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Studies on method validation of ethion 50 EC on GC-FPD and its decontamination behavior from edible cabbage heads

CG Sawant, Guru PN, SG Mundhe, CS Patil, L Devarishi Sharma and Lalhmingsanga

Abstract
Studies on method validation of ethion 50 EC on gas chromatography equipped with a flame photometric detector (FPD) and the decontamination behavior of ethion 50 EC insecticide in cabbage heads were undertaken. Residues were estimated by following the series of quality tests involving the quantitation of an analyte in a specific solvent and sample matrix using a specific laboratory procedure and measurement system. The limit of quantification was 0.05 µg ml^{-1}. R^2 values for linearity and matrix match studies were 0.99. Percent recoveries at the fortification levels of 0.05, 0.25, and 0.50 mg kg^{-1} were between 70-120 percent depicting the validity of the methods used for the present studies. Among various culinary food processes used to decontaminate ethion residues from cabbage heads, close pan cooking was found the most effective process which was recorded 40.74 to 61.53 percent reduction in 3 and 5 days sample, respectively followed by open pan boiling to the tune of 29.63 to 53.84 percent in 3 and 5 days sample, respectively.

Keywords: Method validation, chromatography, ethion, recovery, decontamination

Introduction
Cabbage and cauliflower are preferred hosts of *P. xylostella* all over the world. These vegetables are high-value crops with high cosmetic standards; therefore, effective and economical management of the pest is necessary. To control *P. xylostella* not only as effective but also from an economic point of view, insecticides are the most common strategy adopted by farmers. Farmers need new effective insecticides due to *P. xylostella* long history of eventually becoming resistant to every insecticide used extensively against it.

Pesticides, as a key component of integrated pest management, play an important role in increasing agricultural production, but their indiscriminate and unwise use has led to the environmental problems including health hazards (Akbar *et al*., 2010) [1]. A huge amount of pesticides are used on vegetables and their irrational and continual use has the reason resulted in the accumulation of pesticide residues in the primary agricultural products as well as soil. (Baig *et al*., 2009) [3]. The occurrence of residues of insecticides in vegetable crops is a major concern as they are consumed fresh. Therefore it is unwise to use chemical insecticides alone to manage insect pests. An insecticide should be effective and economic but it should not leave toxic residues.

Organophosphorus insecticides like ethion are being used for the control of insect pests in vegetable crops but they are not recommended for cabbage by Central Insecticide Board and Registration Committee. Recently residues of ehion insecticides have been reported in different vegetables including cabbage, cauliflower, brinjal and tomato (Beena Kumari *et al*, 2002; Singh and Gupta, 2002) [4, 23]. In some cases, the residues of these insecticides exceeded its tolerance limit. The degradation or dissipation of insecticide is influenced by climatic condition, types of application, plant species, dosage interval between application and time of harvest (Khay, *et al*., 2008) [13].

The behaviour of organophosphate pesticides in agriculture produce are of great importance, since the disappearance and persistence of compound determines its usefulness or its effects on the environment. So, we need to decrease the health risk to human and environment from the exposure of pesticides. Considering the toxicity of various pesticides, it is necessary to determine effective methods to remove or to minimize the residue levels in food and keep them below maximum residue limits through traditional processing.

Washing by tap water, washing with brine solution, washing with tamarind water, boiling and cooking have a cumulative effect on the reduction of residues of pesticides in vegetables. Therefore, it is necessary to study the method validation and culinary processs to decontaminate or remove the non-recommended insecticides for consumption of cabbage heads.

Materials and Methods

Insecticide Standards

The Certified Reference Material (CRM) of Ethion (Sigma-Aldrich) with a purity of 97.8 percent was made available by Pesticide Residue Laboratory, AINP on Pesticide Residues, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. An accurately weighed 10 mg of an analytical grade ethion standard was dissolved in a 10 ml volumetric flask using toluene to prepare the standard stock solution to 1000 mg kg^{-1}. The standard stock solution was further diluted to obtain immediacy and working concentrations of 100 and 10 mg kg^{-1}. From intermediate standards, working standards of 1.00, 0.50, 0.40, 0.25, 0.10, and 0.05 mg kg^{-1} were prepared by suitably diluting the stock solution in ethyl acetate and used as a standard check-in in residue determination.

