https://doi.org/10.33307/entomon/v48i3/937
ENTOMON 48(3): 357-364
Article No. ent. 48304

Growth dilution and its effect on pesticide dynamics in okra

G.T. Pradeepkumar[1], V. Vijayasree[2] and K.P. Subhash Chandran[3]

[1]Research and development centre, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.
[2]College of Agriculture, Kerala Agricultural University, Vellayani 695522, Kerala, India.
[3]Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad, Kochi 682506, Kerala, India.
Email: pradeepgt2000@gmail.com

ABSTRACT: Dissipation studies at single and double doses of chlorantraniliprole, thiamethoxam (25 and 50 g ai ha^{-1}), and imidacloprid (20 and 40 g ai ha^{-1}) were conducted on okra fruits following field application, and the residues were estimated using LC-MS/MS. The initial deposit of 0.42 and 0.80 mg kg^{-1} of chlorantraniliprole dissipated below quantitation level on the tenth day at single and double dosages. For thiamethoxam, the initial deposits of 0.42 and 0.71 mg kg^{-1} reached below quantitation level on tenth day at single dosage and on fifteenth day at double dosage; and for imidacloprid, the initial deposits are 0.10 and 0.16 mg kg^{-1} which dissipated below quantification level on fifth day. Growth dilution plays a significant role in the rate of dissipation of thiamethoxam when compared with imidacloprid and chlorantraniliprole when simulated dissipation rate due to growth dilution was calculated. The half-life of chlorantraniliprole, thiamethoxam and imidacloprid were 1.94 and 1.72 days, 1.88 and 1.99 days, 1.13 and 1.05 days, respectively for single and double dosages. Chlorantraniliprole, thiamethoxam and imidacloprid were found to be the safer insecticides with calculated waiting period of less than one day for single dosage.

KEYWORDS: Chlorantraniliprole, thiamethoxam, imidacloprid, dissipation, half-life, waiting period

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop of the tropical and subtropical regions of the world. However, the crop growth and yield is reduced by the attack of a number of pests like *Earias vittella* (Fab.), *Helicoverpa armigera* (Hubner) *Bemisia tabaci* Gennadius, *Phenacoccus solenopsis* Tinsley and so on. Hence application of pesticide is needed for the control of pests on this crop. The indiscriminate use of these pesticides will result in serious implications to man and his environment. Awareness of the dissipation dynamics of any insecticide is essential for determining their fate in the environment. Chlorantraniliprole, an anthranilic diamide class of insecticides, activates ryanodine receptors and stimulates calcium ion release from muscle cells causing paralysis and death in chewing insect pests. Neonicotinoids viz., thiamethoxam and imidacloprid with neurotoxic mode of action exhibit a variety of lethal and sublethal effects on insect feeding, oviposition and fecundity in Lepidoptera, Coleoptera and Hemiptera. Chlorantraniliprole is a reduced-risk pesticide which can be used to control lepidopteran pests while the neonicotinoids can be used for controlling beetles and sucking pests in

* Author for correspondence

okra. The combined application of pesticides with different action mechanisms can prolong the service life of pesticides and can reduce the generation of resistance (Wei *et al.,* 2020).

The rate of dissipation of insecticides is dependent on several factors including degradation, plant absorption, environmental factors, mode of application, plant species, growth dilution among which growth dilution plays a significant role. The effect of growth dilution in total dissipation is least studied and hence the present study was undertaken to gather quantitative information on the effect of growth dilution on dissipation behaviour of the commonly used insecticides chlorantraniliprole, thiamethoxam and imidacloprid on okra fruits.

MATERIALS AND METHODS

Chemicals and reagents: Analytical standards of chlorantraniliprole (purity 97.84 %), procured from Dr.Ehrenstorfer, Germany, thiamethoxam (purity 99.3%) and imidacloprid (purity, 99.9 %) were purchased from Sigma Aldrich. The formulations were obtained from E.I. DuPont India Pvt Ltd, Bayer Crop Science India Ltd and Insecticides (India) Ltd. Solvents like acetonitrile, anhydrous sodium sulfate, and anhydrous magnesium sulfate (ACS reagent grade) were obtained from Merck Germany and primary secondary amine (PSA) was obtained from Agilent Technologies, USA.

