



A Comparative Study of Effect on Brassica Oleracea (Cauliflower) Using Different Washing Methods W.s.r. to Estimation of Pesticide Residue

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ABSTRACT

The potential deleterious effects of environmental chemical exposure caused serious and high concern threat to the life which indeed has gained considerable interest for research works. Cauliflower (Brassica oleracea var. botrytis L.), is one such vegetable where the maximum spraying of pesticides is in practice. There are undeniable evidences regarding the hazardous effects of the sprayed pesticides. Ayurveda has explained the purification measures for all kinds of articles which are poisoned or mixed with chemical. Three samples of Cauliflower were randomly collected from the field which were cut into 4 equal parts. One part was kept as control and remaining three were applied with washing techniques by washing with Tap water, Boiled water and Boiled haridra water respectively. Those all parts were then extracted and subjected to GC-MS for evaluation of concentration of the sprayed pesticides viz. Chlorpyrifos, cypermethrin, profenofos and indoxacarb. Hence, Washing with Boiled haridra water was found more efficient method of washing, which is cost effective and easily practicable.

KEYWORDS : Cauliflower, Pesticide residues, Boiled Haridrawater, Shodhana

INTRODUCTION

The potential deleterious effects of environmental chemical exposure have caused high concern threat to the life, which indeed has gained considerable interest for research works. Their easy availability and reckless use during last few decades has resulted in their build up in ecosystem to carcinogenic levels(1). The organochlorine pesticides widely used before 1970s were banned as they were reported to be toxic to flora and fauna (2). It causes hazardous effects on human health.(3). Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the most important members of the cole crops and has originated in the mediterranean region. It belongs to family *brassicaceae*. (4). Most common pesticides which are sprayed over cauliflower are Chlorpyrifos, Cypermethrin, Profenofos and Indoxacarb. *Garavisha* (artificial poisoning)(5), an Ayurvedic concept which projects the hazardous effects produced by consumption of food substances mixed with the poisons or chemicals. *Garavisha*, refers to the substances which have tendency to accumulate in the body when consumed in smaller quantities. It will produce many diseases and syndromes after the accumulated quantity being capable to produce the illness. Pesticide residues in the cauliflower or other vegetables perhaps constitutes *Garavisha*. These residues if not degraded before consumption they will turn deleterious.

Commonly followed methods to remove these pesticides are washing with water and boiling. Washing with water removes externally adhered pesticides. *Boiling* the vegetables in water also contributes to vaporize some of the pesticides but the deep seated and non degradable pesticides will be retained in the vegetables even after boiling. Hence there is need of the substance which can easily degrade and nullify the effect of pesticide. In this study comparative evaluation between washing with tap water, boiled water and boiled turmeric water. There are plenty of articles regarding the illness caused by the pesticide residues but articles regarding treatment of cauliflower, which reduces the concentration of pesticide, are countable. In this present study an attempt was made to appraise the concentration of the pesticide residues before and after washing with boiled turmeric water in comparison with the normal tap water and boiled water.

MATERIALS AND METHODS

Materials

Collection of Cauliflower

Medium sized well grown 15 cauliflower samples were randomly collected from the village named Kadrolli, (Taluka: Kittur; Dist: Belgaum) from a farmer's field. The authentication of samples was done in AYUSH approved ASU drug testing laboratory. The bottles of pesticides sprayed to them in that field were also collected and the pesticides and their proportion were noted

Methodology

Each cauliflower was divided into four parts (Picture-3, 4 & 5) and coded as mentioned below. Then each part was cut into small fragments. See table no. 1

Total number of samples – 12.

Washing methods:

1. 500gms of each raw sample (AR, BR & CR) were extracted as it is without submitting to any washing method which were considered as controls for respective groups.

2. Second part of cauliflower (500gms) was washed in 175 ml of tap water by rubbing and rinsing for 5 min. Then they were kept on tissue for soaking, and used further for extraction procedures. (Picture. 06).

