The Pharma Innovation Journal 2022; 11(12): 5825-5829

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 5825-5829

www.thepharmajournal.com
Received: 01-09-2022
Accepted: 06-10-2022

CG Sawant
Assistant Professor, Multi-Technology Testing Centre and Vocational Training Centre, Central Agricultural University, Imphal, Thenzawl, Mizoram, India

Guru PN
Scientist, ICAR-Central Institute of Post-Harvest Engineering and Technology, Ludhiana, Punjab, India

SG Mundhe
Subject Matter Specialist, Department of Agrometeorology, Krishi Vigyan Kendra, Durgapur, Amravati, Maharashtra, India

CS Patil
Professor and Head, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Rahul Sadhukhan
Assistant Professor, Multi-Technology Testing Centre and Vocational Training Centre, Central Agricultural University, Imphal, Thenzawl, Mizoram, India

Corresponding Author:
CG Sawant
Assistant Professor, Multi-Technology Testing Centre and Vocational Training Centre, Central Agricultural University, Imphal, Thenzawl, Mizoram, India

Method validation and dissipation behavior of ethion 50 EC insecticide in winter cabbage

CG Sawant, Guru PN, SG Mundhe, CS Patil and Rahul Sadhukhan

Abstract
Studies on method validation of ethion 50 EC on gas chromatography equipped with a flame photometric detector (FPD) and the dissipation 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 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. Dissipation studies were undertaken following two foliar applications of ethion 50 EC at recommended and double the recommended doses (500 and 1000 g a. i. /ha) at the 50 percent head formation stage. Residues of ethion dissipated with a half-life of 3.11 and 2.52 days at both the doses, respectively. The residues reached below the quantification limit (BQL) on the 7^{th} day at the recommended dose. Considering this, a seven-day pre-harvest interval (PHI) can be suggested for ethion 50 EC with a reduced risk of residues in cabbage.

Keywords: Validation, chromatography, ethion, recovery, dissipation, half-life, pre-harvest interval

Introduction
Cabbage (*Brassica oleracea var. capitata* Linn.), a member of the cruciferous family, is one of India's most valuable vegetable crops and is chiefly popular for its culinary value and nutritional purpose in the Indian kitchen. Due to its nutritional value has been a staple dietary vegetable in our daily diet since ancient times. Cabbage is grown mostly in the winter season during December-January and has occupied an important position in meeting the nutritional requirements of most people of the world. An enlarged terminal bud is an edible portion of cabbage, which is a rich source of Ca, P, Na, K, S, Vit A, Vit. C and dietary fiber. Cabbage also supplies essential vitamins, proteins, carbohydrates, and vital minerals.

India holds the second position in the production of cabbage and cauliflower after china. The area under cabbage cultivation in India is about 399 thousand hectares and production of 9095 thousand metric tons. Only cabbage cultivation covers 3.95 percent of vegetable area with a contribution of 4.89 percent to the total vegetable production in India (Anon., 2018).

Cabbage as being a winter vegetable attacked by several insect pests, which cause up to 80 percent yield losses (Devjani and Singh, 1999) [4]. Among different pests, *Pieris brassicae* Linnaeus (cabbage caterpillar); *Plutella xylostella* Linnaeus (diamondback moth); *Thysanoplusia orichalcea* Fabricius and *Autographa nigrisigna* Walker (cabbage semi-looper); *Spodoptera litura* Fabricius (tobacco caterpillar); *Crocidolomia binotalis* Zeller (cabbage leaf webber); *Hellula undalis* Fabricius (cabbage borer) and *Phyllotreta cruciferae* Goeze, *P. chotanica* Duviv, *P. birmanica* Harold, *P. oncera* Maulik and *P. downesi* Baly (cabbage flea beetles) are the major importance in the tropical and subtropical country (Atwal and Dhaliwal, 2002) [3]. Among the above pests, diamondback moth, *P. xylostella* causing great and irreparable damage (Mahla *et al*., 2005 and Kumar *et al*., 2007) [6, 5] and that's why it is considered a major limiting factor for the successful cultivation of cabbage and cauliflower resulting not only in the loss of the quality but also in production (Patil *et al*., 1999) [8]. The young caterpillar scraps and makes zigzag mines on the lower surface of leaves and later on feed on exposed leaves. It produces shot holes in the leaves; sometimes, it also feeds on unexposed heads and causes huge and irreparable damage to the crop.