Preparation of standard solution: Method validation was carried out at 0.01, 0.1 and 0.5 mg kg^{-1} levels initially to test the efficiency of extraction and clean up procedures and to standardize the procedure for residue estimation. Standard stock solutions of chlorantraniliprole, thiamethoxam and imidacloprid (1000 ìg ml$^{"1}$) were prepared in LC-MS grade methanol and were serially diluted to obtain the solutions required for preparing a calibration curve (0.50, 0.25, 0.10, 0.075, 0.05, 0.025, and 0.0l ìg ml$^{"1}$).

Instrument Parameters: The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reverse phase Atlantis C-18 (2.1 mm × 100 mm, 5-micron particle size) column. The operation of the LC gradient involved the following two eluent components: A) 10 per cent methanol in water/ 0.1 per cent formic acid/5 m M ammonium acetate; B) 10 per cent water in methanol + 0.1 per cent formic acid + 5 m M ammonium acetate. The gradient elution was: 0 min. isocratic 20 per cent B, 0.0–4.0 min. linear from 20 to 90 per cent B, 4.0–5 min. linear from 90 to 95 per cent B, and 5–6 min. linear from 95 to 100 per cent B, with 6–8 min. for initial conditions of 20 per cent B. The flow rate was kept constant at 0.8 mL min^{-1}. and injection volume was 10 µL. The column temperature was kept at 40°C. The effluent from the LC system was introduced into AB Sciex API 3200 MS/MS system equipped with an electronspray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600°C; ion source gas (GS1) 50 psi, ion source gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi. The retention time for chlorantraniliprole, thiamethoxam and imidacloprid were 2.98, 0.91 and 1.1 minute, respectively.

Field experiment: Okra was raised in a farmer's field at Maranalloor, Thiruvananthapuram district and the trial was laid out in randomized block design plots of size 25 m^2 with three replicates. The crop was sprayed with chlorantranilirole (Coragen 18.5 SC) at 25 and 50 g ai ha^{-1}, thiamethoxam (Arrow 25 WG) at 25 and 50 g ai ha^{-1} and Imidacloprid (Confidor 17.8 SL) at 20 and 40 g ai ha^{-1} during the fruiting stage.

Extraction and clean up (QuEChERS method): The insecticide residues were estimated after collecting 500 g of tender fruits randomly on 0, 1, 3, 5, 7, 10, and 15 days after insecticide spray. The extraction and clean up was done as per the modified QuEChERS method (Anastassiades *et al.* (2003)). 250 g of the chopped samples per replicate were macerated in a blender. Ten grams of the ground samples were then taken from each replicate in a 50 ml centrifuge tube, 20 ml of HPLC grade acetonitrile was added and the sample was homogenised in a high speed homogenizer (Heidolph Silent Crusher-M) at 14000 rpm for 3-4 min. The sample was then mixed for 2 min on a rotospin after adding 4.5 g sodium chloride (activated) and was then centrifuged for 5 min at 2,500 rpm. 12 ml clear upper layer of the sample was transferred into a 50- ml centrifuge tube prefilled with 5 g pre-activated sodium sulphate, and vortexed for 2 min. The extract was cleaned up by dispersive solid phase extraction (DSPE). Upper layer (8 ml) was transferred into a test tube containing 0.125 g primary secondary amine (PSA) and 0.8 g anhydrous magnesium sulfate and was again vortexed for 2 min, and centrifuged for 5 min at 2,500 rpm. Five mL of the supernatant liquid was evaporated to dryness under a gentle stream of nitrogen at 40°C and 7.5 psi flow rate. The residue was then reconstituted in 2 mL of methanol and filtered through a 0.2-micron filter for UPLC-MS/MS analysis.

