

Deltamethrin Pesticide Residues: Extraction by QuEChERS Method and Analysis by GC-MS/MS

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ABSTRACT

To ensure low pesticide levels in food the present studies was undertaken wherein samples of pigeonpea seeds procured from the field of experiments at the time of harvest of kharif crop of 2013-14 were studied for the residues of deltamethrin, a widely used insecticide in Vidarbha belonging to class synthetic pyrethroid. Pesticide residues were extracted using QuEChERS method and then analyzed through GC-MS/MS. Residues of deltamethrin in pigeonpea seeds in the samples of crop sprayed with 0.0014, 0.0028 and 0.0042 per cent deltamethrin were found below detectable limit (BDL) of 0.01 ppm for all the three concentrations. Considering the maximum residue limit (MRL) 1 mg/kg for pulses as specified by CODEX, the evaluated spray treatments of deltamethrin 2.8 EC can be considered most safe to the consumers.

Keywords: Deltamethrin, GC-MS/MS, Insecticides, MRLs, QuEChERS, Residues

I. INTRODUCTION

Pesticides are used by farmers to fight insects, increase the yield and improve the quality of food crops [1]. Pigeonpea (*Cajanus cajan*) is one of the important pulse crop grown in Vidarbha region of Maharashtra. Due to heavy infestation by pod borer *Helicoverpa armigera*, farmers use deltamethrin which is prominent and widely used insecticide belonging to class synthetic pyrethroid recommended against a broad spectrum of insect pests [2]. For the control of *Helicoverpa armigera*, deltamethrin 2.8 EC @ 1ml/lit is recommended [3]. When good agricultural practices are not followed during pesticide application, residues can reach plant parts. Since dietary intake of pesticides is one of the main routes of exposure to different pesticides, analysis and monitoring of pesticide residues is tremendously an important process to secure consumers. In India, the Food Safety and Standards Authority of India (FSSAI) is responsible for setting the MRLs which have been registered by Central Insecticides Board and registration committee (CIBRC). Many international organizations such as the Codex Alimentarius Commission and European Union as well as different countries have issued their own pesticide maximum residual limits (MRLs) in food commodities [4]. The MRL is the maximum level of a pesticide residue (expressed in mg/kg) which is legally permitted in or on food or feed of animal [4]. In the absence of

established MRLs, CODEX Alimentarius MRLs are followed. Exceedance of MRLs could pose threat to population health.

There is a dearth of studies related to these issues in India especially on pigeonpea in Vidarbha hence in present investigation the pesticide residue content in pigeonpea seeds were analysed for deltamethrin .Pesticide residues were extracted and cleaned up by QuEChERS i.e.Quick, Easy, Cheap, Effective, Rugged and Safe method developed by [5] and then cleaned up residues were analyzed through GC-MS/MS to estimate the possible health risk on consumers.

II. MATERIAL AND METHODS

2.1 Field operations for raising the crop

The field experiments were conducted in Nagpur area during kharif season for consecutive two years. Four treatments including control with three replications were taken up using Randomized Block Design (RBD). Each treatment plot was of 3 x 3m with inter plot and inter replication distance of 1.2 and 1.8 m respectively (Fig.1).Deltamethrin 2.8 EC was evaluated against *H.armigera* pod borer on pigeonpea at the concentration of 0.0014, 0.0028 and 0.0042 per cent. Two sprays were applied at an interval of 15 days, first spraying at 50 per cent flowering stage of the crop and another 15 days thereafter. Thus two sprays were applied. Residue study was conducted on the samples of crop produce from the field trials conducted for studying the bioefficacy of deltamethrin 2.8 EC during kharif season 2013-14.

