



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2021; 9(1): 110-116

© 2021 JEZS

Received: 08-10-2020

Accepted: 06-12-2020

Ratnamma

Pesticide Residue and Food
Quality Analysis Laboratory,
University of Agricultural
Sciences, Raichur, Karnataka,
India

Harischandra Naik R

Pesticide Residue and Food
Quality Analysis Laboratory,
University of Agricultural
Sciences, Raichur, Karnataka,
India

Prabhuraj A

Department of Entomology,
University of Agricultural
Sciences, Raichur-584 104,
Karnataka, India

Nagaraj M Naik

Pesticide Residue and Food
Quality Analysis Laboratory,
University of Agricultural
Sciences, Raichur, Karnataka,
India

Bheemanna M

Pesticide Residue and Food
Quality Analysis Laboratory,
University of Agricultural
Sciences, Raichur, Karnataka,
India

Corresponding Author:**Ratnamma**

Pesticide Residue and Food
Quality Analysis Laboratory,
University of Agricultural
Sciences, Raichur, Karnataka,
India

Determination and dissipation of acetamiprid using LC-MS/MS in okra

Ratnamma, Harischandra Naik R, Prabhuraj A, Nagaraj M Naik and Bheemanna M

Abstract

The sensitive analytical QuEChERS (quick, easy, cheap, effective, rugged and safe) method with liquid chromatography tandem mass spectrometry was developed and validated as per European Commission's guidelines and used for the determination of acetamiprid in okra. This method was validated for linearity, limits of detection, quantification, matrix effect, specificity, trueness, repeatability and reproducibility. The LOQ of the developed method was 0.002 mg kg⁻¹ for acetamiprid. The recovery was 74.79-94.71% at LOQ, 5 LOQ and 10 LOQ level. The % RSD values for intraday and interday precision study were ≤ 20%. For the dissipation study acetamiprid 20% SP was applied at recommended (10 g a.i. ha⁻¹) and double the recommended (20 g a.i. ha⁻¹) dose in okra. The initial deposit of acetamiprid was 2.03 and 3.11 mg kg⁻¹ at recommended and double the recommended doses, respectively. The studied insecticide reached below detectable limit within 30 days after spray.

Keywords: Acetamiprid, dissipation, LC-MS/MS, Okra, QuEChERS

Introduction

Okra, *Abelmoschus esculentus* (L.) is a vegetable, known as bhindi in India, Brazil, West Africa; valued for its edible green nutritive seed pods. It is rich in proteins, minerals and vitamins (A, B, C) and helpful for controlling genito-urinary disorders, spermatorrhoea and chronic dysentery [1, 2]. About 72 insect pests are recorded in okra from germination to harvest [3]. Leafhopper (*Amrasca bigutulla bigutulla* Ishida) causes damage upto the growth period of the crop [4], Whitefly (*Bemisia tabaci* Gennadius) is the vector of vein clearing disease and shoot and fruit borer (*Earis vitella* Fabricius) [5] were collectively cause 36-90% of yield loss [6]. Therefore 10-12 sprays of pesticides in okra being taken up which led to the problem of resistance, resurgence, residues and creation of environmental pollution and decimation of useful fauna and flora [7].

The use of pesticides has increased rapidly over the last two decades at the rate of 12% per year on which India stands seventh rank in pesticide usage [8, 9]. Residues of pesticide finds their way into the human body through food, water and the environment. Commonly fresh vegetables and fruits sold at local markets were usually not analyzed for pesticide residues. Thus, analysis of pesticide residues fruits and vegetables have become essential requirement for consumers, producers and food quality control authorities [10]. Acetamiprid is a synthetic derivative of nicotinoids that have been introduced as an alternative to organophosphate N-methyl carbamates and pyrethroid insecticides and are more widely used among the farmers due to their easy application as foliar spray, soil drench and seed treatment in vegetables against sucking pest [11, 12]. These systemic insecticides are not susceptible to ultraviolet light, degradation, ozonisation with the result that their residues posed a potential risk to consumers. Keeping in view the present study was undertaken to develop and validate a method for determination of acetamiprid in okra.

