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Research Article

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 dravya* 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* (anti-toxic 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, Fenvalarate 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 insectide is acetylcholinestrase 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.

Commonly used methods for cleaning this vegetable are washing with tap water, warm water, or salt water. In *Ayurveda* some *Vishaghna dravya* are mentioned. So, the study was planned to observe whether the toxic residues are nullified or reduced in the solution of the *Vishaghna dravyas*.

Haridra (Curcuma longa) is a drug that draws the attention because of its multi-pronged utility influencing the human life. Although *Ayurvedic* classics instruct of its high utility in treating the symptoms due to *visha* or the venomous poison and various discrete references point out its utility in various diseases also.

AIMS & OBJECTIVES

- To analyze the residues of pesticide Profenofos on Lady finger before and after *Dhavan by Haridra-jal.*
- To compare the efficiency of *Dhavan by Haridra-jal* with tap water.

PESTICIDE REVIEW

Classification

Based on chemical composition

- Alkyl phosphates- tetraethyl pyrophosphate (TEPP), demeton, sulfotepp, etc
- Aryl phosphates- parathion, paraoxon, diazinon, profenofos, etc

Based on toxicity

- Highly toxic - TEPP, parathion
- Moderately toxic - trichlorfon, runnel, profenofos
- Low toxicity – malathion, Tik-20 [1]

PROFENOFOS- Profenofos is a broad spectrum organophosphate insecticide and acaricide. Profenofos has

been classified as a moderately hazardous (Toxicity class II) pesticide by the World Health Organization (WHO).

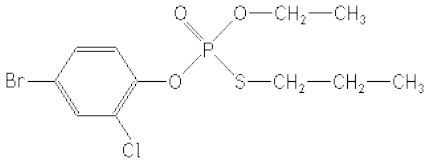
Product name – Profenofos 50% EC

Chemical name - O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate

Type – Insecticide

Molecular formula – $C_{11}H_{15}BrClO_3PS$

Structure -



Physical properties

- **Appearance** - Light yellow to brown color liquid
- **Odour** - Pungent, like garlic or cooked onions
- **Flammability** - Flammable
- **Specific gravity** - 1.455 (20^0 C)
- **Solubility** - In water 20 mg/L (20^0 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 developing 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]

✍ MATERIALS

The following vegetable was selected for the study.

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. [5]

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- H_2O

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, Vishaghna, Kaphapittanut, Pramehanashaka*

Therapeutic uses - *Pandu, Prameha, Vrana, Visha vikara, Kusthha, Tvagroga, shitapitta, Pinasa.* [7]

Pharmacological Actions:

It is antiinflammatory, cholagogue, appetizer, haematinic, hepatoprotective, bloodpurifier, antioxidant, detoxifier and regenerator of liver tissue, antiasthmatic, antitumour, anticutaneous, antibacterial, antifungal, antiprotozoal, stomachic and carminative. [8,9]

CHEMICALS AND REAGENTS

1) 1% acetic acid in Acetonitrile 2)Magnesium sulphate ($MgSO_4$)

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.

(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.

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

Sample	Control group	Experimental (trial) group	
	Without washed (200gms)	Washed with Tap water (200gms)	Washed with *Haridra-jal* (200gms)
Sample 1	15gms	15gms	15gms
Sample 2	15gms	15gms	15gms
Sample 3	15gms	15gms	15gms

METHOD OF EXPERIMENTAL STUDY [10]

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 $MgSO_4$ 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 $MgSO_4$, 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

Sr.	Group	Result (in ppm)			Mean value (in ppm)
		Sample 1	Sample 2	Sample 3	
1.	Without washed (grp 1)	0.5426	0.5497	0.5960	**0.562**
2.	Water washed (grp 2)	0.3251	0.3498	0.3668	**0.347**
3.	*Haridra-jal* washed (grp 3)	0.1243	0.1623	0.1415	**0.142**

Table 3- Comparison of Mean pesticide residue level in between without washed and water washed groups

Group	N	Mean	% of change
without washed	3	0.562	38.26%
washed with water	3	0.347	

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

Group	N	Mean	% of change
without washed	3	0.562	74.74%
washed with *Haridra-jal*	3	0.142	

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

Group	N	Mean	% of change
washed with water	3	0.347	58.23%
washed with Haridra-jal	3	0.142	

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*

Fig.2 Cut into small pieces

Fig.3 Grind in a mixer

Fig.4 15gms of sample in 50 ml tube

Fig.5Than 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.8After shaking the sample was centrifuged at > 1500 rpm to shake vigorously separate the solid material

Fig.9Then 6.0 ml supernatant from the centrifuged sample was transferred to a tube and shacked for 30 sec

Containing Fig.10 300mg PSA + 900mg $MgSO_4$

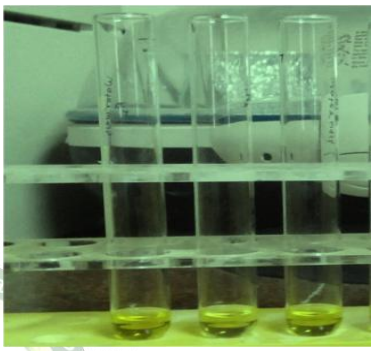


Fig.11Again centrifuged for 1 min

Fig.12Then 2.0 ml supernatant from sample was withdrawal

Fig.13 Evaporates to dryness by evaporator

Fig.14 Dry sample make up to 2.0 ml. with Hexane: Acetone (3:1)

Fig.15 Then injected into GC Fig.16 Gas Chromatography

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