has a lot of medicinal values. And also it is easily available.

But at the time of cultivation the pests like – Aphids, Flea beetles, Leaf miners, Sting bugs, Fruit worms, Blister beetles, Loopers, Horn worms, White fly etc. affect the production, so insecticide like profenofos is sprayed. So okra and profenofos was chosen for the study.

The drug Haridra selected for this analytical study of pesticide residue is one of important drug mention in Ayurveda. Haridra is very important drug, which is described from ancient time. Aacharya Charak has included 10 herbs in Vishaghna Mahakashaya including Haridra. So it was taken as a Dhavan dravya.

CONCLUSION

The following conclusions can be drawn from present work:

- The mean pesticide residue level was found on okra is 0.562ppm.
- The present study reveals that Tap water reduces the concentration of pesticide residue of profenofos on okra but only 38.26%.
- Haridra-jal also reduces the concentration of profenofos and it was max74.74%.
- Comparison b/w Water washed sample and Haridra-jal washed sample shows 58.23% of change, means reduction of concentration of pesticide residues was more in Haridra-jal washed sample.
- It means that the okra or any other vegetable and fruits should be washed before eating or before processing in kitchen with the Haridra-jal minimum one time thoroughly which will help to minimize the residue within edible thing for okra.
- In the absence of Haridra-jal due to its unavailability we should wash the vegetables and fruits by simple running water, which will also help to minimize the pesticide residue in edible but the Haridra-jal is more beneficial than water.

PICTURE

![Fig.1 Abelmoschus esculentus](image1)

![Fig.2 Cut into small pieces](image2)
Fig. 3 Grind in a mixer

Fig. 4 15gms of sample in 50 ml tube

Fig. 5 Than kept in deep freezer overnight

Fig. 6 Then next day 15ml of 1% Acetic acid in Acetonitrile was added to the 15gms sample

Fig. 7 6.0gms MgSO$_4$ anhydrous and 1.5gms sodium acetate anhydrous was added and for 10 min by hand

Fig. 8 After shaking the sample was centrifuged at > 1500 rpm to shake vigorously separate the solid material
Then 6.0 ml supernatant from the containing centrifuged sample was transferred to a tube and shaken for 30 sec.

Again centrifuged for 1 min

2.0 ml supernatant from sample was withdrawn.

Evaporates to dryness by evaporator.

Dry sample make up to 2.0 ml with Hexane: Acetone (3:1)

Then injected into GC

Gas Chromatography
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