Chemical and reagents

Certified reference material (CRM) of acetamiprid having 99.00% purity was procured from Dr. Ehrenstorfer, Augsburg, Germany. LC-MS grade acetonitrile and methanol (purity ≥ 99.9%) were procured from J. T. Baker (NJ, USA), Ammonium formate and formic acid (≥ 90.00% purity) were purchased from Empart, Hyderabad India. Ultrapure water of 18.2 MΩ was obtained using a Milli-Q water purification system (Merck Millipore, Mumbai India). Anhydrous magnesium sulphate (purity ≥ 99.90%), anhydrous sodium sulphate, anhydrous

sodium chloride (purity $\geq 99.90\%$) was obtained from Himedia (Bangalore, India). The primary secondary amine (PSA, 40 μm particle size) was obtained from Agilent Technologies, (USA).

Instruments and apparatus

LC-MS/MS (Shimadzu; LCMS 8040[®]), analytical balance (Make-Sartorius[®], model-BSA224S-CW), centrifuge tube (Make-Tarsons[®]), distilled water (Make-Milli-Q[®]), high volume homogenizer (Make-Robot coupe), low volume homogenizer (model: IKA[®] T18 digital Ultra Turrax), centrifuge (model: Gyrozen-high speed centrifuge 2236R), vortexer (Make-REMI[®]) and nitrogen flash evaporator (Make-TurboVap[®]) were used for extraction, cleanup and quantification of acetamiprid in okra.

Standard preparation

Acetamiprid standard stock solutions (1000 $\mu\text{g ml}^{-1}$) were prepared using CRM (99.00%) weighing accurately 9.35 ± 0.1 mg and transferred into a 10 mL cleaned, calibrated volumetric flask and dissolved with 10 ml of methanol (LC-MS grade). Intermediate stock solution of 400 $\mu\text{g mL}^{-1}$ by transferring primary stock solution of 4.321 in a 10 mL volumetric flask and volume was made up with methanol. The working standard solutions were prepared from intermediate stock solution by serial dilution technique using methanol. All the prepared standard solutions were stored at -20°C . The matrix match standards at the same concentrations were prepared by using control okra sample extract obtained through sample preparation procedure.

Field Experimentation and sample collection

The dissipation kinetics experiment was conducted at Agricultural Entomology Block of Main Agricultural Research Station, UAS, Raichur in Randomized Block Design (RBD), where in 3 treatments and 8 replications. The treatment plot size was $10 \times 32 \text{ m}^2$ and a variety ANKUR-1 was used. Application of acetamiprid 20% SP at 10 and 20 g a.i. ha^{-1} as recommended and double the recommended dose, respectively. Two foliar sprays were given at 15 days interval during fruit formation stage. Okra fruits were drawn on 0 (2 hr after spraying), 1, 3, 5, 7, 10, 15, 21, 25 and 30 days after second spray. The collected samples were extracted according to modified QuEChERS method (SANTAE/11813/2017) and the extracted samples were injected into LC-MS/MS and calculated the residue of acetamiprid at different days.

Extraction of acetamiprid from okra

QuEChERS method and its modification [13, 14] were adopted for extraction and cleanup of acetamiprid in okra fruit samples. Whole laboratory samples (500g) were grounded thoroughly using high-volume homogenizer (Robot Coup). About 10 g of grounded sample was weighed in analytical balance and transferred into 50 mL centrifuge tube. 20 mL of acetonitrile was added and further allowed to stand for 30 min. The sample mixture was then homogenized at 10000-12000 rpm for 3 min. Then 3 g of NaCl was added and vortexed immediately for 2 min. The homogenized sample mixture was centrifuged at 12000 rpm for 5min. at 10°C . After centrifugation, 15 mL of upper organic layer was collected in a test tube and added 9 g of sodium sulphate. Further, the 11 mL of extract was transferred from test tube into a 15 mL centrifuge tube containing 0.4 g of primary

secondary amine (PSA) and 1.15 g of magnesium sulphate and then vortexed the mixture for one min. Centrifuge the supernatant with added reagent at 12000 rpm for 5 min. Then, 1mL of supernatant was filtered using 0.22 μm PTFE nylon filter in to LC vials.

Instrumentation (LC-MS/MS)

A LC-MS/MS system equipped with Shimadzu 1200 series UHPLC and LCMS 8040 with triple quadrupole detector (TQD) was used. Lab Solution[®] Version 1.5 software was used to instrument control, data acquisition and processing. Separation of the analyte was attained on a Shim-pack XR-ODS C18 column (150 x 2 mm i.d.) with 40°C column oven temperature. The flow rate was 0.4 mL min^{-1} ; injection volume 2 μL and column temperature was 40°C . Acetamiprid separated with the following gradient programme: 40% B and 60% A at the beginning for 13 minutes followed by 100% B upto 1 minute and then 40% B for 1 minute. A full scan mass spectrum of both insecticides with electro-spray ionization positive mode (ESI+) was documented to choose the most intense m/z value. Further, the parent ion ((M+H)⁺) was identified and selected as the precursor ion. The transitions of multiple reaction monitoring (MRM) along with acquisition parameter were optimised for the high abundance of selected ions with ESI positive mode. The MS source parameters used were as follows; interface voltage of 4.5 kV, desolvation temperature of 250°C , heat block temperature of 400°C , desolvation gas (N₂) of 2.9 L/min and drying gas at 2.9 L/min. Then collision with argon gas (230 kpa) was done and different collision energies were optimised.

