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Risk Mitigation Methods for Removal of Pesticide Deposits in Spinach for Reducing Residues Content

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A B S T R A C T

Studies were undertaken to establish decontamination methods of commonly used insecticides Triazophos 40% EC, Deltametrin + Triazophos 36% EC sprayed at recommended dose in open field situations. The zero day samples from two various treatments were collected separately in large quantities and made into 2 sets, each in 4 replications. One set of sample from each treatment (in 4 replications) was analyzed for deposits of the pesticide. The remaining sets of samples of zero day from each treatment samples were subjected to various (Tap water wash, soaking in 2% salt solution for 10 min followed by tap water wash and hot water cooking of leafy vegetable for ten min) decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in the removal of pesticide residues from the spinach samples. Among various decontamination methods tested, hot water treatment wash was found to be very effective in removing pesticide residues to an extent of 50-90% varying with type of pesticides followed by common methods *i.e.*, 2% salt solution wash was also effective method removing residues in the range of 30-65%, tap water in the range of 13-55%.

Introduction

Spinach (*Spinacia oleracea* L.) belonging to the family Chenopodiaceae, originated in ancient Persia. It is a green-leafy vegetable often recognized as one of the functional foods for its nutritional, antioxidants and anti-cancer constituents, is a super-cold-hardy vegetable, a tender crop that can be planted in winter (*rabi* season). It is higher in iron, calcium and one of the best sources of vitamins A, B, and C than most cultivated greens. They are rich in mineral and hence can be called as "Mines of minerals". Vitamin A and C are present in abundant quantities. The popular palak growing states include Uttar Pradesh, West Bengal, Maharashtra and Gujarat. Though it is not very popular in South India, it is extensively cultivated in Telangana region (Hyderabad, Rangareddy, Medak, Nalgonda districts). Spinach is infested by caterpillars (Lepidopterous Pests *i.e., Spodoptera exigua*), green peach aphid (*Myzus persicae*), potato aphid (*Myzus persicae*), leafminers (*Pegomya hyoscyami*), spinach crown mite (*Tyrophagus dimidatus*) and wireworms (*Agriotes segetus*) resulting in ragged appearance that diminishes the beauty of the garden to wilted or dead plants. Leafy and bitterly delicious, conventional spinach

might have made strong, but it could also damage our health due to residues. It often contains a pesticide called permethrin, a neurotoxin. Symptoms of high exposure include tremors, loss of coordination, elevated body temperature and behavioral problems.

The pesticide has also been labelled as a carcinogen because it causes lung tumors in female mice and liver tumors in mice of both sexes. This is in addition to the potential exposure to 48 other harmful pesticides. Besides, unguided use of pesticides leads to pesticide residues in leafy vegetables and also contaminates the environment which may lead to deleterious impacts not only on human health but also on other biota (Sreelatha and Diwakar, 1997). As consumption of leafy vegetables has been increasing in recent times and peri urban cultivation of leafy vegetables is on the rise, it is essential to know the extent of usage of different pesticides and also monitor their residues. Since much study was not done on this crop about pesticide usage pattern, farmer perception and different decontamination studies for the safety of the consumer.

Materials and Methods

The zero day samples from two various insecticide treatments were collected separately in large quantities and made into 2 sets, each in 4 replications. One set of sample from each treatment (in 4 replications) was analyzed for deposits of the pesticide. The remaining sets of samples of zero day from each treatment samples were subjected to various decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in the removal of pesticide residues from the spinach samples. The following decontamination / risk mitigation methods were selected for evaluation of efficiency in removal of pesticide residues from spinach.

T_1 (Tap water wash)

Four litres of tap water was taken into the plastic tub of 7 litres capacity and 1 kg of spinach leaves were dipped in the tub for 30 sec, further the leaves were kept for air drying on tissue paper for 5 min, followed by analysis.

T_2 (Soaking in 2% salt solution for 10 min)

Four litres of 2 percent salt solution was prepared by mixing 80 g of table salt in 4 litres of water in plastic tub of 7 litres capacity and 1 kg spinach leaves were dipped in the tub for 10 min, further the leaves were kept for air drying on tissue paper for 5 min, followed by analysis.