3. Third part of cauliflower (500gms) was washed in 175ml of Boiled water for 5 min, rinsed and soaked on tissue paper and further taken for extraction procedure (Picture. 07).

4. Initially Boiled *Haridra* water was prepared by boiling 2gm of *Haridra* (Turmeric) powder in 175ml of water for 5 min. The fourth part of Cauliflower (500gms) was added to Boiled *Haridra* water, rinsed for 5 min and then soaked on tissue paper which was taken for extraction procedure (Picture. 07 & 08).

Extraction of cauliflower sample

The raw samples and other samples were chopped in small pieces. From 500gm of cauliflower, 50 gm of each parts of cauliflower were taken and macerated with 7.5gm of anhydrous sodium sulphate in blender to make a paste. Macerated sample were extracted with 100 ml of acetone on mechanical shaker for 1 hour (pic. 12). After that extract were filtered, concentrated up to 40 ml and subjected to liquid - liquid partitioning (pic 13 & 14) with ethyl acetate (50, 30, 20 ml) after diluting 4-5 times with 100 ml 10% aqueous NaCl solution then concentrated the organic phase up to 10ml on rotatory evaporator. Concentrated part of each sample which is 10ml in concentration was then cleaned up.(6)

Analysis of Pesticides using Gas chromatography

Further the samples were concentrated using Rota Vapor. These extracts were sent to "Center for food testing laboratory", Pune, Maharashtra, there before injecting the sample in gas chromatography machine they done sample clean up. For sample clean up, 2ml from each sample were taken then added 275 mg of PSA (Primary secondary Amines). After adding, PSA samples were kept on vortex mixer for 2 minutes. It was then centrifuged on refrigerated centrifugation

machine on 7000 rpm for 5 minutes. Then 10ml supernatant solution were collected in test tube. This solution was evaporated in low volume concentrator. This procedure of evaporation took 1 to 2 hour. 1ml of Ethyl acetate was added and shaken well, then it was filtered with 0.22um filter paper and the samples were filled in vials. Now the sample was ready to inject in Gas Chromatography – Mass Spectrophotometry machine (Agilent technologies 7890 A G C System). CFT Code for center for food testing laboratory was CFT code- CFT/1 02. The samples were injected into the machine according to SOP's of the instrument. Retention time, area of response and final concentration of pesticide residue were analyzed by the graph. (6)

RESULTS

Maximum reduction in concentration of Indoxacarb (79.244%), moderate reduction in Chlorpyrifos (67.162%), Prefenofos (54.354%), and very minimal reduction cypermethrin (6.991%) was observed by following traditional procedure i.e. washing with tap water.

Washing with boiled water has significantly reduced Prefenofos (97.379%) and Indoxacarb (95.5886%). Chlorpyrifos (55.019%) was moderately reduced and minimal reduction was found in cypermethrin (29.841%).

The group, which was washed with **boiled haridra water**, has significantly reduced 95.72% of the concentration of chlorpyrifos (0.1743 ± 0.151); 96.631% of indoxacarb (0.683 ± 0.147), 100% of **prefenofos** (0.00 ± 0.00) and 23.260% of **cypermethrin** (149.86 ± 15.26).

Tap water considerably reduced 79% of indoxacarb and 67% of chlorpyrifos. In other samples there was no considerable change. It can be noted that all the washing methods have reduced more than 50% of the all the pesticides except Cypermethrin. Mean difference between the groups has revealed that raw sample and haridra boiled water has got highest difference among all, which indicates significant reduction in the concentration of all pesticides after washing with haridra boiled water.