Method validation

The extraction and clean-up to remove co-extractives with PSA and MgSO₄, identification and quantification of acetamiprid from okra sample was optimised and validated according to the SANTE/11813/2017 [15, 18] by ascertaining the different parameters. Linearity of the method was assessed in both solvent and matrix matched by injecting the standard concentrations at 0.001, 0.002, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 $\mu\text{g mL}^{-1}$ and calculated the coefficient of determination. The matrix effect was calculated by comparing the angular coefficients obtained by the calibration curves in the solvent and in the matrix according to the following equation,

$$\text{Matrix effect (\%)} = (b_m - b_s) / b_s \times 100$$

Where b_m and b_s are the angular co-efficient of the curve in the matrix and in the solvent, respectively [15, 16].

The LOD was calculated by preparing different solutions with low concentration that is expected to produce a response that is 3 times baseline noise. LOQ in the same manner and selected as the concentration of pesticide that gives an S/N ratio of 10 and recovery of lowest spike level within the limit of 70-120% with RSD of $\leq 20\%$. Trueness of the developed method was evaluated by estimating the average recovery for each spiked level tested. Recovery experiments were carried out at 3 fortification levels by spiking the okra sample with the mixture of analytical standard solution of acetamiprid. Spiking was done such that the fortification level is at LOQ level, 5 times LOQ and 10 times LOQ with six replications for each level. The spiked samples of six replications at each level were then kept at room temperature of 25°C for 2 hours to attain sample stability. The fortified samples were further analyzed by following the previously

described sample extraction. After the extraction of pesticides from fortified okra samples were injected to LC-MS/MS and the area under the peaks of the known amount of analytes in the spiked matrix prior to extraction and with sample extract spiked near the chromatographic analyses (matrix matched standards) was compared to calculate recovery percentage. The method precision was ascertained with regards to the repeatability relative standard deviation (RSD_r) of the six replicates exactly similar extractions of blank okra matrix spiked with acetamiprid at the same fortification levels and RSD with respect to reproducibility (RSD_{wR}) by fortification at two different dates with six replications.

Data analysis

The half-life ($t_{1/2}$) was determined as $DT_{50} = \log 2/k$. $t_{1/2}$ is the insecticide half-life in okra.

Half-life (RL 50) was mathematically calculated

$$t_{1/2} = \frac{e}{b} = \frac{0.301}{b} \quad (e = \log 2 = 0.301).$$

The waiting periods or Pre-Harvest Interval were calculated by the following formulae,

$$T_{tol} \text{ (days)} = \frac{[a - \text{Log } tol]}{b}$$

Where,

T_{tol} = Minimum time (days) required for the pesticide residue to reach below the tolerance limit.

a = Log of apparent initial deposits obtained in the regression equation, ($Y = a + bx$)

tol = Tolerance limit of the insecticide (MRL)

b = Slope of the regression line^[17].

Results and Discussion

Optimization of LC-MS/MS parameters for acetamiprid and thiamethoxam in okra

In order to develop the method for determination and quantification of acetamiprid in okra the different acquisition parameters of mass spectrometer were optimized. Initially full scan mass spectrum of the selected insecticides were recorded in array to choose the m/z value having most abundance. The parent ion ($M+H$)⁺ of acetamiprid 223.05 was identified and selected as a precursor ion. Based on the known molecular ion, multiple reaction monitoring (MRM) transformation with different collision energies (CE) *viz.*, -22, -23, -17. The related acquisition conditions *viz.*, nebulizing gas flow (2.90 L min⁻¹), drying gas (15.00 L min⁻¹), Desolvation line temperature (250 °C), heat block temperature (400 °C), flow rate of mobile phase (0.40 mL min⁻¹) with binary gradient programme of 3.50 min were standardized. The daughter ions of 126.05, 56.50, 99.05 were selected for further quantification and confirmation, with ESI positive mode (Fig 1). After determination of the MRM transitions, found out the chromatographic conditions for better determination of the studied insecticide. Where, it should be emphasized that, the total ion chromatogram (TIC) had good separation resolution. The present developed MRM positive mode accustomed more sensitiveness and accurate conditions for the detection at low concentration of 0.0005 mg g⁻¹ in okra matrix. Under the developed method acetamiprid found to eluted at retention time of 1.358 ± 0.1 min (Fig 2). The developed method can identify the study analytes within a short period of time programme of 3.50 minutes.

