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Method validation and residue estimation of thiamethoxam in raw banana fruit, male bud and peduncle using QuEChERS method in LC-MS/MS

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ABSTRACT

Thiamethoxam, a new generation neonicotinoid insecticide, is recommended for use in vegetables, fruit crops, paddy and banana against a spectrum of insects. Its systemic nature, formulation and unit packaging make its use convenient to farmers. Owing to the awareness among the consumers and exports, pesticide residues in the agricultural produces have to be estimated with cheap, easy and fast methods. QuEChERS is a quick method of pesticide residue estimation and in the present study this method was validated for estimating thiamethoxam residue in banana such as inflorescence or male bud, fruit and inner core. The method was applied to find out harvest time residues in samples collected from the field after treatment with thiamethoxam. Study revealed that QuEChERS method was valid for thiamethoxam estimation in banana and no detectable residue could be recorded from the samples treated with thiamethoxam in the field for pest management.

Keywords: Thiamethoxam, Residue, Pesticide, Validation, Banana

Introduction

Pesticides are essential for keeping a crop healthy. But improper, injudicious and indiscriminate use of pesticides leads to accumulation of residues in the final produces. Different extraction and quantification methods are developed by various scientists for estimation of multi class pesticide residues in several agricultural produces.

Banana (*Musa* sp.) is a major fruit crop in India. Several insects and pathogens were reported to affect the crop growth and yield (Uma, 2007) [1]. Farmers depend on several pesticides to tackle the issue (Sivakumar *et al.*, 2016). Thiamethoxam was found effective in managing certain pests of banana (KAU, 2011) [2]. CODEX Alimentarius has fixed MRLs of various pesticides for banana. CODEX has fixed MRL of thiamethoxam in banana as 0.02 mg kg⁻¹. Thiamethoxam which is a systemic insecticide, it is essential to estimate residues in consuming parts of banana. So an effective, fast and cheap residue estimation method of thiamethoxam in banana has to be developed.

QuEChERS method and its modified versions of monitoring the pesticide residue was tried in finding residues in banana. Curbelo *et al.* (2011) [3] used QuEChERS in combination with GC-NPD for residue analysis in banana leaves and found that this was reliable, rapid and simple. Pesticide, pymetrozine in banana could accurately be measured using QuEChERS by Wang (2013) [4]. Banana plant parts contain large percentage of water. Validation of multi residue method for 90 pesticides in high water containing matrices using QuEChERS was also successful (Madureira *et al.*, 2012) [5].

So a study was conducted to validate thiamethoxam residue estimation in banana using QuEChERS method as well as estimation of residues at the time of harvest in edible plant parts treated with thiamethoxam for pest management.

Materials and methods

Chemicals and reagents

Analytical standard of thiamethoxam (purity 99.7 %) was purchased from M/s Sigma-Aldrich (Steinheim, Germany). The formulation of thiamethoxam (Tagxone 25WG) was procured from M/s. Tropical Agrosystem (India) Pvt. Ltd., Chennai, India. Analytical grade sodium chloride, sodium sulfate, anhydrous magnesium sulfate, HPLC grade water, HPLC gradient grade acetonitrile and ACS grade formic acid was obtained from Merck (Mumbai, India); Methanol LiChrosolv grade from Merck (Darmstadt, Germany). The solid reagents such as NaCl, Na₂SO₄, MgSO₄ were activated at 450 °C for 4 hours before use. The sorbents such as primary secondary amine (PSA), endcapped C18 and graphitized carbon black (GCB) were procured from M/s Agilent Technologies (USA). Polyvinylidene fluoride (PVDF) syringe filters (13 mm, 0.2 µm)

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were obtained from M/s Rankem, New Delhi, were used to filter the extracts. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

Standard solution preparation

Standard stock solution, 400 mg L⁻¹ of thiamethoxam was prepared in methanol and stored at -20 °C. An intermediate stock solution standard (10 mg L⁻¹) was prepared from 400 mg L⁻¹. The working standards of 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 mg L⁻¹ were prepared by serial dilution with methanol from the intermediate stock standard solution for LC-MS/MS analysis and were used for studying the linearity of the system, preparing matrix matched calibration standard and spiking for recovery studies. The calibration solutions were stored at 4 °C. Matrix-matched calibration standards were prepared by adding the molecule under this study to the extract of blank samples, in the range of 0.025–1 mg L⁻¹.