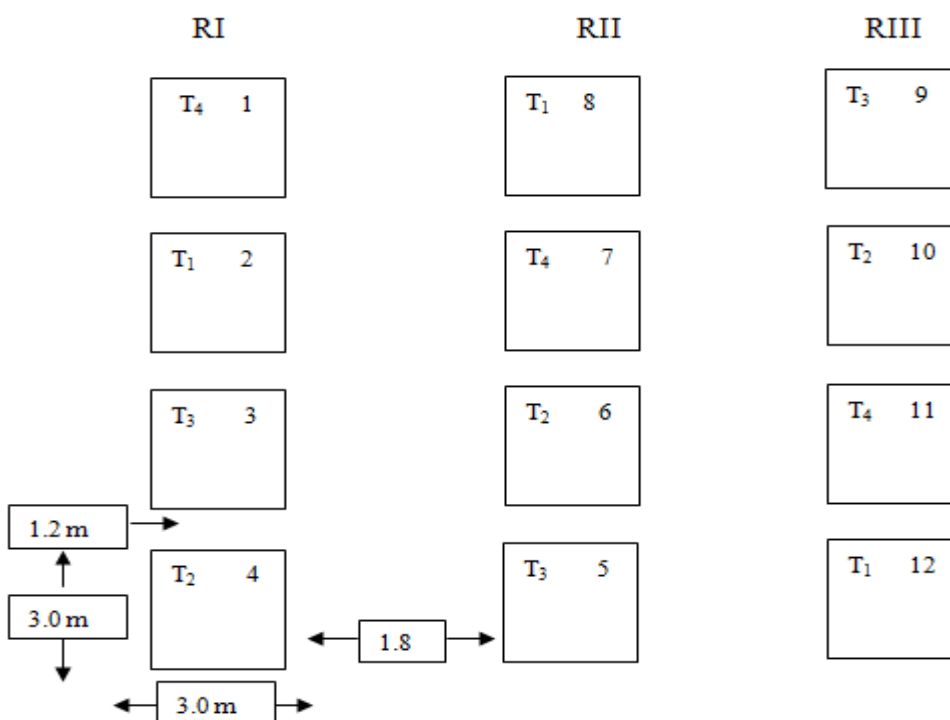


Figure 1: Plan of Layout of Pigeonpea (Year: 2013-2014)

Treatments
T ₁ - Deltamethrin 0.0014%
T ₂ - Deltamethrin 0.0028%
T ₃ - Deltamethrin 0.0042%
T ₄ -Untreated control

2.2 Residues analysis of deltamethrin in pigeonpea seeds

The pesticide residue content in pigeonpea seeds were analysed for deltamethrin .Pesticide residues were extracted and cleaned up by QuEChERS method and then cleaned up residues were analyzed through GC-MS/MS. The flow chart for residue analysis is shown in (Fig: 2)