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's* long history of eventually becoming resistant to every insecticide used extensively against it. Farmers also use a substantial amount of non-recommended organophosphates insecticides throughout crop growth and sometimes even at the fruiting stage. Indiscriminate use of non-recommended Ethion 50 EC, an organophosphorus insecticide particularly during fruiting stage, and non-adoption of safe waiting period results in accumulation of residues in consumable heads pose a serious threat to the health of cabbage consumers. Hence, it is imperative to study the persistence and dissipation behavior of Ethion 50 EC on the edible portion of cabbage to ensure that the levels of harvest time residues are within prescribed limits in domestic as well as international trade. Keeping this in view, the investigation was undertaken to study the method validation and dissipation behavior of Ethion 50 EC insecticide in/on winter 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.

Field experiment for dissipation study

A supervised field experiment for residue studies was conducted at Mahatma Phule Krishi Vidyapeeth, Rahuri. Cabbage crop (var. *Saint*) was raised with a plot size of 3 m X 3 m using the standard package of practices issued for the zone, except plant protection measures. The experiment was laid in RBD with three replications. Overall, two sprays were given at an interval of 10 days, initiating the first spray at the 50 percent head formation stage.

Sampling and sample preparation

The cabbage heads (1 kg each size) were collected randomly from each replicate at the regular time interval like 0 (2 hrs after spraying), 1, 3, 5, 7, 10, and 15 days after the second spray. The collected samples were brought to the laboratory under a controlled box. The samples were then grinded, separately. Homogenized samples were kept at -20 °C in a deep freezer until analysis.

Extraction and clean-up

Modified QuEChERS method

The cabbage samples were extracted and cleaned up using the modified QuEChERS method (Sharma, 2013). 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.

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

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

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}

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) [9].

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.

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 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

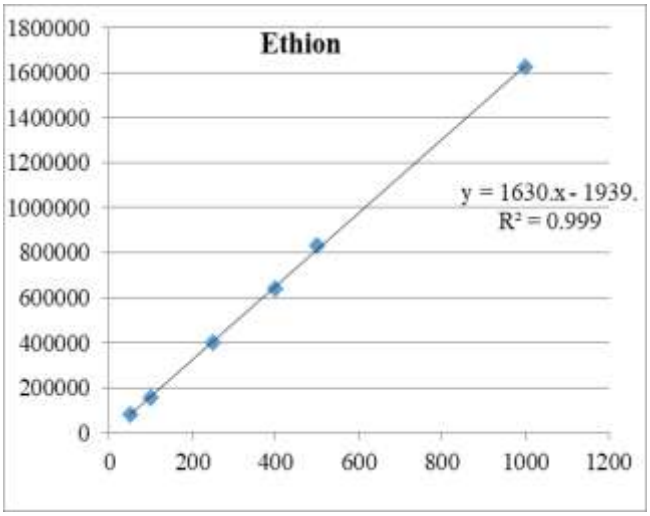

Fig 1: Linearity of ethion standard in ethyl acetate

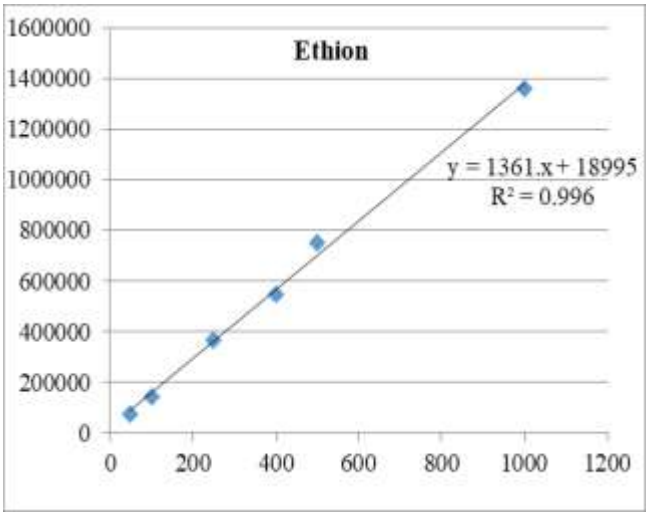

Fig 2: Linearity of ethion standard in cabbage matrix

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.

Dissipation behavior of ethion 50 EC in cabbage heads

Dissipation of insecticide residues in the plant depends on different climatic conditions, type of application of particular insecticides, dosage, and intervals between insecticide application and time of harvest. The results obtained in dissipation revealed a reduction in residue levels of ethion in cabbage with time. Initial deposits were recorded as 0.30 and 0.53 mg kg^{-1}. The ethion residues were below the quantification limit (BQL) on the 7th and 10th day. The ethion residues dissipated to 80.00 and 90.57 percent (Table 5) at the recommended dose and double the recommended dose, respectively. The half-life (RL50) values of ethion for cabbage heads were 3.11 and 2.52 days at the recommended and double the recommended dose, respectively. The samples taken from the control plot contained no detectable amounts of ethion residues.

