DELTAMETHRIN PESTICIDE RESIDUES DETERMINATION BY GC-MS/MS EMPLOYING QuEChERS METHOD FOR EXTRACTION ON SOME CONSUMABLE PRODUCE OF PIGEONPEA CROP

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Abstract

Deltamethrin, a synthetic pyrethroid insecticide effectively limits insect pests of high-value crop pigeonpea. The development of deltamethrin has involved an iterative process of structural modification of pyrethrins which is extracted from Chrysanthemum flowers. Since deltamethrin is widely used in Vidarbha against pod borer on pigeonpea ,there is always a risk of these residues occurring in green pods of pigeonpea which may affect human health and also there is a risk of these pesticides being ingested by livestock through residues on pod covers used as fodder for animals .To ensure low level of residues on green pods the present study was conducted on the samples of green pods from the field trials conducted for studying the bioefficacy of deltamethrin 2.8 EC against pod borer on pigeonpea. QuEChERS method was used for extraction followed by analysis using GC-MS/MS. Residues in green pod samples collected from Mahurzari farm sprayed with 0.0014, 0.0028 and 0.0042 per cent deltamethrin were found to be 0.025,0.037 and 0.055 mg/kg respectively, which were below the prescribed limit.

Keywords - pesticide residues , deltamethrin, green pods , fodder, QuEChERS, GC-MS/MS

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I.INTRODUCTION

Pigeonpea (*Cajanus cajan L.*) is one of the important kharif pulse crop grown in Vidarbha region of Maharashtra. Pigeonpea pods and seed are consumed as a green vegetable and also as dried split seeds while the pod covers are used as animal fodder. Due to heavy infestation by pod borer *Helicoverpa armigera*, farmers use various insecticides belonging to different classes for the management of this pest. Indiscriminate use of pesticide, non-observance of prescribed waiting period, treatment of fruits and vegetables with persistent and non-recommended pesticides, use of sub-standard or misbranded pesticides are some of the cause of pesticide residues in/on the food. In particular, inappropriate use of pesticides results in residues in food commodities which may have adverse effects on human health (Sharma *et al*., 2021).The consumption of contaminated animal fodder by the food producing animals further leads to occurrence of their residues in animal products like milk, meat and eggs. Finally, these residues can be transferred to humans via the food chain leading to long-term human health implications (Stefanelli *et al*., 2009). In Vidarbha region of Maharashtra, deltamethrin 2.8 EC is one of the prominent and widely used insecticides belonging to class synthetic type II pyrethroid insecticide recommended @ 1ml/lit against control of *Helicoverpa armigera* pests (pest guide, 2013).

Concerning the presence of residues above maximum residue limit (MRLs) in green pods and in animal feed, the present study was conducted on the samples of crop produce from the field trials conducted for studying the bioefficacy of deltamethrin 2.8 EC against pod borer on pigeonpea during kharif season (Urkude & Kochhar ,2017).There is need for analytical methods to determine pesticide residues to ensure the safe consumption (Urkude *et al.*,2019). The pesticide residue content in green pods of pigeonpea 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 (Anastassiades *et al.*, 2003) before their instrumental analysis by GC-MS/MS.

II.ORIGIN OF DELTAMETHRIN PYRETHROID FROM NATURAL PYRETHRINS

Synthetic pyrethroids are derived from natural pyrethrins, that refers to six related insecticidal compounds found in natural pyrethrum extracted from the flowers of Chrysanthemum *cinerariaefolium*. Natural pyrethrins resulted from esterification of an acid and an alcohol. The acid moiety of the molecule is either chrysanthemic acid or pyrethric acid .The alcohol moiety is pyrethrolone, cinerolone or jasmolone. The major drawback of pyrethrum as an insecticide was its instability in light and air, which limited their effectiveness as insecticide as a result, structure of natural pyrethrins were modified to enhance their insecticidal activity, increase photostability at the same time retaining the potent and rapid insecticidal activity and low mammalian toxicity. Most synthetic pyrethroids were discovered by the sequential replacement of structural elements of the pyrethrins with novel structural moieties that were selected to conserve the molecular shape and physical properties of the template structure.