Validation of the analytical method

Method validation and residue estimation studies were conducted at Pesticide Residue Research and Analytical Laboratory (PRRAL), College of Agriculture, Vellayani. For validation, samples of male bud, raw fruits with peel and peduncle (inner core) were taken from plants which were not treated with any pesticides. Each sample was cut into small pieces and ground to a fine paste. Five replicates of 25 g representative samples of the inflorescence or male bud, fruit and peduncle were taken in 50 ml centrifuge tubes and spiked at 0.05, 0.25 and 0.5 mg kg⁻¹ levels with the pure analytical standards of the insecticide, thiamethoxam. The extraction and clean-up was done following the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.*, 2003) [6] and quantified using UPLC-MS/MS under optimized conditions. The Limit of Quantification (LOQ) of this method was 0.05 mg kg⁻¹. The blended sample (25 g) was taken from each replicate, homogenized at 14,000 rpm using a tissue homogenizer for two minutes after adding 50 mL acetonitrile. The samples were shaken for one minute and 10 g sodium chloride was added. The sample was centrifuged for five minutes at 2500 rpm. Supernatant (16 mL) was transferred into 50 mL centrifuge tube containing 6 g anhydrous Na₂SO₄ and mixed well for 2 min using high speed vortex shaker. A 12 mL extract was transferred to a 15 mL centrifuge tube containing 0.2 ± 0.01 g PSA sorbent and 1.2 ± 0.01 g anhydrous MgSO₄. The sample was shaken at 2500 rpm speed and centrifuged for about three minutes. Using turbopap, five mL of supernatant was evaporated and made up with two mL using methanol for LC-MS/MS analysis.

Instrumentation

The chromatographic separation was achieved using Waters Acquity UPCL system which was equipped with a reversed phase Atlantis C-18 column. A gradient system with the following two eluent components: A: 10% methanol in water + 0.1% formic acid + 5 mM ammonium acetate; B: 10% water in methanol + 0.1% formic acid + 5 mM ammonium acetate was used as mobile phase for the separation of residues. The gradient elution was zero min isocratic 20% B, 0.0 – 4.0 min linear from 20 to 90% B, 4.0 – 5.0 min linear from 90 to 95% B, and 5 – 9 min linear from 95 to 100% B, with 9 – 12 min for initial conditions of 20% 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 eluent from the LC system was introduced into API 3200 tandem mass spectrometry (MS/MS) system (AB Sciex) equipped with an electron spray ionization interface (ESI), operating in the positive ion mode. The source parameters were as follows: temperature-600 °C, ion source gas (GS1) 50 psi, ion source gas (GS 2), 60 psi; ion spray voltage- 5500 V and curtain gas, 13 psi. The retention time for thiamethoxam under the above conditions was 0.88 min. The quantitative and qualitative ion pairs selected were 292.0 > 211.0 and 292.0 > 181.0 respectively and other instrument parameters were listed in Table 1.

Thiamethoxam residue at the time of harvest was estimated using samples collected from banana plants that were previously treated

with thiamethoxam 25WDG. Thiamethoxam was applied using two methods such as leaf axil filling (0.01%) and stem injection (0.03% @ 10 mL/plant) at five and six months after planting. A sub-sample consists of 500 g each for fruit, male bud and peduncle were prepared taken from the thiamethoxam treatment plots by quartering and commingling. Methods for clean up, extraction and detection were followed as explained earlier.

Residues (mg Kg⁻¹) = Sample area × Conc. of std. (µg g⁻¹) × Final Vol. of extract (mL) / Standard area × Weight of sample (g)

The residue data were subjected to first order kinetics ($\log C_t = \log C_0 - \frac{Kt}{2.303}$), where C_t is concentration after a lapse of time t ; C_0 is apparent initial concentration and K is the dissipation constant. The values of K were obtained from regression equation $y = ax + b$; where $y = \log C_t$, $a = \log C_0$, $b = \frac{K}{2.303}$ and $x = t$. Using the formula $t_{1/2} = \frac{0.693}{K}$, half-life values were calculated (Hoskin, 1961) [7].