T_3 (Hot water cooking10 min)

Spinach leaves were cooked (boiled) in the cooker upto 10 min, with sufficient water, further the boiled material were kept for air drying on tissue paper for 5 min, followed by analysis.

T_4 (Control)

In this, 1 kg of spinach leaves was not treated with any of the decontamination methods and was preceded for the analysis.

Method validation

The spinach leaves (5 kg) collected from field were brought to the laboratory. The sample was homogenized using Robot Coupe Blixer (High volume homogenizer) homogenized sample of each 15 g was taken in to 50 ml centrifuge tubes, 30 ± 0.1 ml acetonitril was added to the tube, it was silent crushed (to get fine sample), 3gm sodium chloride was added, centrifuged for 3 min, the supernatant of 16 ml was taken into 50 ml centrifuged tubes into it added 9 gm anhydrous sodium

sulphate, from this 8 ml was taken into 15 ml centrifuged tubes into which 1.2 gm magnesium sulphate and 0.4 gm PSA was already added, kept on vertex 30 min, centrifuged for 5 min, 2 ml of it was transferred into vials.

Then these samples were added for GC and LC analysis of chemicals (for LC Analysis 1 ml of centrifuged sample were further taken, directly transferred into vials and for GC analysis caliper is set, 2 ml of centrifuged sample was made to evaporate completely, then 1 ml oh hexane was added to the evaporized and was filtered), these fortification levels are selected to know the suitability of the method to detect and quantify pesticides in spinach below Maximum Residue Limits (MRLs) of Codex Alimentarius Commission (CAC). The AOAC official method 2007.01 (Pesticide Residues of Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate) was slightly modified to suit to the facilities available at the laboratory and the same was validated for estimation of LOQ (Limit of Quantification) of above mentioned pesticides in spinach matrix.

The final extract of the sample i.e., 2 ml equal to 1 g of the sample was evaporated using turbovap and made up to 1 ml (equal to 1 g sample) using suitable solvent for analysis on GC, while for LC analysis, filtered 1 ml final extract (equal to 0.5 g sample) was directly injected in LC and the residues of pesticides recovered from fortified samples were calculated using the following formula

Details of GC parameters (TRIAZOPHOS)

Spinach samples fortified with Triazophos at 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg were analyzed and the mean recovery of the residues using the method was 93.02%, 118.4% and 110.9%, respectively and the results show that the method is suitable for the analysis of Triazophos residues up to 0.05 mg kg^{-1}, and the limit of quantification (LOQ) is 0.05 mg kg^{-1}**.**

Spinach samples fortified with deltamethrin + triazophos at 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} were analyzed and the mean recovery of the residues using the method was 103.7%, 124.4% and 124.1%, respectively and the results show that the method is suitable for the analysis of Chlorpyriphos residues up to 0.05 mg kg^{-1}, and the limit of quantification (LOQ) is 0.05 mg kg^{-1} (Table 1).

Results and Discussion

The spinach samples were collected from various plots treated with recommended doses of triazophos and deltamethrin + triazophos to estimate pesticide residues and the efficiency of different test decontamination methods was evaluated through quantification of their residues after subjecting to risk mitigation methods.

Triazophos

Various decontamination methods were evaluated to know their efficiency in removing triazophos residues from spinach leaves. Results revealed that all the treatment solutions significantly differed among each other in their efficiency in removing triazophos residues. Hot water cooking for 10 min was found to be most effective (54.72%) than other treatments. Next promising treatment was dipping in 2% salt solution for 2 min followed by tap water wash for 30 sec (43.78%). Other treatment like tap water wash (19.88%) is also found to remove significant amount of residues from spinach leaves.

Percentage removal of triazophos residues due to various decontamination methods is depicted in figure 1.