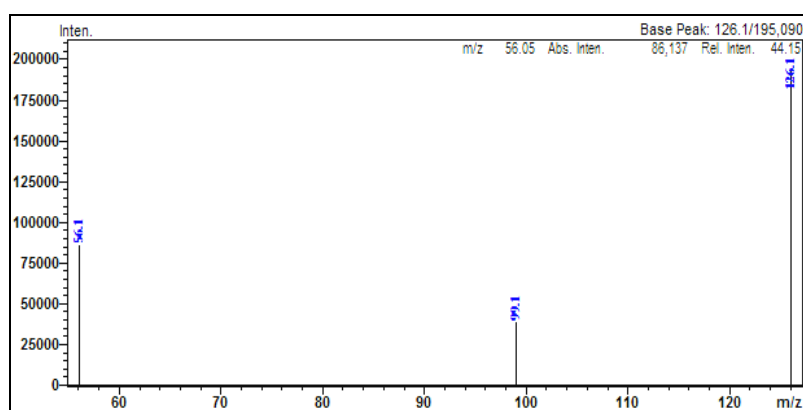


Fig 1: Product ion mass spectra of acetamiprid

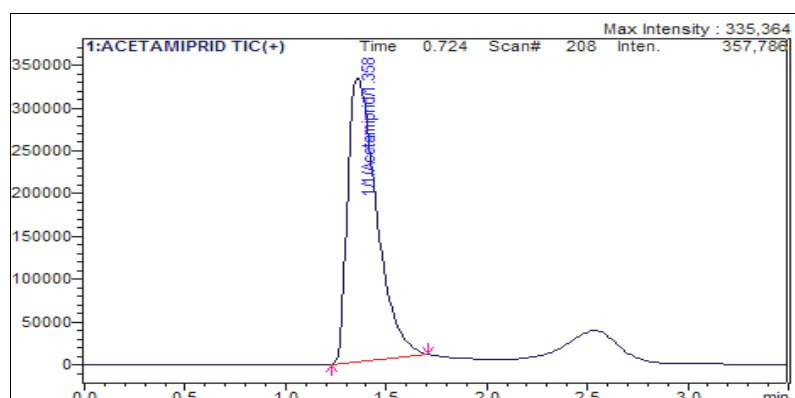


Fig 2: Total ion chromatogram of acetamiprid standard

Method validation

The standardized method in the present study was validated according to SANTE/11813/2017, guidelines. The blank okra sample was collected and used as matrix during method validation (Fig. 3). After the matrix extraction, it was injected to LC-MS/MS. Linearity for acetamiprid were studied in okra matrix as well as in methanol solvent in the calibration range

of 0.001 to 0.1 $\mu\text{g g}^{-1}$ (Fig. 4). A good linearity and strong correlation between concentrations of peak area in terms of residuals obtained at $\pm 20\%$ with the coefficient of determination (R^2) higher than 0.998. The LOD of 0.0005 $\mu\text{g mL}^{-1}$ for acetamiprid in methanol solvent was identified. The LOD of 0.0005 mg g^{-1} was selected (Table 1).