Deltamethrin was developed in 1974 and was the first pyrethroid containing the alpha cyano-3-phenoxybenzyl moiety (Elliot *et al.*, 1974) also among the first photo stable synthetic pyrethroid. Deltamethrin, the only single isomer pyrethroid of the group is around 750 times more active than natural pyrethrum. Deltamethrin kills insects through contact and stomach action or distrupts the insect's nervous system giving a quick knockdown effect .In the natural environment, deltamethrin can be degraded through several possible processes, including volatilization, photolysis, hydrolysis and biodegradation (Liu, *et al*., 2010) .WHO has classified deltamethrin as moderately hazardous (class II).

III.MATERIAL AND METHODS

3.1 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 3m x 3m with inter plot and inter replication distance of 1.2m and 1.8 m respectively (**Figure 1**).The experimental field was prepared by undertaking all necessary field operations. The tilth was prepared for sowing the crop of pigeonpea. 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.

From each plot as per plan of layout five plants from net plot were selected randomly and tagged as observational plants .After waiting period , 20 green pods were randomly collected from each observational plants. Thus total 100 green pods were collected from five observational plants from each plot. Residue study was conducted on the samples of green pods from the field trials conducted for studying the bioefficacy of deltamethrin 2.8 EC during kharif season.

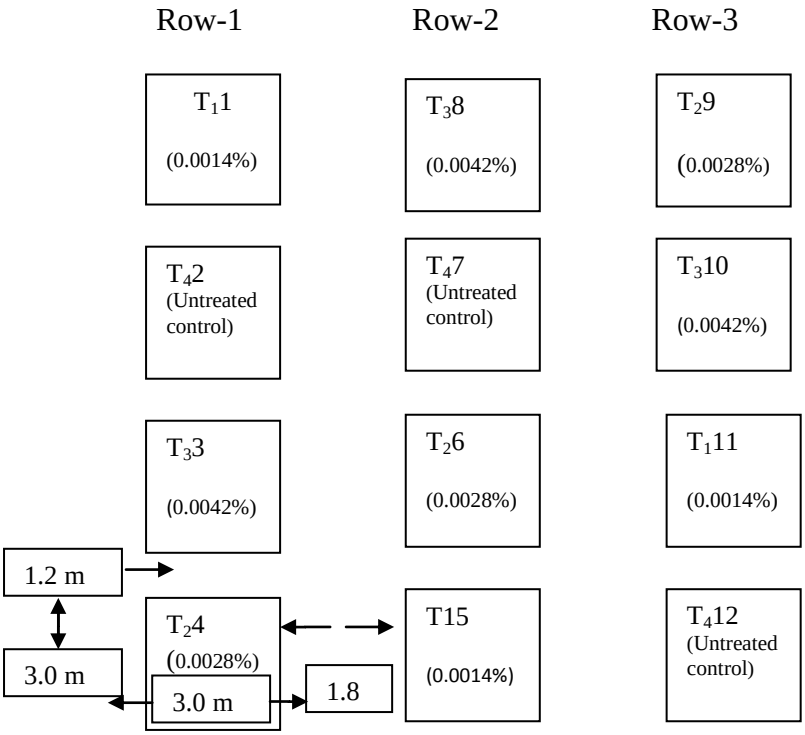


Figure 1: Plan of Layout of Pigeonpea

3.2 Residues analysis of deltamethrin in green pods

Residue analysis involved three steps, viz., extraction, clean-up and estimation.

Step 1: Sample preparation and extraction

QuEChERS method was used for extraction and clean up of deltamethrin from green pods. **(Figure 2)** shows the schematic layout of the QuEChERS procedure. Samples of green pods were ground 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 .