Table.1 Details of GC parameters (Triazophos)

Gas Chromatograph	Gas Chromatography- SHIMADZU -5010
Column	Phenomenex Zb5 ms column 30 Mx 0.25
Column Oven (^{0}C)	Initial 150^{0}C 5 min hold 5 ^{0}C up to 200 ^{0}C and then 5 min hold, 2^{0}C min up to 280 ^{0}C total 60 min
Detectors	Electron Capture Detector (ECD) Thermionic Specific Detector (TSD)
Detector Temperature (^{0}C)	300
Injector Temperature (^{0}C)	260
Injector Status	Split Ratio: 1:10
Carrier Gas	Nitrogen, Iolar II, Purity 99.999%
Carrier Gas Flow (ml min-1)	1 ml min-1
Make-up Flow (ml min-1)	25 ml min-1
Retention time (min)	Triazophos 37.60 min
Total run time (min)	60 min

Recovery of Triazophos residues in spinach

Details	**Recoveries of Triazophos from fortified spinach samples**					
	Fortified level (mg kg^{-1})					
	0.05 mg kg^{-1}		0.25 mg kg^{-1}		0.50 mg kg^{-1}	
	Calculated Level (mg kg^{-1})	Recovery %	Calculated Level (mg kg^{-1})	Recovery %	Calculated Level (mg kg^{-1})	Recovery %
R1	0.044	89.08	0.302	121.6	0.56	113.5
R2	0.046	92.87	0.292	116.8	0.54	108.0
R3	0.048	97.10	0.293	117.3	0.55	111.1
Average		93.02		118.4		110.9
SD		4.00		2.34		2.78
RSD		4.30		1.98		2.51

Details of GC parameters (Deltamethrin + Triazophos)

Gas Chromatograph	Gas Chromatography- SHIMADZU -5010
Column	Phenomenex Zb5 ms column 30 Mx 0.25
Column Oven (^{0}C)	Initial 150^{0}C 5 min hold 5 ^{0}C up to 200 ^{0}C and then 5 min hold, 2^{0}C min up to 280 ^{0}C total 60 min
Detectors	Electron Capture Detector (ECD) Thermionic Specific Detector (TSD)
Detector Temperature (^{0}C)	300
Injector Temperature (^{0}C)	260
Injector Status	Split Ratio: 1:10
Carrier Gas	Nitrogen, Iolar II, Purity 99.999%
Carrier Gas Flow (ml min-1)	1 ml min-1
Make-up Flow (ml min-1)	25 ml min-1
Retention time (min)	Deltamethrin 22.15 min Triazophos 15.31 min
Total run time (min)	60 min

Recovery of Deltamethrin residues in spinach

Details	**Recoveries of Deltamethrin from fortified spinach samples**					
	Fortified level (mg kg^{-1})					
	0.05 mg kg^{-1}		0.25 mg kg^{-1}		0.50 mg kg^{-1}	
	Calculated Level (mg kg^{-1})	Recovery %	Calculated Level (mg kg^{-1})	Recovery %	Calculated Level (mg kg^{-1})	Recovery %
R1	0.045	89.78	0.242	96.76	0.516	103.25
R2	0.045	90.73	0.222	88.72	0.527	105.39
R3	0.046	91.56	0.247	98.79	0.522	104.39
Average		90.69		94.56		104.34
SD		0.89		5.33		1.07
RSD		0.98		5.02		1.02

Fig.1 Per cent removal of Triazophos residues from spinach by Various decontamination methods