Results

The QuEChERS method for pesticide residue estimation was validated by finding the recovery percentage of chemical after fortifying with fixed quantities of pesticide as elucidated above. Linear response of the thiamethoxam at different concentration in various matrices was checked by plotting calibration curve (Fig. 1 a-c).

The results showed that 80.58 to 98.2 % recovery percent was obtained from different matrices and relative standard deviation (RSD) ranged from 0.793 to 8.41. Green fruits of banana fortified with 0.05 mg Kg⁻¹ recorded a recovery percentage of thiamethoxam as 95.88 ± 1.035 per cent with RSD 1.08 (Table 2). The higher concentrations *ie.*, 0.25 and 0.5 mg Kg⁻¹ also showed a recovery percentage of 95.84 ± 2.809 per cent (RSD 2.931) and 98.2 ± 1.78 per cent (RSD 1.812), respectively.

Similarly tests with inflorescence/ male bud showed thiamethoxam recovery of 86.84 ± 2.435 per cent with RSD 2.804 for 0.05 mg Kg⁻¹ while concentrations of 0.25 and 0.5 mg Kg⁻¹ recorded 92.4 ± 7.767 per cent and 97.8 ± 1.086 per cent, respectively (Table 2).

The peduncle or inner core which is edible and extracted after fruit harvest, had a maximum recovery of thiamethoxam 96.76 ± 0.767 per cent at 0.05 mg Kg⁻¹, meanwhile at higher level *ie.*, 0.5 mg Kg⁻¹, per cent recovery was reduced to 86.96 ± 4.949. RSD values ranged from 0.793 to 5.691 for this experiment (Table 2). In these tests, no detectable thiamethoxam residue was found on any tested consumable banana plant parts. All tested concentrations registered RSDs well below the acceptable limit of 20.

Discussion

The validation results establish that the QuEChERS method is well-suited for determining thiamethoxam residues across different banana matrices. The results on validation demonstrated mean recoveries spanning from 80.58% in the pseudostem (at the 0.5 mg/kg fortification level) up to 98.24% in the fruit (at the same 0.5 mg/kg fortification level). These values comfortably satisfy established guidelines, which require recoveries between 70% and 120% alongside relative standard deviations under 20% (SANCO, 2011) [8]. Given that the QuEChERS approach successfully fulfills these validation criteria for quantifying thiamethoxam residues across banana fruits, peduncles, and male buds, it can be reliably implemented for all related analytical work.

All the evaluated matrices tested negative for thiamethoxam residues, indicating that regardless of concentration used, or delivery method, the compound failed to leave any detectable traces in the edible components of treated banana crops. Specifically, no residues were identified in male buds sampled just two months post-application. Although administering a higher concentration (0.03%) via stem injection successfully warded off pests, it still did not result in detectable residues on any edible parts. This absence of residue paired with effective pest control is likely aided by the high moisture content of the banana pseudostem, which promotes dilution of the chemical, alongside a

prolonged pre-harvest interval of two to three months that allows the insecticide to degrade into metabolites or drop beneath the detection threshold. These outcomes align with research by Kumar *et al.* (2014) [9], who observed zero thiamethoxam residues in potato tubers 90 days post-application, as well as with Sharma *et al.* (2005) [10], who noted rapid dissipation in okra within 7 to 10 days of the final treatment. Furthermore, the findings are strongly supported by Karmarkar *et al.* (2006) [11], whose previous work demonstrated a short soil half-life of only 11 to 26 days for thiamethoxam.