Method validation

Method validation is used to confirm the analytical procedure employed for a specific test is suitable for its intended use. Parameters *i.e.* the limit of detection (LOD), the limit of quantification (LOQ), specificity, linearity, matrix match, recovery, repeatability, and reproducibility studies were performed to validate the method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) of Ethion was determined by considering a signal-to-noise ratio of three concerning the background noise obtained for the blank sample. The limit of quantification was (LOQ) determined as two and a half times of LOD.

Specificity

Specificity studies were performed by spiking the cabbage sample and reagent blank with working standards of ethion at the concentration of 0.05 mg kg^{-1}. The area of cabbage sample and reagent blank was compared with spiked matrix match area.

Linearity studies

Six linear concentrations (0.05, 0.10, 0.25, 0.40, 0.50, and 1.00 mg kg^{-1}) of the working standard of ethion were injected three times, and the linearity lines were drawn.

Recovery studies

The analytical method for the estimation of residues of ethion in cabbage was validated by conducting recovery studies using cabbage samples from control samples. 10 (Ten) g each of control samples of cabbage was taken in separate 50 mL centrifuge tubes in three replicates; each was spiked separately with ethion at the required fortification levels *i.e.* LOQ, 5 x LOQ and 10 x LOQ, adding an appropriate volume of working standard of 10 mg kg^{-1}. This mixture was then shaken, to attain a proper homogeneity of insecticide in the samples. The extraction and clean-up were followed as per QuEChERS method as described below. The percent recovery was calculated by using the following formula.

$$\text{Percent recovery} = \frac{\text{Quantity of insecticide recovered}}{\text{Quantity of insecticide added}} \times 100$$

Repeatability

A repeatability study or retest reliability was performed to check the variation in measurements taken by the same person on the same instrument on the same item under the same conditions. Standards of ethion were separately spiked into the control samples of cabbage at the required fortification levels *i.e.* LOQ, 5 x LOQ and 10 x LOQ.

Reproducibility

A reproducibility study was performed to test the ability of an entire analysis of an experiment by another person on the same instrument on the same item under the same conditions. For reproducibility study, standards of ethion were separately spiked into the control samples of cabbage at the required fortification levels *i.e.* LOQ, 5 x LOQ and 10 x LOQ.

Extraction and clean-up

Modified QuEChERS method

The cabbage samples were extracted and cleaned up using the modified QuEChERS method (Sharma, 2013) [20]. The pre-grinded sample of 1 kg was macerated thoroughly in a mixer and grinder (Robot coupe), and approximately 10 g homogenized sample weighed in a 50 ml polypropylene tube which was kept in a deep freezer for 10 min. Homogenized samples were extracted with 10 ml ethyl acetate in the presence of 10 g anhydrous Na_2SO_4 and centrifuged at 3500 rpm for 5 min. Two ml supernatant was transferred to a 15 ml polypropylene tube containing 50 mg Primary Secondary Amine (PSA). The content was mixed well centrifuged at 2500 rpm for 2 min. The supernatant was filtered through a 0.2-micron filter, and GC analysis was carried out.