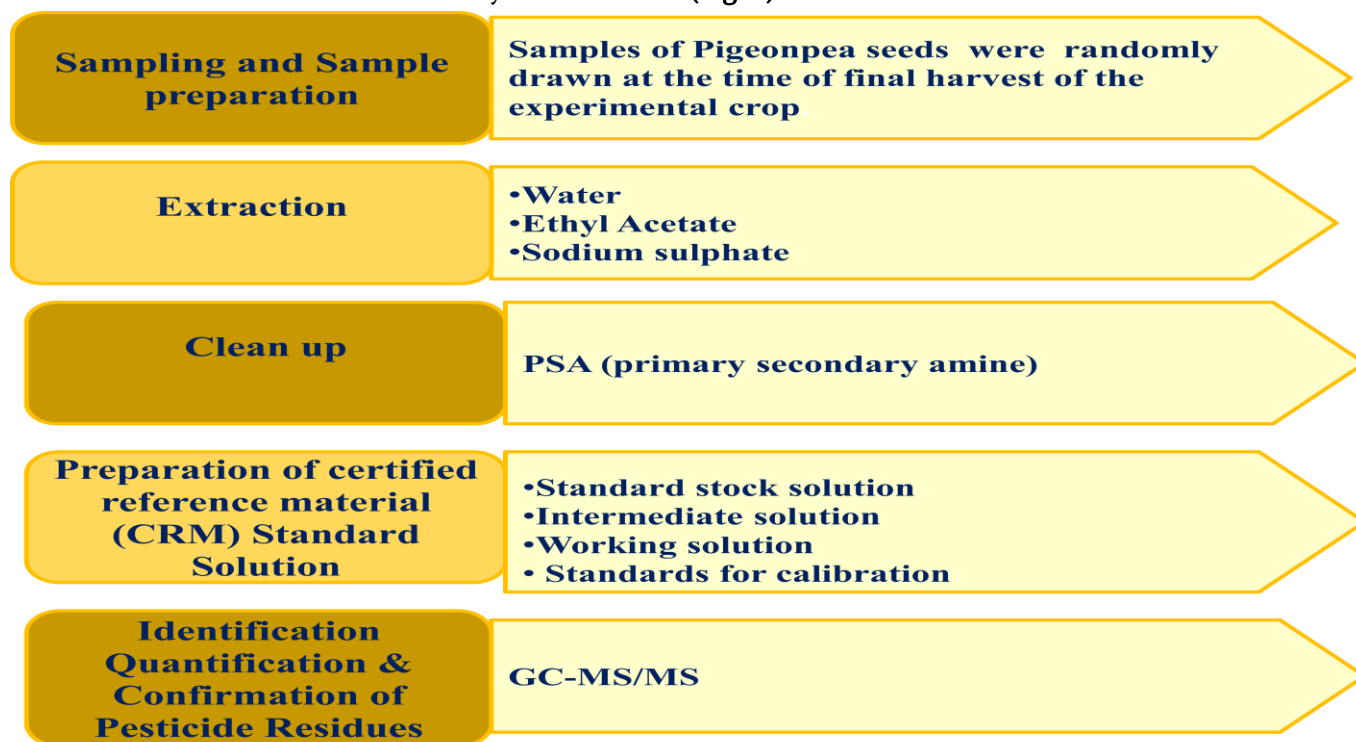


Figure 2: Residue analysis of deltamethrin in pigeonpea seeds

2.2.1. Procedure

Step 1: Sample preparation and extraction

Samples of seeds were randomly drawn at the time of final harvest of the experimental crop. After collection of samples in polythene bags it was brought to the laboratory for further processing. Samples of pigeonpea seeds were ground to powder using mixer at high speed .Out of 1Kg homogenized sample, 200 g representative samples were homogenized for 2 minutes after which samples were kept in deep fridge (-21°C) for 5 minutes. Then 10 grams of each sample was taken in 50 ml centrifuge tube. To this, chilled water (5 ml), Ethyl Acetate (10 ml) and sodium sulphate (10 g) were added and the mixture was homogenized for 5 minutes at 4000 rpm using high speed homogenizer. This led to phase separation.

Blank was also prepared by taking water (5 ml), Ethyl acetate (10 ml) and Sodium sulphate (10 g). The mixture was homogenized at 4000 rpm for 5 minutes

Step 2: Sample extract cleanup

The supernatant extract (1ml) was transferred to 2ml Eppendorf tube containing 25 mg PSA (primary secondary amine). The tube was centrifuged at 4000 rpm for 5 minutes and passed through 0.2µm sized pore PTFE membrane filter. 1µl extract was injected into the GC-MS/MS for deltamethrin analysis.

Step 3: Estimation

The residues of deltamethrin were estimated using GC-MS/MS operated under the following conditions. Residues were estimated by comparison of peak area of the standards with that of the unknown or spiked samples run under identical conditions.

The samples were injected at the following GC conditions:

GC Conditions

Column	: HP-5MS, (30m x0.25mm x 0.25 micron)
Oven temp	: Oven temp:70°C hold 2.0 min -15°C/min to 160°C -3.0°C/min to 200°C hold 1.0 min -2°C/min to 230°C hold 1.0 min -8°C/min to 285°C, hold 6.0 min
Carrier Gas -He-1.2 ml/min	: He
Carrier flow rate	: 1.2 ml/min
Injection mode	: Pulsed spitless
Injection port temperature	: 120°C
Transfer line temp:	: 280°C
Detector Source	: EI Positive
Scan Type	: MRM
Sample injection volume	: 1µl

MS Conditions

Ionization mode	: ESI
Polarity	: positive

Identification and confirmation of target analytes

The identification of the pesticides was based on the retention time that was defined as per the injection made of the certified reference material. Blank sample and sample spiked at LoQ level i.e. 10ppb (0.01ppm) were injected and was compared against the spectra obtained on injection of the certified reference material.

Identification and Quantification

The pesticide was identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of calibration curve drawn from chromatographic experiments with standard solutions. The standard solutions for the calibration curve were prepared in control matrix. For quantification an external calibration curve with five different concentrations of each pesticide, with matrix match were made.