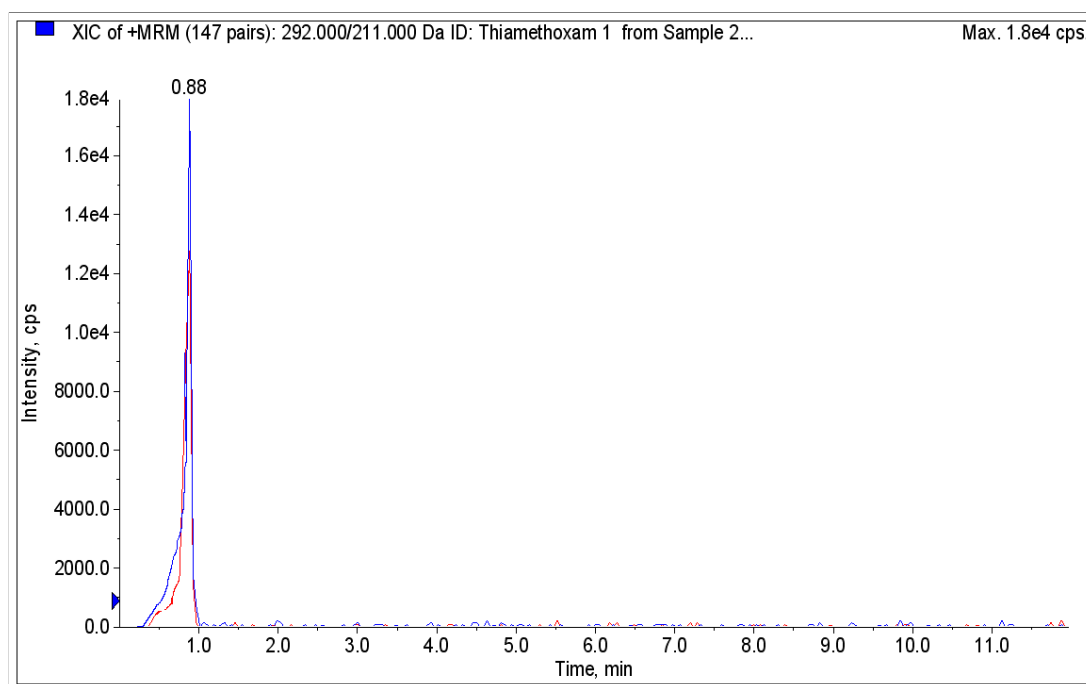
The QuEChERS procedure for insecticide residue estimation proved to be a rapid, simple and efficient approach for quantifying thiamethoxam in edible banana tissues. Because the insecticide leaves zero detectable residue at harvest, even when applied at elevated concentrations, it stands out as a safe and reliable option for managing

key banana pests, most notably the rhizome weevil (*Cosmopolitus sordidus*) and the pseudostem weevil (*Odoiporus longicollis*).