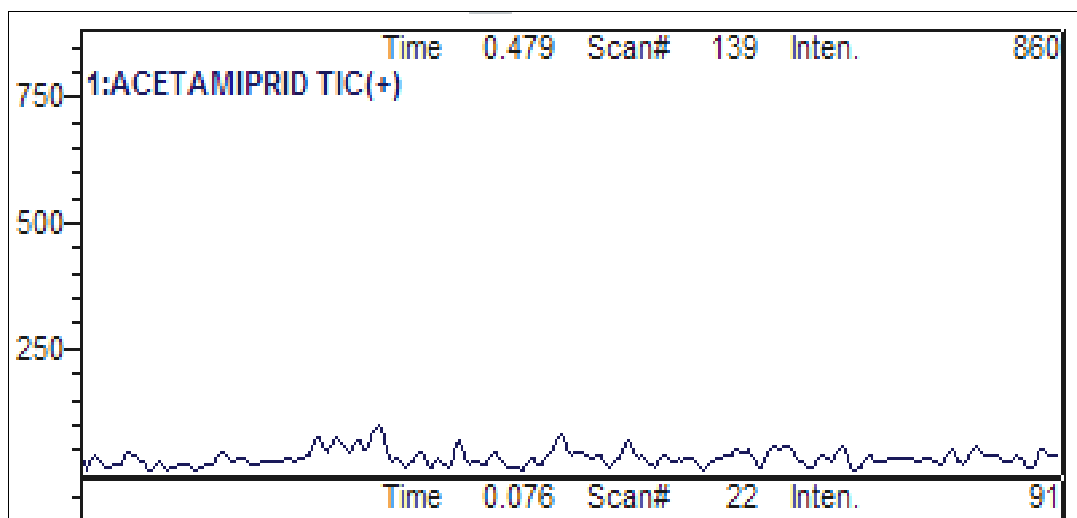


Fig 3: The chromatograms represent the okra blank matrix free from acetamiprid

Table 1: Linearity, Limit of detection, limit of quantification and matrix effect of acetamiprid in okra

	Regression equation	R^2	LOD	LOQ	Matrix effect (%)
Solvent	$Y = 5322x - 20575$	0.998	$0.0005 \mu\text{g L}^{-1}$	$0.0010 \mu\text{g L}^{-1}$	-
Matrix	$Y = 5119x - 8326$	0.999	$0.0005 \mu\text{g g}^{-1}$	$0.0020 \mu\text{g g}^{-1}$	-3.81

R^2 -coefficient of determination, LOD- Limit of detection, LOQ-Limit of quantification

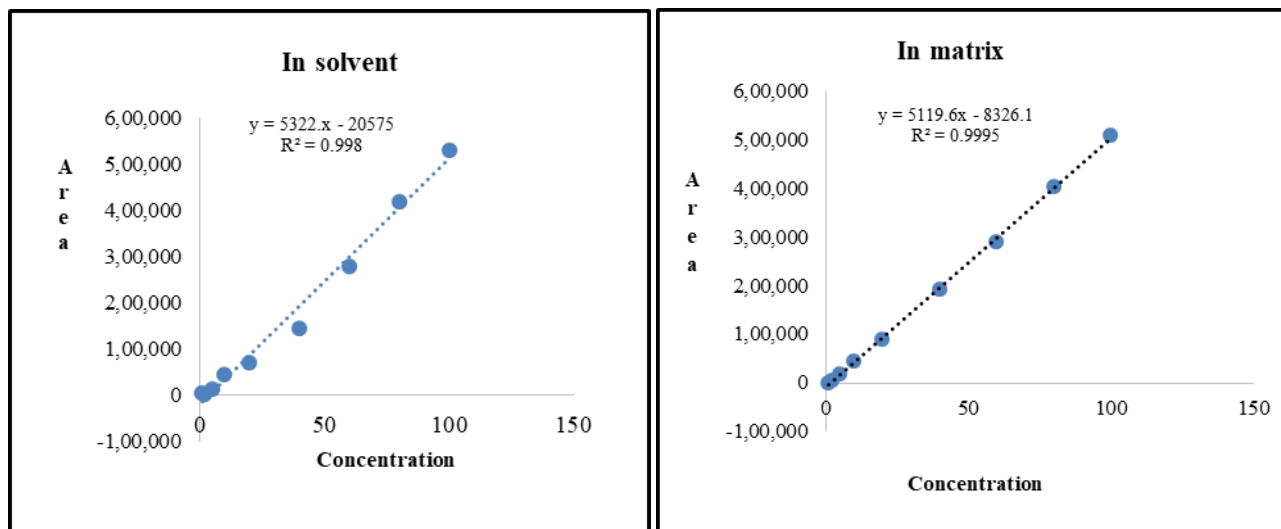


Fig 4: Linearity of acetamiprid in solvent (methanol) and okra matrix

The LOQ of the method, which was defined as the minimum spiked concentration with compliance recovery of 70 to 120% was 0.001 $\mu\text{g mL}^{-1}$ in methanol solvent were identified similarly LOQ of 0.002 $\mu\text{g g}^{-1}$ in okra matrix, which were well below the maximum residual limit (MRL) of 0.20 $\mu\text{g kg}^{-1}$ in okra [15].