Residue Determination

Residue estimation of ethion was performed using a Gas Chromatograph (Shimadzu 2010 plus) equipped with a Flame Photometric Detector (instrument parameters listed in table 1). Identification of residue was accomplished by retention time (RT) and compared with known standard (CRM) at the same conditions. The quantities of residues were calculated on a peak area basis by using the following formula.

$$\text{Residues (mg kg}^{-1}) = \frac{\text{Area of sample}}{\text{Area of standard}} X \frac{\mu l \text{ of sample injected}}{\mu l \text{ of standard injected}} X \frac{\text{Conc. of standard (ppm)}}{\text{wt .of sample}} X \text{ Final volume (ml)}$$

$$\text{Wt. of sample} = (g) \frac{\text{Sample Wt. (g) X Aliquot taken (ml)}}{\text{Volume of solvent added (ml)}}$$

Table 1: Gas Chromatographic Parameters

Column	DB-1, 30 m x 0.25 µm × 0.25 mm
Column Temperature	170 °C 3 min hold @ 6.5 °C/min 220 °C 2 min hold @ 10 °C/min 280 °C 6 min hold
Injector Temperature	250 °C
Column Temperature	170 °C
Detector Temperature	300 °C
Injection Volume	1 µl
Column flow	0.96 ml min^{-1}
Hydrogen Flow	90 ml min^{-1}
Air Flow	120 ml min^{-1}

Experiment on decontamination of ethion 50 EC in cabbage

Impact of different food processes commonly followed at home before consumption of cabbage were studied by subjecting the cabbage heads to different treatments. For this study, one foliar spray of ethion 50 EC at recommended dose was given at 50 percent head formation stage of cabbage. After 0 (2 hrs), 3, and 5 days of application, the samples were collected and processed. Analytical methods earlier described for estimation of residues of ethion 50 EC were used for the unprocessed and processed samples. The difference in residue levels obtained in such samples were used to calculate the effect of food processes on the percent reduction of residues deposited on the cabbage head exposed to spray treatment. Different decontamination processes followed during the study are given as below.

1. Washing with tap water

The cabbage head sample was taken under running tap water and gently rubbed twice or thrice by hand for about two minutes. Water was decanted and sample was subjected to residue analysis.

2. Washing with 2% sodium chloride (NaCl)

The cabbage head sample was dipped in a beaker containing 2 percent sodium chloride solution (NaCl). After 5 min, the sample was gently rubbed by hand in salt solution and the salt water was decanted. Then, the sample was washed in tap water and subjected to residue analysis.

3. Open pan boiling

The chopped cabbage sample was boiled by placing 1 litre of water in an open pan. The sample (500 g) was added immediately to boil in an open pan for 5-10 min. The water was decanted and subjected to residue analysis.

4. Steam cooking

The chopped cabbage sample was cooked by placing 1 litre of water in a closed pan. Cabbage (500 g) was added immediately to cook in a close pan for 10 min. Water was decanted, and then the sample was subjected to residue analysis.

5. Washing with 2% tamarind water

The cabbage head sample was dipped in 2 percent tamarind solution for 5 min and the water was decanted. Then, the sample was subjected to residue analysis.

6. Unprocessed

Cabbage sample was taken from insecticide treated plot as such without any process and subjected to residue analysis.

Percent removal of pesticide

$$\text{Percent removal} = \frac{\text{Initial deposit - Residues after treatment}}{\text{Initial deposit}} \text{X } 100$$

Statistical Analysis

The mean residues, standard deviation, regression equation, R^2 value, and half-life were calculated in the Microsoft excel program. Analyzed samples were quantified with Lab Solution GC-Solution software of SHIMADZU®.

Results and Discussions

Limit of detection and limit of quantification

The limit of detection (LOD) of the tested insecticides was 0.020 mg kg^{-1} and derived by considering a signal-to-noise ratio of the compound with reference to the background noise obtained for the blank sample. The limits of quantification (LOQ) determined in cabbage of a given compound giving a response that could be quantified with RSD lower than 20 percent, and that was 0.05 mg kg^{-1} for ethion.

Specificity

The area of the cabbage sample and reagent blank were compared with the spiked matrix match area, which was manually quantified during the matrix match study. The acceptable range of specificity was ± 30 percent variation (Table 2).