The above findings are in corroboration with Singh, 2018 which recorded initial deposits of ethion on cabbage heads when sprayed @ 500 g a.i. ha^{-1} and 1000 g a.i. ha^{-1} was found to be 0.970 mg kg^{-1} and 1.670 mg kg^{-1}, which persisted up to 7 and 10 days, respectively. The ethion residues were dissipated to 90.98 and 94.87 percent at single and double the recommended dosages, respectively. Parmar *et al*. (2012) [7] reported that the average initial deposit of ethion in okra is 0.254 mg kg^{-1} which is in line with the present investigation. However, Singh *et al*. (2007) [11] studied the persistence of ethion on cucumber and reported the higher mean initial deposit of ethion on cucumber fruits i.e. 2.40 and 4.97 mg kg^{-1} following three applications at 10 days interval at 375 and 750 g a. ha^{-1}. Arora *et al*. (2004) [2] also estimated residues of ethion on pear fruits following its application at 0.05 percent and 0.1 percent i.e. Average initial deposits were observed to be 1.53 and 3.11 mg kg^{-1}, respectively. These residues were less than the MRL value of 2.0 mg kg^{-1} of ethion on pear fruits. The half-life of ethion was observed to be 1.95 and 2.21 days at single and double the dosage, respectively.

Table 5: Dissipation behavior of ethion 50 EC in cabbage heads

Interval between the last application and sampling	Recommended dose @ 500 g a.i. ha-1		Double the recommended dose @ 1000 g a.i. ha-1	
	Mean residues (mg kg-1)	Dissipation (%)	Mean residues (mg kg-1)	Dissipation (%)
0 day (2 hrs)	0.30	-	0.53	-
1 day	0.24	20.00	0.40	13.01
3 day	0.11	63.33	0.29	45.28
5 day	0.06	80.00	0.10	81.13
7 day	BQL	-	0.05	90.57
10 day	BQL	-	BQL	-
15 day	BQL	-	BQL	-
Regression equation	$y = -0.096x - 0.611$ $R^2 = 0.967$		$y = -0.119x - 0.306$ $R^2 = 0.974$	
RL 50 (days)	3.11		2.52	

Acknowledgement

The authors are thankful to the DST, Ministry of Science and Technology, Government of India for awarding 'INSPIRE Fellowship' and to Pesticide Residue Laboratory, Department of Agricultural Entomology, MPKV, Rahuri for providing necessary facilities to carry out the research.

References

1. Anonymous. National Horticulture Board; c2018-19.
2. Arora PK, Battu RS, Singh B. Bioefficacy of ethion against mite *Eutetranychus orientalis* (Klein) and its residues on pear fruits. Pesticide Research Journal. 2004;16(2):33-35.
3. Atwal AS, Dhaliwal GS. Pests of vegetables. In: Agricultural Pests of Souh Asia and Their Management. Kalyani publishers; c2002 p. 248-253.
4. Devjani P, Singh TK. Field density and biology of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) on cauliflower in Manipur. Journal of Advanced Zoology. 1999;20(1):53-55.
5. Kumar P, Prasad CS, Tiwari GN. Population intensity of insect pests of cabbage in relation to weather parameters. Annals of Plant Protection Sciences. 2007;15(1):245-246.
6. Mahla RS, Singh S, Chaudhary P. Management of diamondback moth, *Plutella xylostella* (L.) larvae by entomopathogenic fungus, *Metarhizium anisopliae*. Indian Journal of Entomology. 2005;67:342-344.
7. Parmar KD, Korat DM, Shah PG, Singh S. Dissipation and decontamination of some pesticides in/on okra. Pesticide Research Journal. 2012;24(1):42-46.
8. Patil BA, Shah PG, Raj MF, Patel BK, Patel JA, Talathi JG. Chlorpyriphos residues in/on cabbage and brinjal. Pest. Res. J. 1999;11(2):195-195.
9. SANTE. Method Validation and quality control procedures for pesticide residue analysis in food and feed. Document No. 11945/201; c2015. p. 1-42.
10. Sharma KK. Pesticide Residue Analysis Manual, ICAR, Gov. of India; c2013. p. 90-91.
11. Singh G, Singh B, Battu RS, Jyot G, Singh B, Joia B. Persistence of ethion residues on cucumber, *Cucumis sativus* (Linn.) using gas chromatography with nitrogen phosphorus detector. Bull. Environ. Contam. Toxicol. 2007;79:437-439.
12. Singh Gagandeep. Persistence and Dissipation Pattern of some Organophosphorus insecticides on Cabbage and Tomato: A Gap Supervised Field study. Ph. D thesis submitted to Department of Entomology, Dr. Y. S. Parmar University of Horticulture & Forestry, Solan, Himachal Pradesh; c2018.