Observations and Calculations

From the chromatograms, residues of deltamethrin in pigeonpea seeds were measured and recorded. The residues of deltamethrin were compared with CODEX MRL [4]

The following formula was used to derive the residues level in test sample

$$\text{Residue } \mu\text{g/g} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Conc of standard in } \mu\text{g/ml}}{\text{Weight of sample in gm}} \times \text{Dilution Factor}$$

III. EXPERIMENTAL FINDINGS

3.1 Harvest time residues of deltamethrin in pigeonpea seed

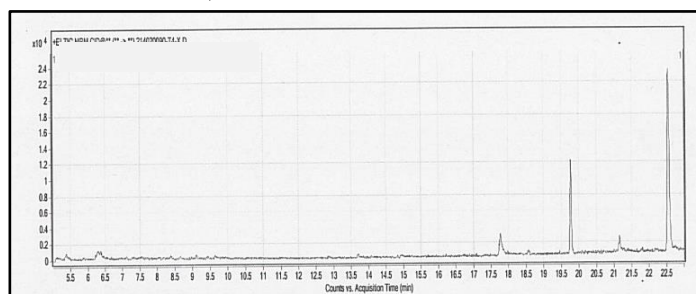
Levels of deltamethrin residues in samples of pigeonpea seeds from the field trial of kharif season 2013-14 is presented in the **table 1**. In pigeonpea seeds from the crops sprayed with 0.0014, 0.0028 and 0.0042 per cent deltamethrin, residues were found below detectable limit of 0.01 ppm.

Table 1. Residues of deltamethrin 2.8 EC in pigeonpea seeds in kharif 2013-14 experiment

Sample Component	RPL	Residue level in ppm			
		Spray concentration of Deltamethrin (% a.i)			
		0.0014	0.0028	0.0042	Control
Seed					
	i	BDL	BDL	BDL	BDL
	ii	BDL	BDL	BDL	BDL
	iii	BDL	BDL	BDL	BDL
	mean (±SD)	BDL	BDL	BDL	BDL

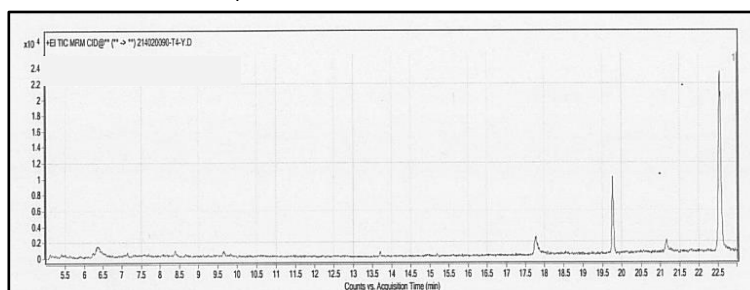
BDL = Below detectable limit 0.01 ppm
RPL =Replication

The chromatograms of residues of deltamethrin 2.8 EC obtained for pigeonpea seeds are presented below
Seed Sample – T1-X (0.0014% deltamethrin)



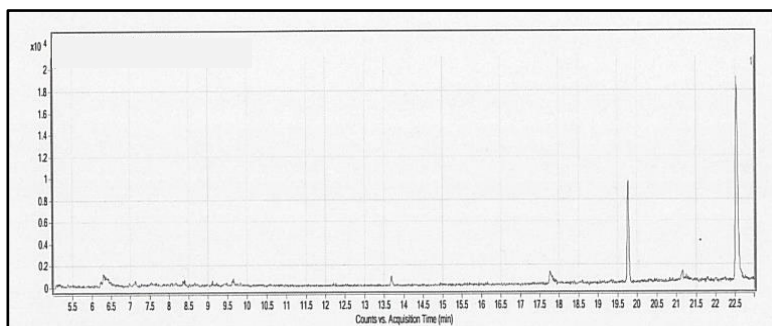
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	36	181.0 - > 152.0	0.001	mg/kg

Seed Sample – T1-Y (0.0014% deltamethrin)