Simulated dissipation rate due to growth dilution for each day is calculated by multiplying initial residue of pesticide with percentage weight gain of okra fruits from fruit setting stage to maturing (Miles et al. (1964)). This simulated data was compared with field data using excel spreadsheet. Half-life of the insecticides was calculated as per the procedure outlined by Hoskins (1961) and was done by Microsoft Excel 2007 spreadsheet.

RESULTS AND DISCUSSION

Efficiency of the method

The fortification at levels of 0.01, 0.05 and 0.1 mg kg^{-1} gave a good recovery percentage of 88 to 115, 92 to 118, 89 to 120 of chlorantraniliprole, imidacloprid and thiamethoxam, respectively (Table1). Hence the recovery and precision confirmed to the acceptable limits (recovery percentage: 70-120 and relative standard deviation values: below 20), establishing the suitability of the method. Matrix matched calibration was done and was found that and compared to the standard in pure solvent, in the calibration range of 0.01 to 0.50 ìg ml"1 (Figs.4, 5). The limit of quantitation (LOQ) for all these three insecticides were found to be 0.01 mg kg^{-1} and the limit of detection (LOD) being 0.005 mg kg^{-1}.

Persistence of chlorantraniliprole, imidacloprid and thiamethoxam

The data on the persistence of chlorantraniliprole (Fig.1), imidacloprid (Fig.3) and thiamethoxam (Fig.2) when applied at standard and double dosage on okra fruits revealed that the chlorantraniliprole and imidacloprid residues persisted for 10 days and

Table 1. Recovery of insecticides from okra

Dose ($mgkg^{-1}$)	Recovery (%)	Precision (RSD)
Chlorantraniliprole		
0.01	88.4	15.6
0.05	106.2	16.2
0.1	115.3	14.8
Thiamethoxam		
0.01	89.7	20.6
0.05	120.0	11.3
0.1	117.6	16.4
Imidacloprid		
0.01	92.7	18.7
0.05	119	15.2
0.1	118.6	16.0

3 days at both dosages respectively, thiamethoxam residues persisted for 7 days and 10 days, at standard and double dosage, respectively. The initial deposit of chlorantraniliprole for single and double dose was 0.42 and 0.80 mg kg^{-1} which dissipated to 0.20 and 0.59 mg kg^{-1} respectively on the first day. However, the simulated values were 0.23 and 0.45 on first day which shows that there are environmental factors also along with growth dilution that will be affecting the dissipation of chlorantraniliprole. From the date of flowering, requires about five to seven days for the okra fruits to mature. Within this short period okra variety Anakomban grows at a faster rate ie., from 1 to 45

Table 2. Dissipation equations, half-life and waiting period of insecticides at two dosages

Dosage (g a.i.ha^{-1})	Regression equation	Half-life ($t_{1/2}$)	Waiting period (days)
Chlorantraniliprole			
25	y=-0.16X+1.59	1.94	0.74
50	y=-0.18X+1.89	1.72	2.36
Thiamethoxam			
25	y=-0.16X+1.58	1.88	-0.7
50	y=-0.15X+1.88	1.99	1.2
Imidacloprid			
20	y=-0.27X+1.00	1.13	-4.8
40	y=-0.29X+1.27	1.05	-3.5

g and showing growth dilution up to 97 per cent that may result in the rapid decline of the insecticide. Hopkins *et al.* (1952) also examined the importance of growth as a factor in the reduction of residues on alfalfa in New York state. The degradation half-life of chlorantraniliprole was less than one day and the waiting period calculated from the residue dissipation data and compared with FSSAI MRL was found to be less than one day for standard dose and 2.4 day for double dose. Similar results were obtained in another dissipation study of chlorantraniliprole (Coragen 18.5 SC) in okra fruits at single and double doses of 30 and 60 g ai ha $^{-1}$, and the initial residues were 0.48 and 0.91 mg kg^{-1}, respectively which reached below detectable level of 0.01 mg kg $^{-1}$ on the 10th day. Half-life of chlorantraniliprole were 0 and 1.20 days, respectively (Vijayasree *et al*., 2015). In another study by Singla *et al*., 2020 it was shown that the residues dissipated to below the limit of quantification (LOQ) of 0.03 mg kg^{-1} after 7 and 10 d of the application of insecticide at the two doses respectively. The half-lives (t1/2) and waiting periods of chlorantraniliprole in okra were calculated to be 2.27 and 2.45 d and 0 and 1d, at the recommended and double the recommended dosages respectively.