Table 2: Specificity studies on ethion standard

Concentration (ppb)	Sample Area		MMS Area	Residue (mg/kg)	LOQ (mg/kg)	Variation (%)	Acceptance criteria (%)
50	R1	58880	58602	0.050	0.05	0	±30
	R2	57586	59526	0.048	0.05	3	±30
	R3	59395	61719	0.048	0.05	4	±30
	Reagent Blank Area		MMS Area	Residue (mg/kg)	LOQ (mg/kg)	Variation (%)	Acceptance criteria (%)
50	R1	63363	58602	0.054	0.05	-8	±30
	R2	62708	59526	0.053	0.05	-5	±30
	R3	62643	61719	0.051	0.05	-1	±30

Linearity

For the linearity studies, a graph of detector response versus concentration of ethion standard was plotted, and correlation equation and coefficients were determined.

The response was linear over the range tested, and the R^2 value was 0.999 (Table 3 and fig. 1). These results indicated that the GC-FPD analysis is a valid method for residue determination of the ethion (SANTE 2015).

Matrix match linearity

Six linear concentrations (0.05, 0.1, 0.25, 0.40, 0.50, and 1.00 mg kg^{-1}) of working standards of ethion were added into the known quantity of sample matrix of cabbage and injected three times, and the linearity lines were drawn (Table 3 and fig. 2). The response was linear over the range tested and the R^2 value was 0.996 for ethion insecticides.

Table 3: Linearity and Matrix match studies on ethion standard

Peak areas of ethion standard at different concentration during linearity study						
concentration	0.05 mg kg^{-1}	0.10 mg kg^{-1}	0.25 mg kg^{-1}	0.40 mg kg^{-1}	0.50 mg kg^{-1}	1.00 mg kg^{-1}
Peak area	80747	158184	401458	641066.9	834178	1623490
concentration	Peak areas of ethion standard at different concentration during matrix match study					
Peak area	74102	142592	368020	548442.3	751493	1359792