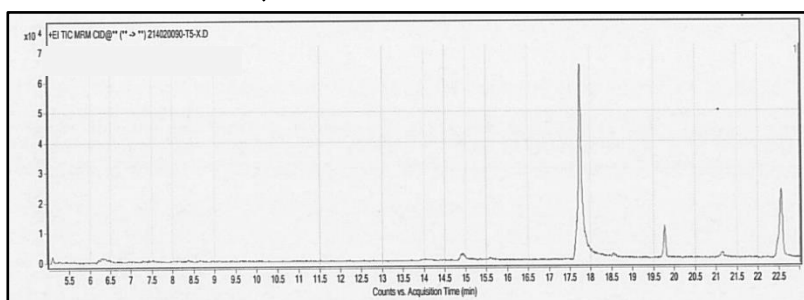
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	75	181.0 - > 152.0	0.001	mg/kg

Seed Sample – T1-Z (0.0014% deltamethrin)



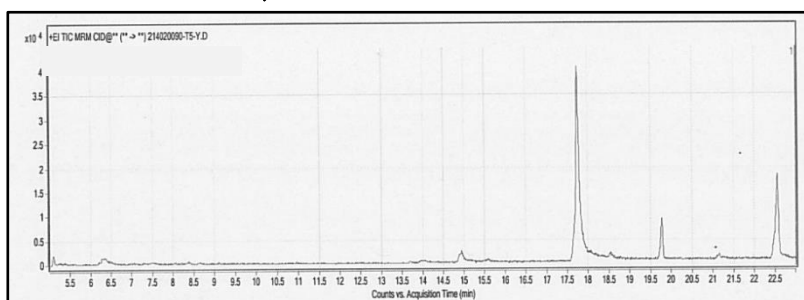
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	132	181.0 - > 152.0	0.003	mg/kg

Seed Sample – T2-X (0.0028% deltamethrin)



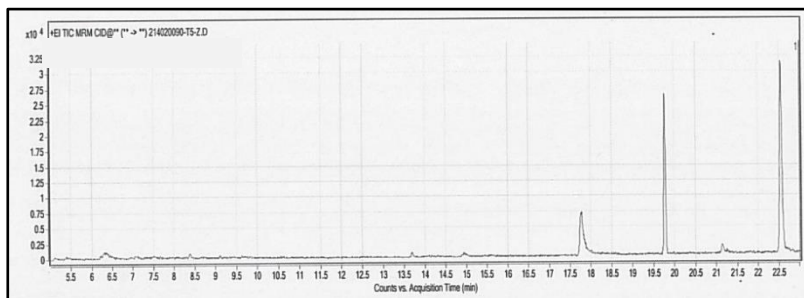
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	154	181.0 - > 152.0	0.001	mg/kg

Seed Sample – T2-Y (0.0028% deltamethrin)



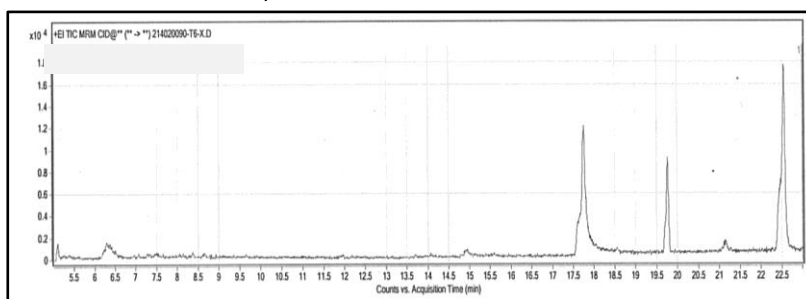
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	261	181.0 - > 152.0	0.003	mg/kg

Seed Sample – T2-Z (0.0028% deltamethrin)



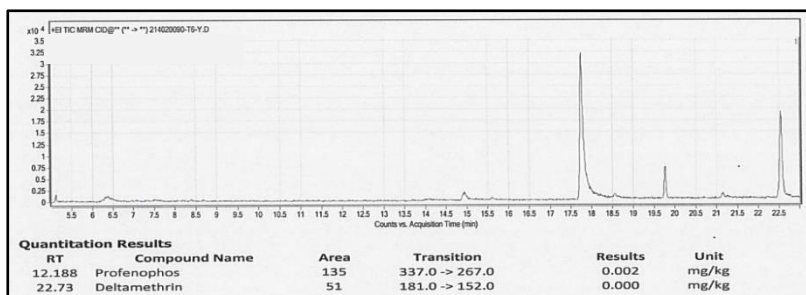
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	154	181.0 - > 152.0	0.000	mg/kg