The initial deposit of imidacloprid was 0.10 and 0.16 mg kg^{-1}, dissipated to the residue concentration of 0.04 and 0.12 mg kg^{-1} respectively whereas the initial concentration of thiamethoxam was 0.42 and 0.71 mgkg^{-1} and 0.24 and 0.66 mg kg^{-1} on first day for single and double dose, respectively. The simulated rate of dissipation due to growth dilution at single and double dosages were 0.23 and 0.40 and 0.06 and 0.09 on the first day for thiamethoxam and imidacloprid, respectively. Hence the growth factor would have significantly contributed for the dissipation of thiamethoxam unlike imidacloprid. The degradation half-life of imidacloprid and thiamethoxam was 1.1 and 1.05 days and 1.88 and 1.99 days, respectively. The waiting period calculated for thiamethoxam was found to be less than one day for single dosage and 1.2 days for

Table 3. Simulated rate of dissipation of insecticide in okra due to growth dilution at two dosages

DAS	EGd (%)	Mean residue (Field) mgkg^{-1}		Residue (Simulated) mgkg^{-1}	
		x	2x	x	2x
chlorantranilprole					
0		0.42	0.80		
1	44	0.20	0.59	0.23	0.45
3	87	0.14	0.15	0.05	0.10
5	97	0.10	0.16	0.01	0.02
thiamethoxam					
0		0.42	0.71		
1	44	0.24	0.66	0.23	0.40
3	87	0.12	0.22	0.05	0.09
5	97	0.05	0.15	0.01	0.02
imidacloprid					
0		0.10	0.16		
1	44	0.05	0.12	0.06	0.09
3	87	0.02	0.03	0.01	0.02
5	97	BDL	0.01	0.00	0.00