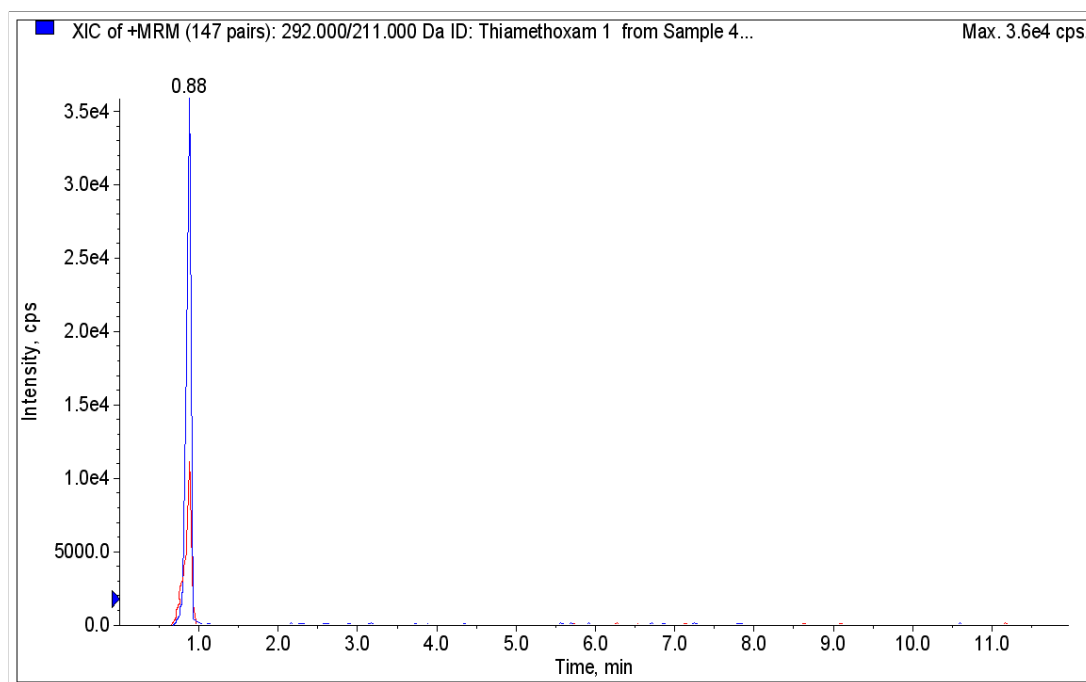
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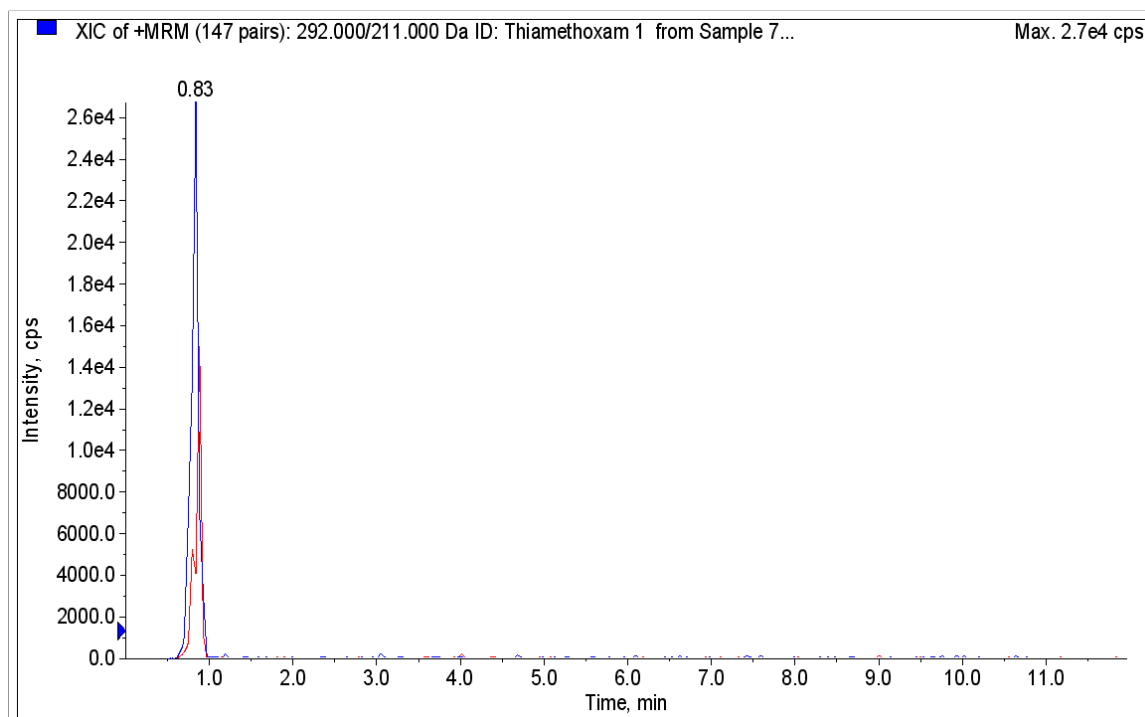
Matrix match std chromatogram of thiamethoxam in (a) Fruit, (b) Bud and (c) Stem @ LOQ (0.05 mg Kg⁻¹)



(a)



(b)



(c)

Table 1. Multiple reaction monitoring (MRM) parameters for thiamethoxam in LC-MS/MS

Compound	Precursor ion m/z	Product ion m/z	DP	EP	CEP	CE	CXP	Quantitative/ Qualitative
Thiamethoxam	292	211	31	7	19	19	2	Quantitative
		181	31	7	19	32	2	Qualitative

Qualitative DP - Declustering potential; EP - Entrance potential; CEP - Collision cell entrance potential; CE- Collision energy; CXP - Collision cell exit potential.

Table 2. Recovery of thiamethoxam from different matrices of banana

Matrix	Fortification level (mg Kg ⁻¹)	Mean recovery (%)	Standard Deviation	RSD
Fruit with peel	0.05	95.88	1.04	1.08
	0.25	95.84	2.81	2.93
	0.50	98.24	1.78	1.81
Male bud	0.05	86.84	2.44	2.80
	0.25	92.40	7.77	8.41
	0.50	97.80	1.09	1.11
Inner core (stem)	0.05	96.76	0.77	0.79
	0.25	80.58	1.08	1.35
	0.50	86.96	4.96	5.69

RSD= relative standard deviation

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