DISCUSSION

Pesticides are widely used in agricultural production to prevent or control pests, diseases, weeds, and other plant pathogens in an effort to reduce or eliminate yield losses and maintain high product quality. Serious concerns have been raised regarding health risks resulting from occupational exposure and from residues in food and drinking water.(7) *Aaharavidhivisheshaaayatana (guidelines for the food preparation and intake)*(8), a concept of Ayurvedic treatise which enlighten the preparation of food and its consumption guidelines, portrays *toya* (contact with water) and *Shoucha* (purification) procedures. These *Sanskara* (refining) procedures either fetch the changes in properties or detoxify the chemicals. *Shoucha* (= *shodhana*, purification), which intends either to remove the toxic principles or to detoxify them by means of washing or processing with antitoxic herbs(8). On the basis of these principles an attempt was made to purify cauliflower in different methods viz. washing with water, boiled water and *haridra* boiled water.

There are references in Ayurveda to purify these polluted/poisoned substances, named, *Shodhana Sanskara* (purification procedures) which perhaps may either bring various changes in properties of substances, or alter the chemical nature of the substances, or detoxify the toxic principles by treating with various antitoxic herbs, which actually do so. One among such procedure is *Shoucha* (Washing)(9). The group, which was washed with Boiled haridrawater, has significantly reduced 100% of **prefenofos**, 96.631% of Indoxacarb (0.683 ± 0.147), 95.72% concentration of Chlorpyrifos (0.1743 ± 0.151); and 23.260% of **Cypermethrin** (149.86 ± 15.26). see graph no.1 and table no.3 Washing the samples with tap water has washed off superficially adhered residues. Based on their concentration on the surface they showed varied reduction. Cypermethrin which is quite stable, has showed very minimal reduction. This might have penetrated into deeper tissues of the flower hence only 6% of reduction was observed after washing with tap water.

Boiled water has higher temperature than the normal tap water which might be responsible for the hydrolysis of residues and thus

more reduction was observed than tap water which has relatively lesser temperature. Boiled *Haridra* water has more significantly reduced the concentrations than other groups. Possibly, due to the temperature of the liquid which has caused oxidation and hydrolysis. The chemical constituents of the turmeric which might have caused the chemical degradation by chemical reaction with the residues, which might have formed a stable compound which was washed off easily by water. Ayurveda mentioned various drugs having multifaceted activity, utilized as medicines, food etc. The appreciated constituents of haridra, Curcumin (50-60%), Curcumenone, Cineole, Camphene, Beta-sitosterol, have been reported as best vishghna (Antitoxic) which may bind or denature the pesticides which are markedly reduced. Several studies have found that surface residues are amenable to simple washing operations whereas systemic residues present in tissues will be little affected. There is evidence for a variety of crops and pesticides that the proportion of residue that can be removed by washing declines with time. This has been interpreted as being due to residues tending to move into cuticular waxes or deeper layers. For example the fractions of fenitrothion or methidathion residues on cauliflower that could be removed by washing or blanching were inversely proportional to the days after spray application(10).