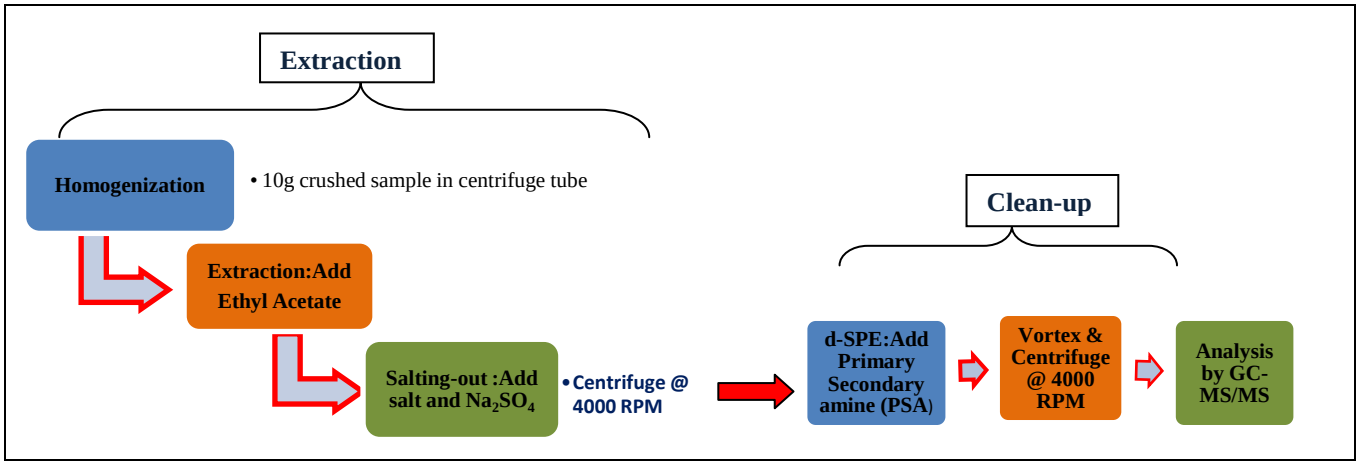


Figure 2: Scheme for QuEChERS Method

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

Preparation of certified reference material (CRM) Standard Solution:

1. Standard stock solution (1000 mg/l): 0.01 g of standard technical grade of deltamethrin was taken in 10ml calibrated volumetric flask and volume was made up to mark with ethyl acetate. The stock solution was stored in dark at 2°C-8°C.

2. Intermediate solution (10mg/l): From stock solution intermediate solution intermediate solution was prepared by dissolving 0.1 ml of stock solution in 10 ml volumetric flask and the volume was made up to mark with ethyl acetate. The solution was stored in dark at 2°C-8°C.

3. Working solution (1mg/l)): From stock solution working solution was prepared by dissolving 1 ml of stock solution in 10 ml volumetric flask and the volume was made up to mark with ethyl acetate. The solution was stored in dark at 2°C-8°C.

4. Standards for calibration (0.150 mg/l) for the GC: 0.150 mg/kg of standard was prepared by withdrawing 0.15 ml of standard working solution and the volume was made to 1 ml with n-Hexane. Respective standards (0.100, 0.050, 0.025, 0.010, 0.005 mg/l) was prepared serially by withdrawing 0.1, 0.05 ml (from 1 mg/l), 0.25,0.1 ml (from 0.1 mg/l) & 0.1 ml (from 0.05 mg/l) of standard working solution and the volume made up to 1 ml with n-Hexane.

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 GC-MS/MS analytical conditions are presented in detail in (**Table 1**).

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 pesticide, with matrix match were made.

3.3 Observations and Calculations

From the chromatograms **(Figure 3)**, residues of deltamethrin in green pods of pigeonpea from the experimental field were measured ,recorded and residues were compared with Codex MRL.

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

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

Table 1. Analytical conditions for residue analysis using GC-MS/MS.

GC Conditions		
Column		HP-5MS, (30m x0.25mm x 0.25 micron)
Oven temp		Oven temp:70^{0}C hold 2.0 min -15^{0}C/min to 160^{0}C -3.0^{0}C/min to 200^{0}C hold 1.0 min -2^{0}C/min to 230^{0}C hold 1.0 min -8^{0}C/min to 285^{0}C, hold 6.0 min
Carrier Gas		He-1.2 ml/min
Carrier flow rate		1.2 ml/min
Injection mode		Pulsed spitless
Injection port temperature		120^{0}C
Transfer line temp:		280^{0}C
Detector Source		EI Positive
Scan Type		MRM
Sample injection volume		1µl
MS Conditions		
Ionization mode		ESI
Polarity		positive

IV. EXPERIMENTAL FINDINGS

4.1 Harvest time residues of deltamethrin in green pods of pigeonpea

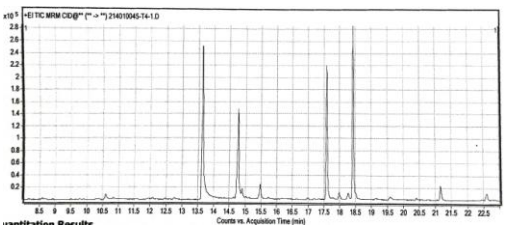
Levels of deltamethrin residues in green pod samples collected from the field trial of kharif season is presented in the (**Table 2).** Residues in green pods in the samples of crop sprayed with 0.0014, 0.0028 and 0.0042 per cent

deltamethrin were 0.025,0.037 and 0.055 mg/kg respectively, which were below the prescribed limit. The Codex maximum residue limits (MRLs) for deltamethrin residues in/on several commodities ranged from 0.01 to 5 mg/kg. (CODEX,2020).