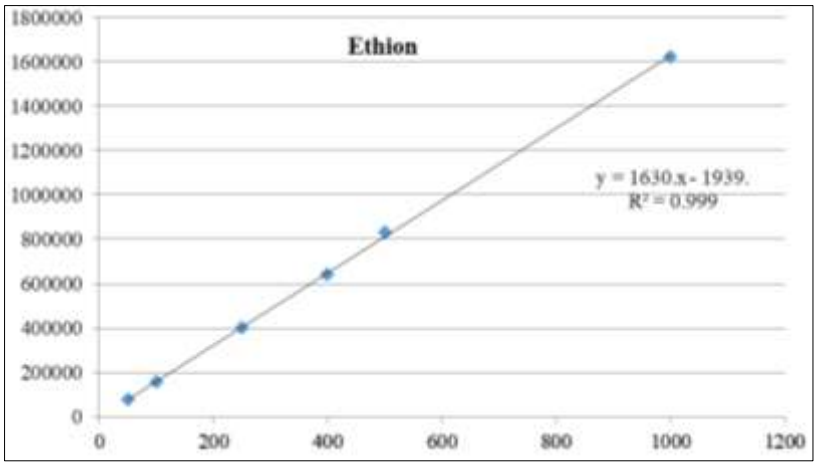

Fig 1: Linearity of ethion standard in ethyl acetate

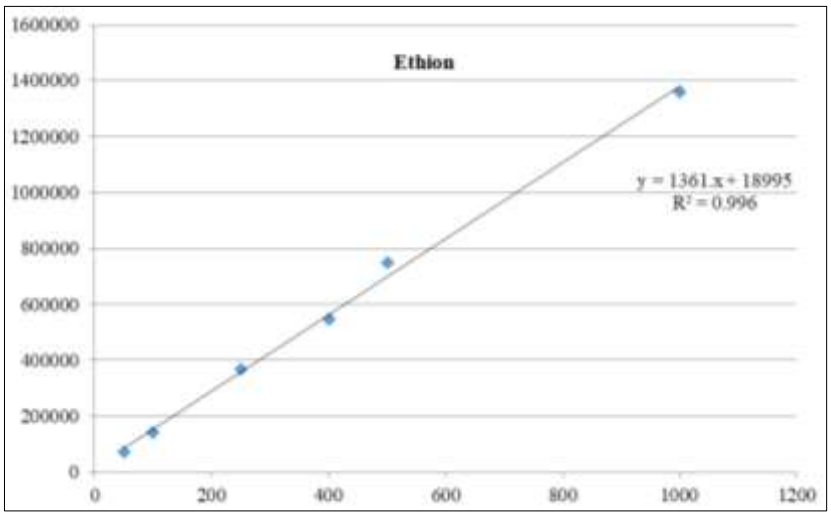

Fig 2: Linearity of ethion standard in cabbage matrix

Recovery, Repeatability, and Reproducibility

Results showed (Table 4) that the QuEChERS method is valid for residue determination of the tested insecticides in cabbage. The analytical method employed for the extraction and clean-up of cabbage samples was found accurate and precise as mean percent recovery under recovery, repeatability and reproducibility study were within 70-120 percent limits with relative standard deviation (RSD) of lower than 20.

Table 4: Recovery, Repeatability, and Reproducibility studies of ethion standard in cabbage matrix

Fortification levels (mg kg^{-1})	Recovery (%)	Repeatability (%)	Reproducibility (%)
0.05	119.33	101.01	95.54
0.25	116.62	98.00	112.23
0.50	109.34	100.09	98.88

The above different analytical parameters with their results recorded and evaluated under method validation follow up the SANTE/19945/2015 guidelines prescribed by European Commission, Directorate-General for Health and Food Safety on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. According to these guidelines, an analytical method which records relative standard deviation (RSD) lower than 20 percent for LOD and LOQ, acceptable criteria of ± 30 percent for specificity, R^2 values of 0.99 for linearity and matrix match study and mean percent recovery of residues in recovery, repeatability and reproducibility studies in the range of 70-120 percent with relative standard deviation (RSD) less 20 percent are accurate and precise.

Decontamination behavior of ethion 50 EC in cabbage heads

Effect of food processing on pesticide residues influenced by physical locations of pesticide residues as well as physico-chemical properties of pesticides such as solubility, volatility, hydrolytic rate of constant, thermal degradation, and hydro-octanol partition coefficient. Therefore in the present investigation, washing with tap water, 2% NaCl solutions, 2% tamarind solutions, open pan boiling and close pan cooking were practiced to check the percent reduction of ethion residues and which were compared with unprocessed samples of cabbage heads at 0 (2 hours), 3 and 5 days after treatment of recommended dose of ethion 50 EC @ 500 g a.i. ha^{-1} on cabbage heads in the field (Table 5).

Washing with tap water

Washing with tap water is the most common household process practiced in home before the preparation and final consumptions by the consumers. Data given in the table 6 revealed that when the treated cabbage heads collected at 0 (2 hours), 3 and 5 days were washed with running tap water for 5 minutes, the ethion residues were reduced to 0.24, 0.10 and 0.06 mg kg^{-1}, respectively and the percent reduction was observed to the tune of 11.11, 23.07 and 16.67 percent, respectively. These results are in conformity with the findings of Singh (2018) who observed; washing of cabbage heads with running tap water recorded 26.07, 25.18 to 27.62 percent reduction of ethion residues at 1, 3 and 5 days, respectively. Thakur (2017) [27] recorded, washing of cucumber fruits with running tap water reduced the residues of ethion up to 29.61 percent. Similarly Aktar *et al*. (2010) [2] reported cabbage heads washing with a running tap was the effective household practice to reduce the quinolphos residues with an average reduction of 27.72-32.48 percent.