Fig.1 Dissipation of chlorantraniliprole in okra

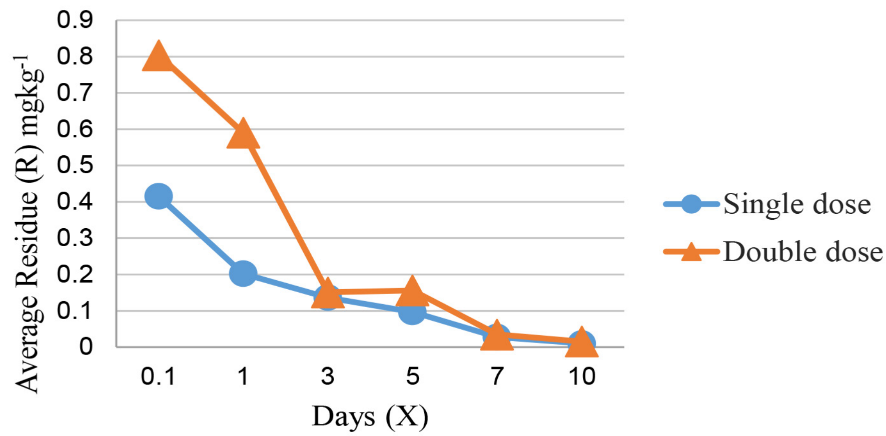


Fig. 2 Dissipation of thiamethoxam in okra

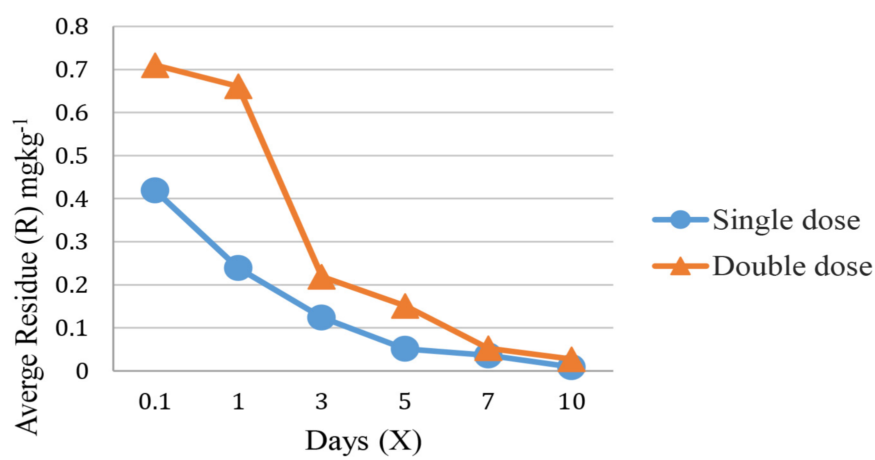
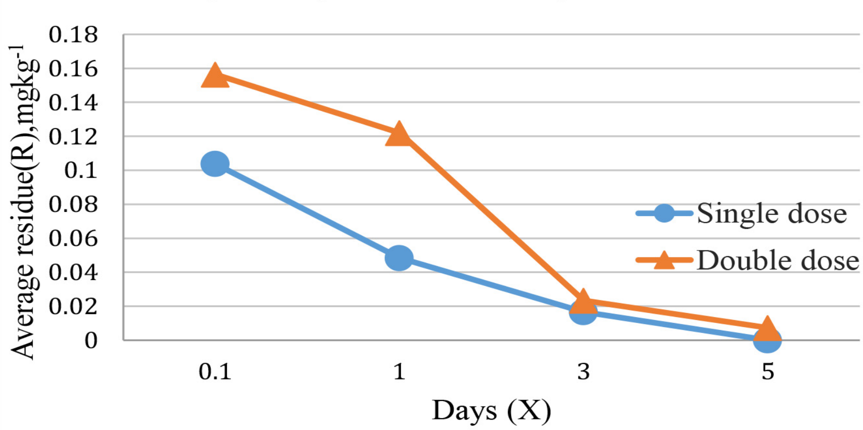