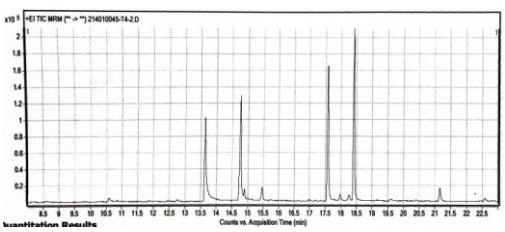
Table 2. Residues of deltamethrin 2.8 EC in green pods of pigeonpea

Sample Component	RPL	Residue level in ppm			
		Spray concentration of Deltamethrin (% a.i)			
		0.0014	0.0028	0.0042	Control
Green Pods					
	i	0.038	0.041	0.050	BDL
	ii	0.018	0.029	0.034	BDL
	iii	0.020	0.042	0.083	BDL
	mean (±SD)	0.025 (0.011)	0.037 (0.007)	0.055 (0.025)	BDL
BDL = Below detectable limit 0.01 ppm					
RPL =Replication					

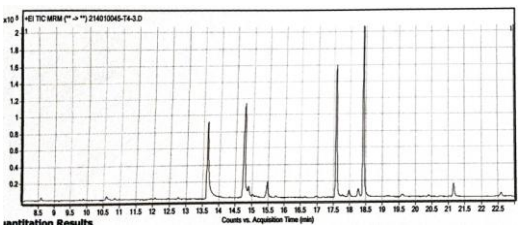
Figure 3:Chromatograms of residues of deltamethrin 2.8 EC in green pods of pigeonpea



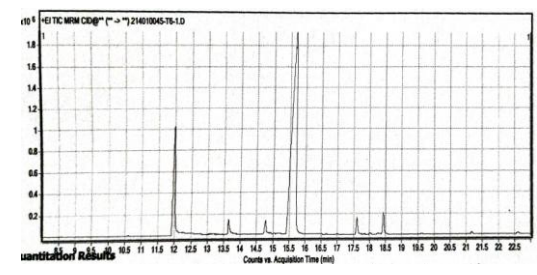
T1X (0.0014%)			
RT	Area	Transition	Results
22.598	16851	181.0 -> 152.0	0.038 mg/kg



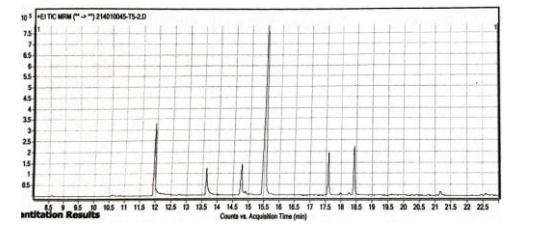
T1Y (0.0014%)			
RT	Area	Transition	Results
22.598	7367	181.0 -> 152.0	0.018 mg/kg



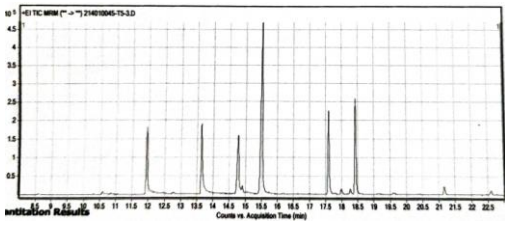
T1Z (0.0014%)			
RT	Area	Transition	Results
22.598	8497	181.0 -> 152.0	0.020 mg/kg



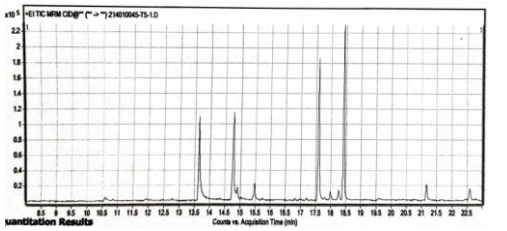
T2X (0.0028%)			
RT	Area	Transition	Results
22.598	18414	181.0 -> 152.0	0.041 mg/kg