Dipping in 2% NaCl water solution

The data pertaining to the effect of washing cabbage heads with 2% NaCl water solution for 5 minutes revealed that the residual deposit were reduced to 0.20, 0.12 and 0.04 mg kg^{-1} at 0, 3, and 5 day sample, respectively and percent reduction was in the range of 25.92, 07.69 and 100 percent, respectively. Ethion treated cabbage heads when processed at 5 days the residues were under below quantification limit of 0.05 mg kg^{-1}. According to the findings of Singh (2018) [25] dipping of cabbage heads with saline water recorded 46.81, 48.74 and 100 percent reduction of ethion residues at 1, 3 and 5 days, respectively. Thakur (2017) [27] who reported dipping of cucumber fruits in 2 percent sodium chloride solution for 10 minutes reduced the ethion residues up to 47.15 to 48.50 percent. Kumar *et al*. (2000) [14] reported that washing green chillies with salt water did not differ significantly from ordinary washing with tap water but their dipping in 2 percent NaCl solution for 10 minutes removed higher amount of OPs insecticides like triazophos and acephate residues which support the outcome of present findings. Geetha (2015) reported 46.87 and 43.78 percent reduction in residues of cypermethrin and triazphos, respectively in spinach by employing 2% salt solution. Begum *et al*. (2016) [6] reported that dipping of brinjal fruits in 2 percent common salt water reduced the quinolphos residues up to 82 percent. Brar *et al*. (2017) [7] also reported 28.65 to 48.48 and 30.21 to 54.54 percent reduction of acephate and triazophos residues in brinjal fruits when processed with 2 percent saline water, respectively.

Dipping in 2% tamarind water solution

The data pertaining to the effect of washing cabbage heads with 2% tamarind water solution for 5 minutes revealed that the residual deposit of ethion were reduced to 0.21, 0.11 and 0.05 mg kg^{-1} at 0, 3, and 5 days sample, respectively and percent reduction was in the range of 22.22, 15.38 and 16.67 percent, respectively. Cherukuri *et al*. (2014) [9] reported that treatment of brinjal fruits with 2% tamarind solution removed the residues of dimethoate, chlorpyriphos, quinolphos, profenophos, phosalone, lambda-cyhalothrin and malathion from 24-65 percent. Similarly, Pallavi *et al*. (2014) [15] reported dipping okra fruits in 2% tamarind solution for 15 min was more effective in removing the residues of malathion, chlorpyriphos quinalphos, profenophos and cypermethrin between 54.46 to 68.92 percent. Tamarind solution (2%) reduced the residues of quinalphos, lambda cyhalothrin and triazophos in tomato in the range of 46.1 to 80.4 percent (Harinathareddy *et al*. 2015) [11].

Open pan boiling

Cabbage heads samples upon boiling in an open pan for 10 min, the ethion residues reduced to 0.19, 0.06 and 0.04 mg kg^{-1} on 0, 3 and 5 days cabbage sample with 29.63, 53.84 and 100 percent reduction, respectively. Cabbage head processed at 5 day showed the residues of 0.04 mg kg^{-1} which were under below quantification limit (BQL) of 0.05 mg kg^{-1}. These findings are in agreement with those of Singh (2018) [25] who recorded cabbage sample subjected to open pan cooking showed 51.10, 51.96 and 100 percent reduction in ethion residues at 1, 3 and 5 days, respectively. According to Chauhan *et al*. (2012) [8] washing + boiling was most effective in tomato which reduced 42.10 and 45.23 percent in bifenthrin after application at 25 and 50 g a.i.ha-1, respectively. Satpathy *et al*. (2012) [19] reported 97.60, 93.90 and 88.40 percent reduction in chlorpyriphos, malathion and methyl parathion, respectively when cauliflower, tomato, brinjal, okra and capsicum were subjected to boiling process. Sachin Kumari (2013) [17] reported maximum reduction of bifenthrin after application of 25 and 50 g.a.i.ha-1 (64.58 to 68.42%, respectively) by washing + boiling in okra. Almost 99 percent monocrotophos, chlorpyriphos and cypermethrin residues were dislodged from treated brinjal and okra fruits by boiling in water (Subhash *et al*. 2015) [26]. Similarly, Beena Kumari (2008) [5] reported boiling is comparatively more effective than washing to dislodge the residues of OPs insecticides. Boiling process reduces 100 percent of OP

insecticides in brinjal followed by 92 percent in cauliflower and 75 percent in okra.