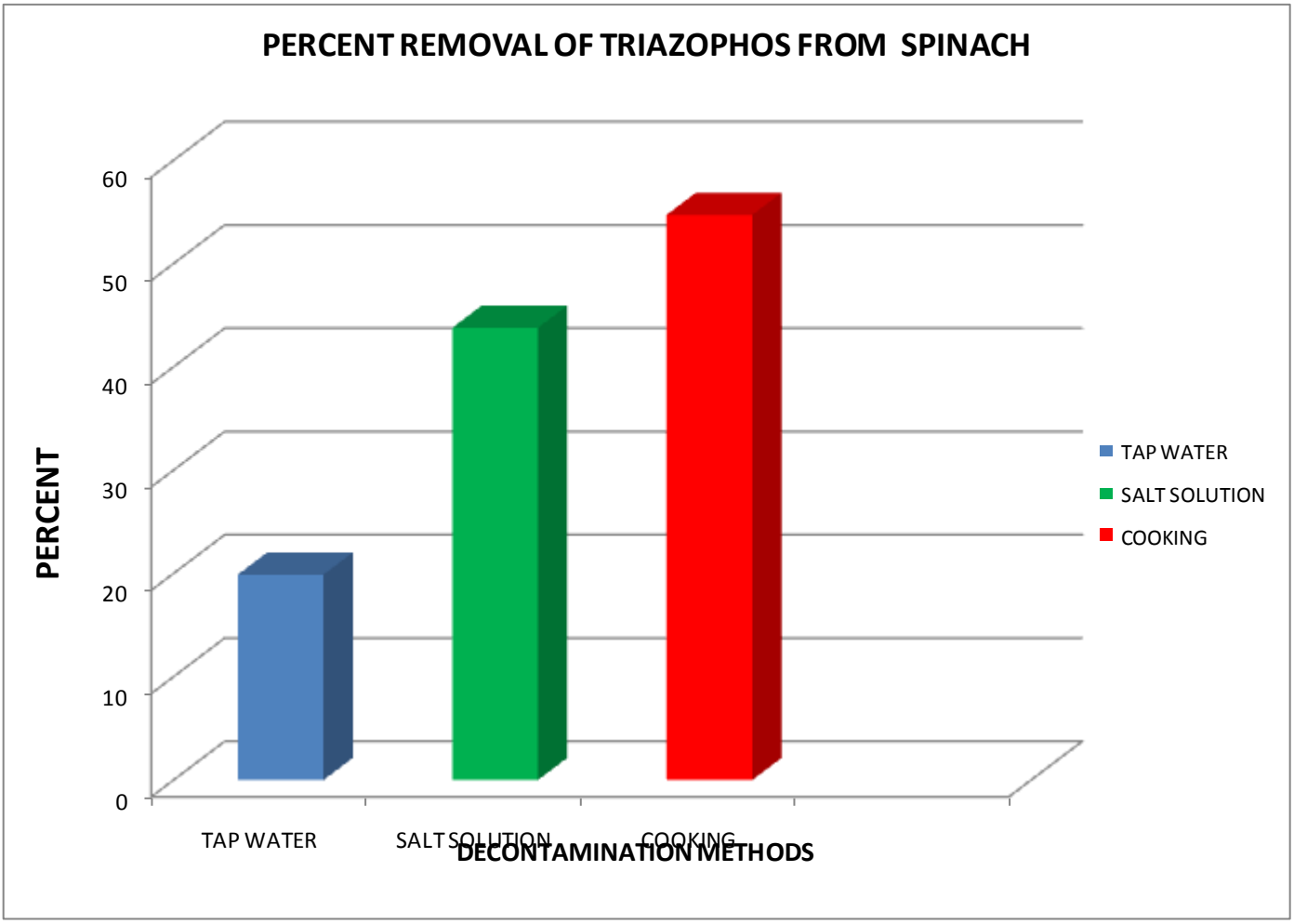
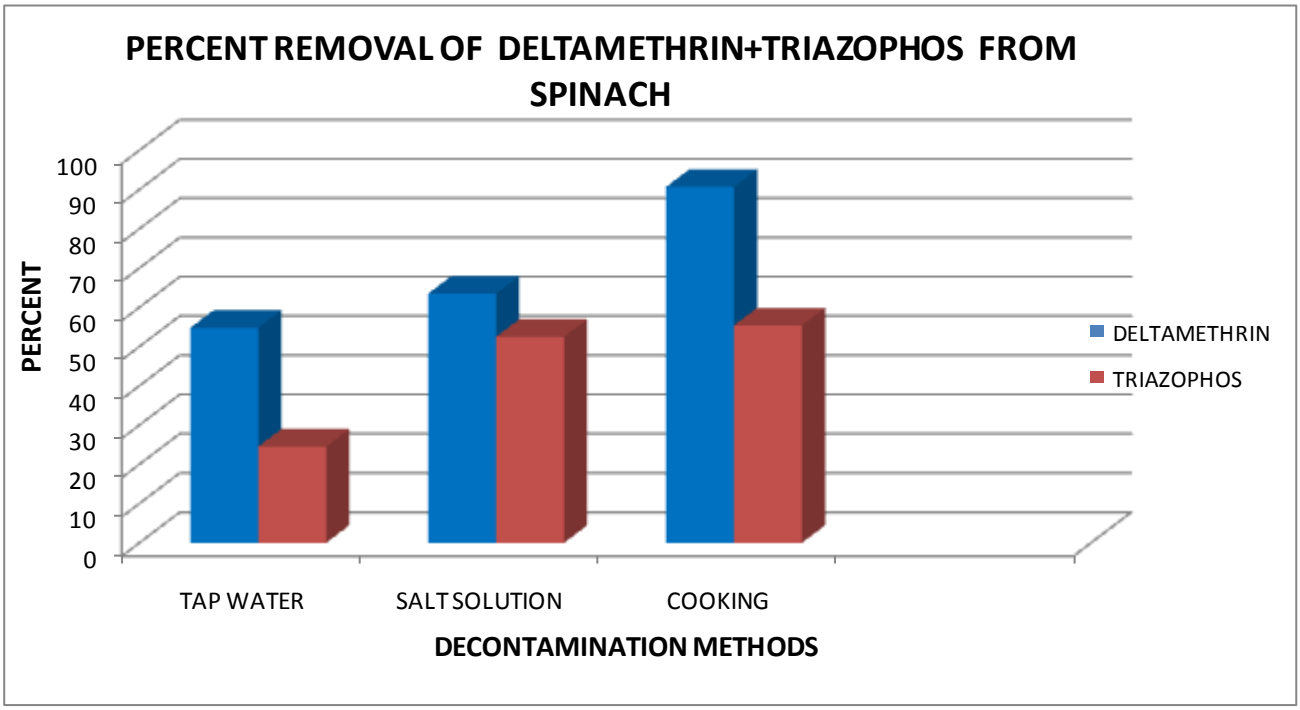