Fig.3 Dissipation of imidacloprid in okra



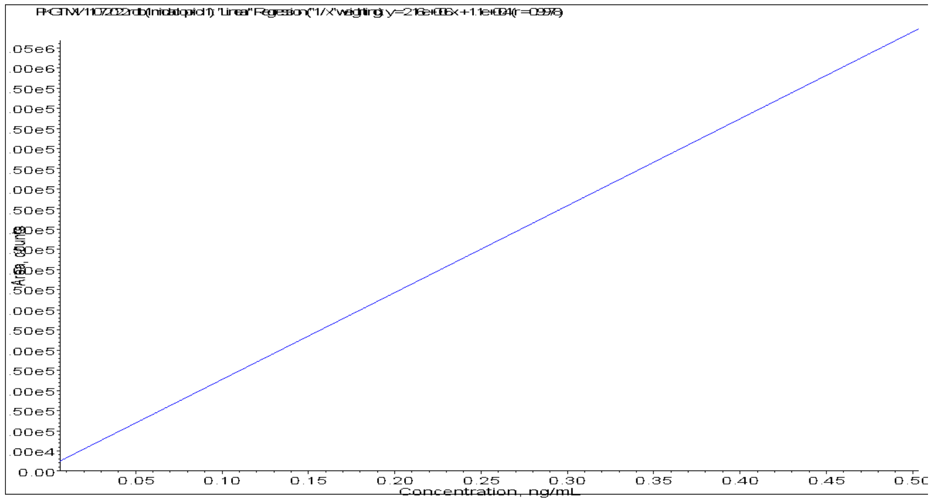


Fig. 4 linearity curve of Imidacloprid in LC-MS/MS

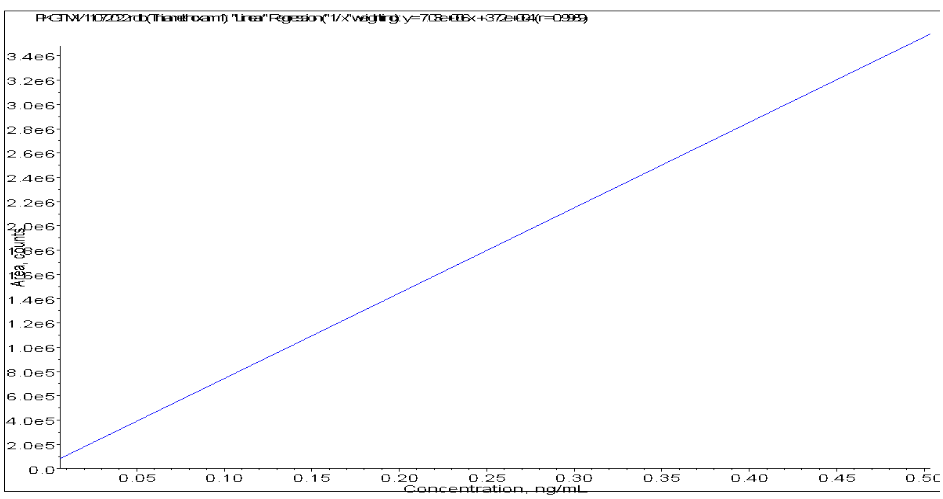


Fig. 5 linearity curve of thiamethoxam in LC-MS/MS

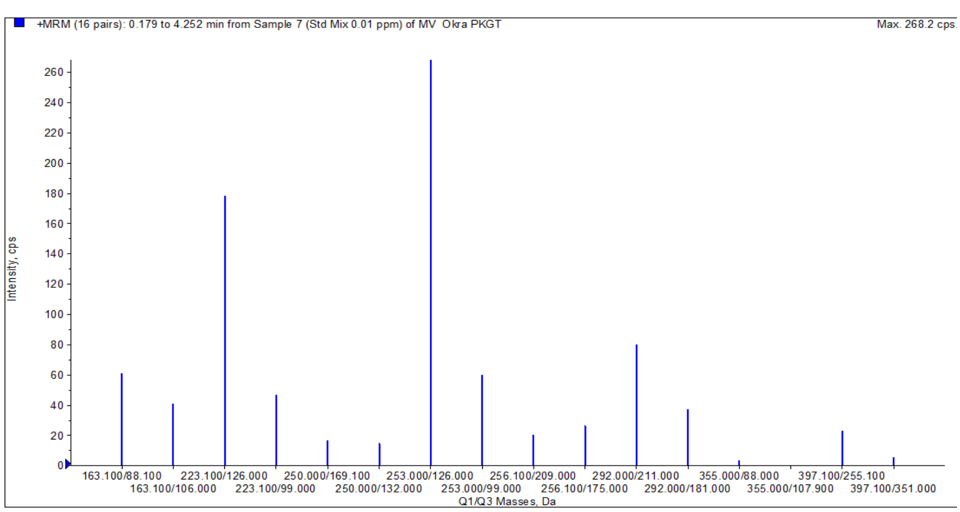


Fig. 6 MRM spectrum of pesticides: Imidacloprid, Thiamethoxam and Chlorantraniliprole

double dosage when compared with FSSAI MRL value (0.5 $mgKg^{-1}$). However, it was less than one day for both the dosages of imidacloprid as the FSSAI MRL value is 2 $mgkg^{-1}$. Dissipation studies by Pandit, 2016 found that the residues of imidacloprid in okra declined to below detection level (BDL) within 7 days in fruits when sprayed at the rate of 24.5 g a.i. ha^{-1} and the half-life (t1/2) ranged between 1.76 - 2.07 days in fruit. Joshi, 2019 reported that the initial deposit of 1.02 mg kg^{-1} residues of imidacloprid applied at dose of 20g a.i. ha^{-1} in okra dissipated in 7 days. According to Sharma, 2016 imidacloprid residues were the highest at the first picking after spray and lowest at the third picking and were in the range of 0.10 to 0.16 ug g^{-1} at the recommended dose of 0.3 mL L^{-1}. According to Chauhan, 2013 dissipation behaviour of thiamethoxam applied at recommended dose of 25 g ai ha^{-1} showed that the initial deposits of 0.245 mg kg^{-1} reached below detectable level of 0.005 mg kg^{-1} at 15 days after application with a half-life period of 1.47 day while Shalaby,2016 reported that okra fruits could be consumed safely after 15 days of treatment with thiamethoxam.