Geisman J *et al* evidenced that hot washing and blanching are more effective than cold washing and the effectiveness may be further improved by detergent(11). Farrow *et al* found that blanching removed 82 percent of methidathion residues from cauliflower and did not show any effect of withholding period as compared to the lower proportion of residues removed by washing. Domestic rinsing is less effective compared to thorough commercial washing. Hot caustic washes used in some commercial peeling operations can efficiently remove and degrade residues of hydrolysable pesticides(12) Boiled *haridra* water being an organic substance, which perhaps may be responsible for binding of the cypermethrin and indoxacarb as Cypermethrin is a synthetic, pyrethroid insecticide that has high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light (Kaufman *et al.*, 1981, and U.S.D.A., 1995). The water solubility of cypermethrin is very low, 4 ppb at 200C (Kollman and Segawa, 1995). Cypermethrin is extremely hydrophobic and will quickly move from an aqueous solution to suspended particulates (Fitzpatrick, 1982). Cypermethrin hydrolyzes slowly in water at pH 7 and below, with hydrolysis being more rapid at pH 9. Under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life of >50 days. It is also stable to photolysis with a half-life of >100 days. In sterile solution in sunlight, cypermethrin photodegrades slowly, with <10% lost in 32 days (Walker and Keith, 1992). In darkness, cypermethrin was fairly stable with 88.7 and 95.6% recovery after 10 days in river water and distilled water, respectively (Takahashi *et al.*, 1985).(13) Indoxacarb is a member of the new oxadiazine class of insecticides. The pesticide is relatively non-volatile with a low vapor pressure and a low Henry's law constant. Consequently, volatilization is not a significant factor in dissipation. The low water solubility, high K_{ow} , and moderately high K_{oc} suggest that indoxacarb will have a moderate to strong tendency to partition from water to soil and therefore be relatively immobile in soil. Indoxacarb undergoes alkaline-catalyzed hydrolysis, photodegradation in water, and microbial mediated degradation. In near-neutral aerobic aquatic systems, indoxacarb degraded with half-lives ranging from 18 to 34 days. (14) Adulteration and spraying of pesticides on the haridra itself is a major concern. Boiled haridra water has effectively degraded all the pesticides at the maximum which may be due to the organic constituents and the temperature of the water. It could be very easy, cost effective and beneficent method of washing cauliflower.

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TABLES

Table no. 1 Sampling method

	Sample A	Sample B	Sample C
Raw,	AR	BR	CR
Tap water washing;	AT	BT	CT
Boiled water washing	AB	BB	CB
Boiled Haridra water washing	ABH	BBH	CBH

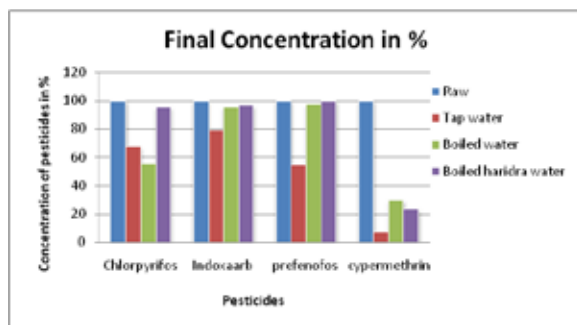
Table no. 2 Concentration of pesticides

Samples	Concentration of pesticides with standard deviation				P value
	Chlorpyrifos	Indoxcarb	Profenofos	Cypermethrin	
Raw	5.91±0.35	20.56±1.57	7.93±0.43	195.03±9.91	<0.0001
Tap water	2.55 ±0.48	4.13±3.59	3.62±0.459	181.48±11.98	
Boiled water	3.20±0.32	0.90±0.22	0.21±0.263	136.35±29.39	
Boiled Haridra water	0.17±0.15	0.68±0.14	0.00±0.00	149.87±15.26	

Table no. 3 Reduction in pesticide residues after processing (in %)

Samples using different washing	Reduction in Percentage of Chlorpyrifos	Reduction in Percentage of Indoxcarb	Reduction in Percentage of profenofos	Reduction in Percentage of cypermethrin
Tap water	67.162%	79.244%	54.354%	6.991%
Boiled water	55.019%	95.5886%	97.379%	29.841%
Boiled Haridra water	95.724%	96.631%	100%	23.260%

Graph no. 1 Graph Showing final concentration in percentage.



CONCLUSION

In this present study the most efficient method of washing for removal of residual pesticide in the cauliflower was evaluated. Boiled haridra water sample was appreciated to be an efficient method of washing, in which significant reduction (100% of profenofos, 96.631% of indoxcarb, 95.72% of chlorpyrifos) in their residual concentration in all the pesticides except cypermethrin (23.260%) was evidenced by the GC-MS. Washing with boiled water has also reduced the concentrations but not as efficient as boiled haridra water washing and only by washing with tap water has moderate effect on the residues. Hence it could be concluded that washing with haridra boiled water was found more efficient method of washing, which is cost effective and easily practiced.

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