Fig.2 Per cent removal of Deltamethrin residues from spinach by Various decontamination methods



Deltamethrin + Triazophos

The percentage removal of deltamethrin and triazophos residues in spinach when subjected to different decontamination methods at 2 hours after spraying showed that hot water cooking for 10 min was found to be most effective removing 90.71% (deltamethrin), 55.42% (triazophos) residues over other treatments.

The next promising treatment was 2% salt solution 63.50 % (deltamethrin), 52.51% (triazophos) and tap water treatment 54.59 % (deltamethrin), 24.54% (triazophos).

Based on the percentage removal of residues, it is noticed that there is a significant difference in the efficiency of different decontamination methods in removing residues of above mentioned pesticides.
Percentage removal of deltamethrin + triazophos residues from various decontamination methods is depicted in figure 2.

In the present study hot water cooking proved to be the most efficient in removing various pesticides. Hot water cooking for 10 min was found to be the most effective treatment compared to salt solution and tap water.

The results are in agreement with the findings of Harinath Reddy *et al.*, (2014) who reported that cooking reduced the residues up to 42.9-83.2% in tomato. Randhava *et al.*, (2007) and Yang *et al.*, (2012) reported that cooking removed maximum residues, which is in conformity with present study.

Two percent salt solution was found to be second effective treatment after the cooking treatment. The results are in compliance with Soliman *et al.*, (2001) who reported that 2% salt solution removed pesticide residues up to 20 – 90%. Washing of tomato fruits with 2%

salt solution removed 82.4% of profenophos residues (Abou-Arab., 1999) and the residues of carbaryl and methomyl were removed up to 39.33% and 91.98% from leafy vegetables by 0.9 % salt solution respectively (Kilnhom *et al.,* 2008).

Tap water wash was the least effective treatment and the findings of present investigations are in conformity with the findings of Panhwar *et al.,* (2013) reported that washing of cauliflower curds with water removed profenophos residues by 14.32%, 10-50% of deltamethrin residues were removed from spinach leaves when washed with tap water (Bonnachere *et al.,* 2012).

Similarly tap water wash for 10 min removed trichlorfon, dimethoate, dichlorovos, fenitrothian and chlorpyrifos residues by 53.7%, 32.6%, 52.4%, 26.7% and 62.9% in cucumber, respectively (Liang *et al.,* 2012) and tap water removed the deltamethrin residues up to 36.25 -52.76% (Singh *et al.,* 2005)

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