Among the different insecticides studied, it was found that growth dilution plays a significant role in the rate of dissipation of thiamethoxam. Even though, the presence of residues of chlorantraniliprole, imidacloprid and thiamethoxam were detected even after three days in okra, the observance of prescribed waiting period is a way of reducing the risk of the residue problems. And it was revealed that chlorantraniliprole thiamethoxam and imidacloprid are safer insecticides that can be used in okra when the waiting period was worked out.

ACKNOWLEDGMENT

The first author wishes to express his gratitude to the Research and Development Centre, Bharathiar University Coimbatore for providing necessary research facilities to conduct the study.

REFERENCES

Anastassiades M., Lehotay S.J., Stajnbaher D. and Schenck F.J. (2003) Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. Journal of AOAC International 86(2): 412–431.

Chauhan R., Kumari B. and Sharma S.S. (2013) Persistence of thiamethoxam on okra fruits. Pesticide Research Journal 25: 163–165.

Fantke R., Gillespie B.W., Juraske R. and Jolliet O. (2014) Estimating Half-Lives for Pesticide Dissipation from Plants. Environmental Science and Technology 48: 8588–8602.

Hopkins L., Nonrox L.B. and Gyntsco G.G. (1952) Persistence of insecticide residues on forage crops. Journal of Economic Entomology 45: 213–218.

Hoskin W.M. (1961) Mathematical treatment of the rate of loss of pesticide residues. FAO Plant Protection Bulletin 9: 163–168.

Joshi S., Srivastava R. M., Ahmad A.H. and Verma M.K. (2019) Dissipation of Imidacloprid residues in okra fruits in Tarai region of Uttarakhand. Journal of entomology and zoology studies 7(1): 1503–1506.

Miles J.R.W., Sans W.W., Wressell H.B. and Manson G.F (1964) Growth-dilution as a factor in the decline of pesticide residues on Alfalfa-grass forage. Canadian Journal of Plant Sciences 44: 37–44.

Pandit G., Gharde S., Chowdhury N. and Ghosh J. (2016) Dissipation of imidacloprid residues in okra leaves, fruits and soil in the northern region of West Bengal. Pesticide Research Journal 28: 20–24.

Shalaby A. (2016) Residues of thiamethoxam and chlorpyrifos on okra in relation to their effects on some internal quality parameters and elements in fruits. Journal of Productivity and Development 21: 349–367.

Sharma D., Krishnamoorthy P.N. and Divakara J.V. (2016) Evaluation of imidacloprid residues in okra fruits by LC-MS/MS. Pesticide Research Journal 28(1): 120–122.

Singla A., Sharma S., Mandal K. and Kaur A. (2020) Persistence and dissipation kinetics of chlorantraniliprole in okra and soil. Pesticide Research Journal 32: 172–178.

Vijayasree V., Bai H., Beevi S.N., Mathew T.B., George T. and Xavier G. (2015) Persistence and effect of processing on reduction of chlorantraniliprole residues on brinjal and okra fruits. Environmental Monitoring and Assessment 187(5): 299.

Wei K., Xu W., Liu Q., Yang L. and Chen Z. (2020) Preparation of a chlorantraniliprole–thiamethoxam ultra low-volume spray and application in the control of *Spodoptera frugiperda.* ACS Omega 5(30): 19293–19303.

(Received March 18, 2023; revised ms accepted July 22, 2023; published September 30, 2023)