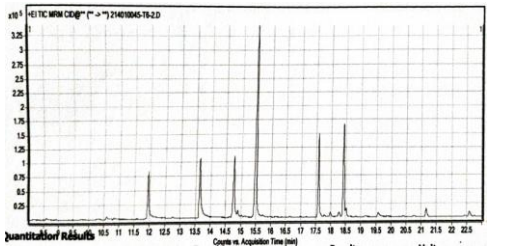
T2Y (0.0028%)			
RT	Area	Transition	Results
22.598	12673	181.0 -> 152.0	0.029 mg/kg



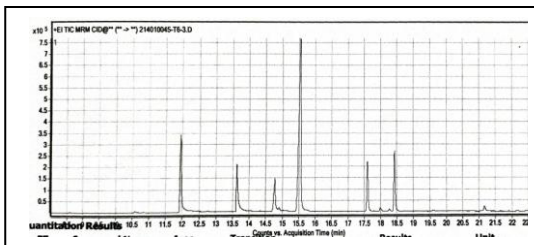
T2Z (0.0028%)			
RT	**Area**	**Transition**	**Results**
22.598	**18936**	**181.0 -> 152.0**	**0.042 mg/kg**



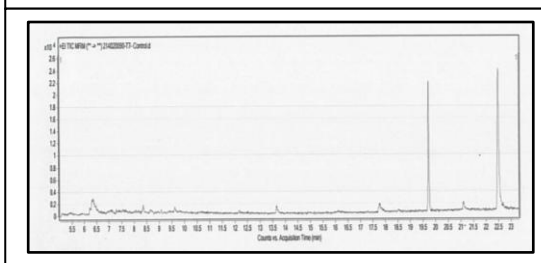
T3X (0.0042%)			
RT	**Area**	**Transition**	**Results**
22.598	**20007**	**181.0 -> 152.0**	**0.050 mg/kg**



T3Y (0.0042%)			
RT	**Area**	**Transition**	**Results**
22.598	**14965**	**181.0 -> 152.0**	**0.034 mg/kg**



T3Z (0.0042%)			
RT	**Area**	**Transition**	**Results**
22.598	**38773**	**181.0 -> 152.0**	**0.083 mg/kg**



T4 Control			
RT	**Area**	**Transition**	**Results**
22.598	**65**	**181.0 -> 152.0**	**0.000 mg/kg**

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es of g r the w thrin residues. QuEChERS method was used for extraction and clean-up while, detection and quantification of residues was undertaken with the help of GC-MS/MS. The observed values of deltamethrin residues in green pod samples sprayed with 0.0014, 0.0028 and 0.0042 per cent deltamethrin were 0.025, 0.037 and 0.055 mg/kg respectively, which were below the prescribed limit. From the above studies it was also found that deltamethrin did not persist in/on pigeonpea crop for longer time therefore there is no risk in serving green pod covers of pigeon pea to animals. keeping in view the level of residues found due to treatments of deltamethrin 2.8 EC the evaluated spray treatments of deltamethrin can be considered safe to the consumers.

These results are corroborating with the findings of researchers, though there are slight variations in the doses or concentration used by them. (EL Bahgy *et al.*, 2018) reported that deltamethrin could be not detected from the 7th day in feed after its application.(Kaur *et al*.,2015) in their studies on 106 samples of fodder revealed the occurrence of deltamethrin residues in 62% of the total fodder samples however residues were within limits after waiting period.(Panickar *et al*., 2005) observed that the initial deposits of deltamethrin (0.0014%) in/on cowpea pods was 0.035 µg g^{-1}, which gradually dissipated to 0.002 µg g^{-1}on the 10th day. (Srivastava *et al*., 2002) reported that residues of deltamethrin at 0.005 per cent resulted in the mean accumulation of 2.21 ppm on pods of chickpea which were reduced to 93.2 per cent within 7 days. (Reddy *et al*., 2001) in studies reported that the residues of deltamethrin (0.004%) were found below the maximum residue limit in grains and pod covers of pigeon pea at harvest in pigeonpea.

VI.CONCLUSIONS

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. Farmers should be properly educated for judicious use of pesticides with appropriate concentration of pesticide in the spray suspension.

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