The specificity study was conducted by comparing the area response of acetamiprid at concentration of 0.01 $\mu\text{g g}^{-1}$ in okra matrix with six replications and observed there is no much

interference of the matrix during analysis and did not found other peak at determined RT and area. Also calculated the response in solvent as well as in matrix and obtained % RSD at RT and area response were less than acceptable limit of $\pm 20\%$ which compliance with SANTE 2017, guidelines. The matrix effect calculated with the angular coefficient of calibration curve was -3.81. The calculated matrix effect was less than 20% for all the studied insecticides, which compliance with method validation criteria.

Table 2: Accuracy of the proposed method for acetamiprid in okra spiked at different levels

Spiking level ($\mu\text{g g}^{-1}$)	Mean recovery% (% RSD)	Precision in terms of repeatability% (% RSD)	Precision in terms of reproducibility% (% RSD)
0.002	74.79 (3.22)	78.25 (6.19)	77.85(5.84)
0.01	94.71 (5.79)	78.25(16.95)	77.85(16.28)
0.02	82.95 (2.78)	78.25(9.82)	77.85(7.42)

RSD-Relative standard deviation

The trueness was evaluated at spiking levels of 0.002, 0.01 and 0.020 $\mu\text{g g}^{-1}$ and the mean recovery was found to be 74.79, 94.71 and 82.95%, respectively (Table 2). Precision of acetamiprid in terms of repeatability (RSD_r) and reproducibility (RSD_{wr}) was found between 70-120% spiked at 1, 5 and 10 times of LOQ level. The limits quantification (LOQ) ranged from 0.13 to 5.9 $\mu\text{g kg}^{-1}$ in okra matrix for imidacloprid, acetamiprid, thiacloprid, thiamethoxam, clothianidin and the matrix-matched standard gave satisfactory recoveries (72.40 -105.10%) and relative standard deviation (2.20 - 20.00%) values in different matrices at three spiked levels (0.01, 0.1, and 1 mg kg^{-1}) for okra [18]. About 86 to 112 per cent average recoveries for the fortification levels of 0.01, 0.05 and 0.1 mg kg^{-1} in tomato, okra, cabbage, cauliflower and grape with 0.01-0.5 mg mL^{-1} linearity response [19].

Dissipation study

The results of dissipation study for acetamiprid in okra are presented in table 3. The average initial deposits of was 2.034 and 4.044 mg kg^{-1} , at recommended and double the

recommended dose, respectively Fig 5. Residue of acetamiprid reported in okra was lower (0.335 mg kg^{-1}), following two application at 75 g a.i. ha^{-1} [20] and on watermelon the initial residue was 1.5 times more when applied at 63 g a.i. ha^{-1} than the recommended dose (42 g a.i. ha^{-1}) [21]. The residues of acetamiprid was much higher than the concentrations reported in chillies (0.02-0.1 mg kg^{-1}) at recommended and double-the-recommended dose [22]. The residues of acetamiprid dissipated from 70-77% at 3 days after second spray and accounting to the loss of 99-99.99% between 25-30 days after spray in okra. The% dissipation of acetamiprid increased with the progress in time and reached 60- 69% for the recommended dose (10 g a.i. ha^{-1}) and 78-89% for double the recommended dose (20 g a.i. ha^{-1}) after seven days of application in chilli [22]. The initial deposits of acetamiprid in okra was dissipated to half of its concentration at 3.11 and 3.29 days at recommended and double the recommended dose, respectively. The half-life values for acetamiprid were 2.3 days in okra [23], 1.02 and 1.59 days in mustard plant [24], 2.24 and 4.84 days in chilli [22].

Table 3: Residues (mg kg^{-1}) of acetamiprid in okra at recommended and double the recommended dose

Days after treatment	Acetamiprid residue at recommended dose		Acetamiprid residue at double the recommended dose	
	Residue (mg kg^{-1}) \pm SD	% dissipation	Residue (mg kg^{-1}) \pm SD	% dissipation
0	2.034 \pm 0.094	-	4.044 \pm 0.105	-
1	0.972 \pm 0.059	52.23	1.931 \pm 0.092	52.25
3	0.490 \pm 0.063	75.92	0.962 \pm 0.24	76.21
5	0.258 \pm 0.010	87.31	0.489 \pm 0.037	87.90
7	0.128 \pm 0.005	93.71	0.258 \pm 0.015	93.62
10	0.067 \pm 0.001	96.72	0.131 \pm 0.019	96.75
15	0.031 \pm 0.010	98.490	0.064 \pm 0.00	98.42
21	0.018 \pm 0.001	(99.14)	0.034 \pm 0.003	99.17
25	0.001 \pm 0.001	(99.95)	0.026 \pm 0.002	99.43
30	BDL	-	0.002 \pm 0.001	99.98
35	BDL	-	BDL	-
Correlation coefficient	0.952		0.966	
Regression equation	$y = 0.627 - 0.092x$		$y = 1.321 - 0.097x$	
Degradation rate constant (day^{-1})	0.092		0.097	
Half life (days)	3.06		3.09	
Safe waiting period (days)	13.52		20.64	
Student t test	T_{cal}	2.924	3.830	
	T_{crit}	2.263	2.863	