Seed Sample – T3-X (0.0042% deltamethrin)



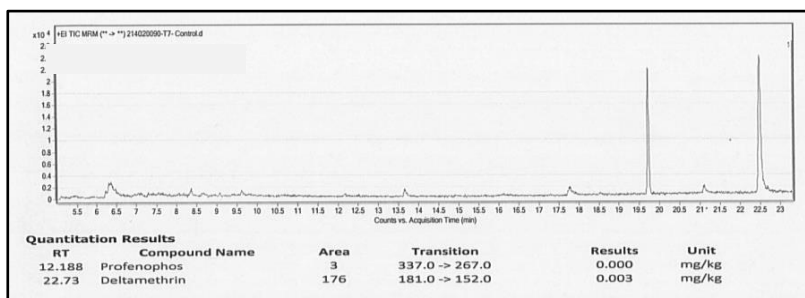
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	36	181.0 - > 152.0	0.001	mg/kg

Seed Sample – T3-Y (0.0042% deltamethrin)



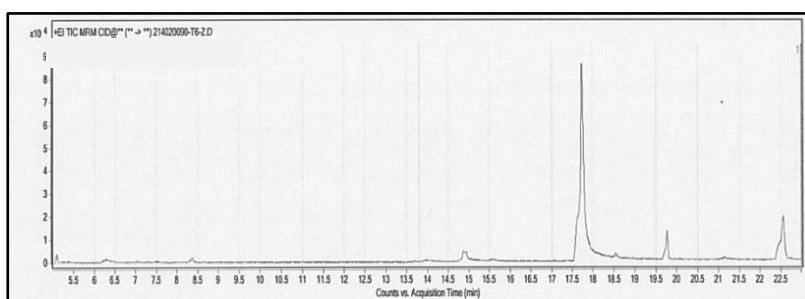
Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	51	181.0 - > 152.0	0.000	mg/kg

Seed Sample – T3-Z (0.0042% deltamethrin)



Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	176	181.0 - > 152.0	0.003	mg/kg

Seed Sample - T4-Control



Quantitation Results					
RT	Compound Name	Area	Transition	Results	Unit
22.73	deltamethrin	146	181.0 - > 152.0	0.000	mg/kg

IV. RESULTS AND DISCUSSION

The samples of seed from the treated crop collected 15 days after final spray were studied for the residues. In the present investigations residues of deltamethrin in pigeonpea seeds were found to be below detectable limit of 0.01 ppm for the spray concentration of 0.0014, 0.0028 and 0.0042 per cent. Considering the maximum residue limit of 1 mg/kg for pulses as specified by CODEX, the evaluated spray treatments of deltamethrin 2.8 EC can be considered most safe to the consumers. Similar studies on other crops with slight variations in the doses or concentration has been used by researchers. Panickar *et al.* (2005) [6] in their studies reported that deltamethrin (0.0014%) on cowpea dissipated to 0.002 $\mu\text{g g}^{-1}$ on the 10th day. Prem *et al.* (2003) [7] found that deltamethrin persisted for 7 days on leaves and fruits of tomato when applied at the rate of 0.0028 per cent. Battu *et al.* (2003) [8] observed that the residues of deltamethrin were not present in cotton seed when deltamethrin was applied as per recommended practices. Reddy *et al.* (2001) [9] reported that the residues of deltamethrin (0.004%) were found below the maximum residue limit in grains of pigeon pea at harvest. The present findings corroborate with the results of these research workers.

V. CONCLUSIONS

It is concluded that pesticide residue monitoring is effective tool to control the quantity of pesticides on food and that the determination of pesticide residues at harvest time is designed to ensure the safe consumption of food. QuEChERS method is the modern extraction technique to analyze a wide range of pesticides residual levels in food. In order to minimize the accumulation of residues in edible plant parts, it is advocated to restrict the spray concentration of deltamethrin at 0.0028 per cent active ingredient which is equal to 28 g a.i /ha using the spray fluid of 500L/ha by manually operated high volume sprayer.

VI. REFERENCES

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