Close pan cooking

The initial residues of ethion in treated control sample at 0 (2 hrs), 3 and 5 day was 0.27, 0.13 and 0.06 mg kg^{-1}, respectively when applied at 500 g a.i. ha^{-1}. In cabbage, minimum amount of ethion residues *i.e.* 0.16 mg kg^{-1} and maximum percent reduction of residues (40.74%) were observed at 0 (2 hrs) days cabbage sample followed by 0.09 with 61.53 percent reduction at 3 days cabbage sample. Whereas, cabbage head sample processed at 5 days recorded residues of 0.04 mg kg^{-1} this was under below quantification limit (BQL) of 0.05 mg kg^{-1}. Present findings are in close agreement with the findings of Singh (2018) [25] who recorded cabbage sample subjected to microwave cooking at 1, 3 and 5 days recorded 60.07, 63.24 and 100 percent, respectively reduction in ethion residues. Parmar *et al*. (2012) [16] who reported the extent of insecticidal removal was 61.88 percent of ethion from okra fruits. He also reported cooking was the best culinary process in which triazophos residues were dislodged from okra fruits up to 66.34 percent. Thanki *et al*. (2012) [28] reported 22.84, 40.00 and 25 percent reduction in quinalphos, cypermethrin and permethrin residues, respectively by the process of cooking. Effectiveness of cooking was also emphasized by Cherukuri *et al*. (2014) [9] in brinjal where direct pressure cooking removed 39.40 and 48.70 percent residues of quinalphos and lambda cyhalothrin, respectively. Harinathareddy *et al*. (2014) [12] reported cooking as an effective decontamination process in tomato with 54.3 percent reduction in residues of triazophos, 47.4 percent of quinalphos and lambda cyhalothrin. Geetha (2015) reported reduction of triazophos and deltamethrin + triazophos residues in spinach by employing different household processes. Hot water cooking for 10 min (54.52 and 90.71% +55.42%) was found to be more effective than tap water treatment (19.88 and 54.59%+24.54%). Effectiveness of cooking was also endorsed by Shashi *et al*. (2015a) [21] in brinjal where direct cooking removed 58.20 and 59.00 percent residues of quinalphos and profenophos, respectively. Further, Shashi *et al*. (2016) [22] reported 39.40, 52.90 and 48.70 percent reduction in residues of quinalphos, profenophos and lambda cyhalothrin, respectively when tomato fruits were subjected to cooking.

Table 5: Decontamination behaviour of ethion 50 EC in cabbage heads.

Treatment Details	Interval (Days)	Ethion @ 500 g a.i. ha^{-1}	
		Mean residues (mg kg^{-1})	Reduction (%)
Unprocessed	0 (2 hrs)	0.27	---
	3	0.13	---
	5	0.06	---
Washing with tap water	0 (2 hrs)	0.24	11.11
	3	0.10	23.07
	5	0.06	16.67
Dipping in 2% salt solution	0 (2 hrs)	0.20	25.92
	3	0.12	07.69
	5	BQL	100.00
Dipping in 2% tamarind water	0 (2 hrs)	0.21	22.22
	3	0.11	15.38
	5	0.05	16.67
Open pan boiling	0 (2 hrs)	0.19	29.63
	3	0.06	53.84
	5	BQL	100.00
Close pan cooking	0 (2 hrs)	0.16	40.74
	3	0.05	61.53
	5	BQL	100.00

*BQL-Below Quantification Limit

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