SD-Standard deviation

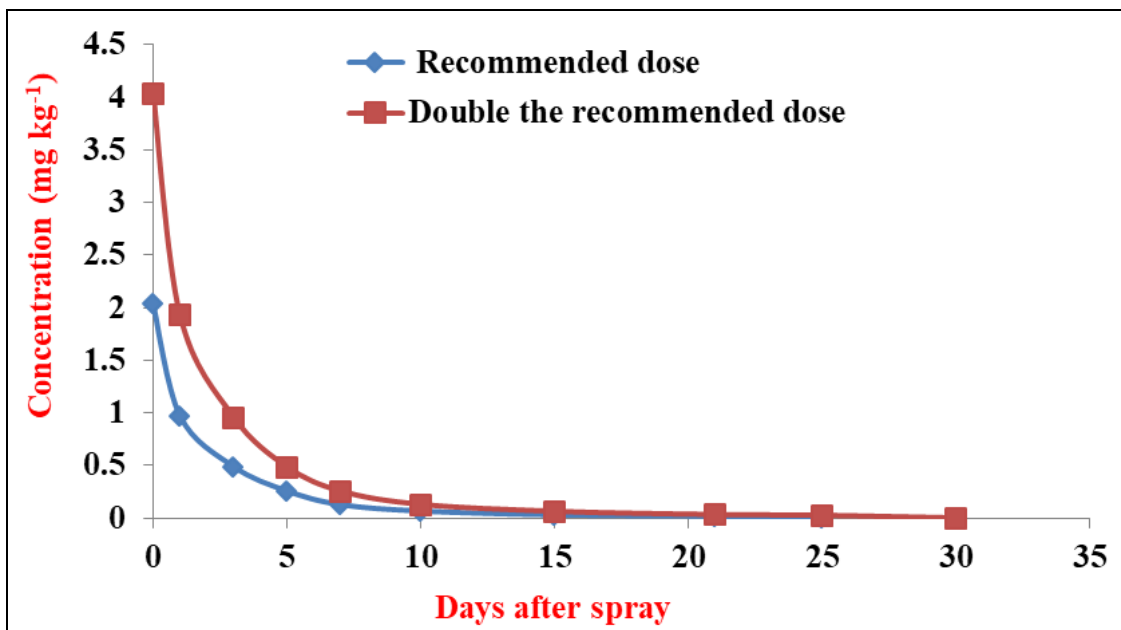


Fig 5: Dissipation curve for acetamiprid at recommended and double the recommended dose in okra

Conclusion

The development and validation of acetamiprid in okra is challenging due to complicated matrix. In this study simple, robust QuEChERS method combined with LC-MS/MS was used. The proposed method has satisfactory LOQ ($0.002 \mu\text{g g}^{-1}$) and accuracy (70-120%) demonstrating the suitability of the method for acetamiprid analysis in okra. The dissipation of acetamiprid recorded the initial deposit of 2.034 and 4.044 mg kg^{-1} with half life of 3.11 and 3.29 days at recommended and double the recommended dose, respectively. This insecticides need to be applied with caution with adequate time gap before harvest to avoid detection of its residues at harvest. The safe waiting period will be useful to farmers to ensure safe consumption.

Acknowledgements

The authors wish to express their gratitude to Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), UAS, Raichur, Karnataka for providing facilities during research work

References

- Nandkarni KM, Naya Prakash. Vegetable Crops. Indian Materia Medica, Nandkarni and Co., Bombay 1927;6:712.
- Singh B, Gupta A, Bhatnagar A, Parihar NS. Monitoring of pesticide residues in farmgate samples of chilli. Pesticide Research Journal 1970;11(2):207-209.
- Pal S, Maji TB, Mondal P. Incidence of insect pest on okra *Abelmoschus esculentus* (L) Moench in red lateritic zone of West Bengal. Journal of Plant Protection and Sciences 2016;5(1):59-64.
- Mahal MS, Brar LS, Singh R, Singh B. Influence of varying magnitude of jassid injury on seed yield of okra and economics of its control. Journal of Insect Science 1994;7(1):129-132.
- Gupta S, Sharma RK, Gupta RK, Sinha SR, Singh R, Gajbhiye VT. Persistence of new insecticides and their efficacy against insect pests of okra. Bulletin of Environmental Contamination and Toxicology 2009;82(2):243-247.
- Misra HP, Dash DD, Mahapatra D. Efficacy of some insecticides against okra fruit borer, *Earias spp.* and leafroller, *Sylepta derogata* Fab. Analytical Plant Protection Science 2002;10(1):51-54.
- Dubey VK, Ganguly RN. Fruit loss in okra due to *Earias vittella* (Fab.) at Raipur. Insect Environment 1998;4(1):25.
- Bempah CK, Buah-Kwofie A, Denutsui D, Asomaning J, Tutu AO. Monitoring of pesticide residues in fruits and vegetables and related health risk assessment in Kumasi metropolis, Ghana. Research Journal of Environmental and Earth Sciences 2011;3(6):761-771.
- Singh M. Production and utilization of pesticides in Asia and Pacific Tokyo. Asian Production and Organization 2002;32:190-202.
- Kumar BA, Ragini K, Padmashri A, Rao KJ, Shashibhushan V. Monitoring of pesticide residues in okra (*Abelmoschus esculentus* L.) Agriculture Update 2017;12(7):1909-1913.
- Stamm MD, Heng-Moss TM, Baxendale FP, Siegfried BD, Blankenship EE. Uptake and translocation of imidacloprid, clothianidin and flupyradifurone in seed-treated soybeans. Pest Management Science 2016; 72:1099-1109.
- Jeschke P, Nauen R, Schindler M, Elbert A. Overview of the status and global strategy for neonicotinoids. Journal of Agriculture and Food Chemistry 2011;59:2897-2908.
- Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. JAOAC International 2003;86(2):412-43.
- Lethotay SJ, Kok AD, Hiemstra M, Bodegraven PV. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Journal of AOAC International 2005;88(2):595-614.
- European Commission, SANTE/11813/2017 of 1st January 2016. Guidance document on analytical quality control and method validation procedures for pesticide

- residues and analysis in food and feed 2017, 1-34.
16. Silva V, Mol HG, Zomer P, Tienstra M, Ritsema CJ, Geissen V. Pesticide residues in European agricultural soil. *Science of the Environment* 2001;653:1532-1545.
 17. Regupathy A, Dhamu KP. Insecticide decay curve. In: *Statistics Work Book for Insecticide Toxicology*. Suriya Desktop Publishers 1990;32:76-84.
 18. Hussan HNM, Xingang L, Fengshou D, Jun X, Yongquan Z. Simultaneous determination of six insecticides in okra (*Abelmoschus esculentus* L. Moench) by UHPLC-MS/MS. *Journal of Chromatography* 2017; 8(3):100-103.
 19. Paramasivam M, Chandrasekaran S. Determination of fipronil and its major metabolites in vegetables, fruit and soil using QuEChERS and gas chromatography-mass spectrometry. *International Journal of Environmental Analytical Chemistry* 2013;93:1203-1211.
 20. Singh SB, Kulshrestha G. Residues of Thiamethoxam and Acetamiprid, Two Neonicotinoid Insecticides, in/on Okra Fruits (*Abelmoschus esculentus* L.). *Bulletin of Environmental Contamination and Toxicology* 2005;75(5):945-951.
 21. Wu J, Wang K, Zhang H. Dissipation and residue of acetamiprid watermelon and soil in the open field. *Bulletin of Environmental Contamination and Toxicology* 2012;89(3):644-648.
 22. Sanyal D, Chakma D, Alam S. Persistence of a neonicotinoid insecticide, acetamiprid on chili (*Capsicum annum* L.). *Bulletin of Environmental Contamination and Toxicology* 2008;81(4):365-368.
 23. Christensen HB, Poulsen ME, Rasmussen PH, Christen D. Development of a LC-MS/MS method for the determination of pesticides and patulin in apples. *Food Additives and Contaminants* 2009;26:1013-1023.
 24. Pramanik SK, Bhattacharyya J, Dutta S, Dey PK, Bhattacharyya A. Persistence of acetamiprid in/on mustard (*Brassica juncea* L.). *Bulletin of Environmental Contamination and Toxicology* 2006;76:356-360.