

DETERMINATION OF PROFENOFOS PESTICIDE RESIDUES IN/ON GREEN PODS OF PIGEONPEA USING GC-MS/MS FOLLOWING DISPERSIVE SPE-QuEChERS METHOD

Sonika Kochhar, Rashmi Urkude and Varsha Dhurvey

ABSTRACT

In the Vidarbha region of Maharashtra, profenofos, a broad-spectrum pesticide from the organophosphate group, is extensively used to combat pod borers on pigeonpea. Due to the widespread use of pigeonpea as food, feed, fodder, forage and medicinal purposes, pesticide residue originating from overuse of pesticides may pose a risk to food quality, environment and human health. Additionally, there is a chance that livestock will consume these pesticides through residues on pod covers used as animal fodder. The current investigation was carried out on samples of green pods from field trials at Mahurzari, Nagpur for studying the bioefficacy of profenofos 50 EC against pod borer *H. armigera* on pigeonpea during *kharif* season of 2019 with the aim of ensuring minimal levels of residues in/on green pods. In this context a novel extraction technique, QuEChERS method was used followed by analysis using GC-MS/MS. QuEChERS technique was the preferred tool for extraction and clean-up of residues because of its adaptability, efficiency and simplicity.

Residues in green pod samples collected from Mahurzari farm sprayed with 0.1, 0.125 and 0.15 per cent profenofos were found to be 0.204, 0.325 and 0.45 mg kg⁻¹ respectively, which were below the prescribed maximum residue limit (MRL). Therefore the evaluated spray treatments of profenofos can be considered safe to the consumers, environment and will not pose any adverse effect on exports to other countries from India.

(Key words: Pesticide residues, profenofos, green pods, fodder, QuEChERS, GC-MS/MS)

INTRODUCTION

One of the major *kharif* pulse crops farmed in Maharashtra's Vidarbha region is pigeonpea. Dried split seeds and green pods from pigeonpea plants are eaten as a vegetable. Since pigeonpea contains polyphenols and flavonoides, it has therapeutic benefits in addition to its nutritional worth. In China, India and certain other countries, it is an essential component of traditional folk medicine Saxena *et al.* (2010). 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 causes of pesticide residues in/on the food. Specifically, inappropriate pesticide use leaves residues in food products that can cause a range of harmful health effects, such as neurological disorders, nervous system disorders, reproduction issues caused by hormone mimicking, and possible carcinogenicity, in addition to concerns about

environmental contamination from pesticide use Sharma *et al.* (2021) and Qin *et al.* (2017). Contaminated animal feed is eventually consumed by food-producing animals, which increases the likelihood of its residues appearing in animal products including milk, meat, and eggs. Lastly, these residues have the potential to enter the food chain and affect human health over the long run Stefanelli *et al.* (2009). Profenofos 50 EC, a broad-spectrum insecticide belonging to organophosphate group is widely used in Vidarbha region of Maharashtra against pod borer on pigeonpea. Profenofos 50 EC @ 2.5 ml l⁻¹ is recommended against control of *Helicoverpa armigera* pests (Anonymous, 2013). Profenofos has been classified as a moderately hazardous (class II) pesticide by the World Health Organization. The acute toxic action of profenofos is the inhibition of the acetylcholine esterase (AChE) enzymes in the insect nervous system.

In order to investigate the bioefficacy of profenofos 50 EC against pod borer on pigeonpea, samples of crop produce from field trials were used in this studies and special interest was to detect any presence of residues above maximum residue limits (MRLs) in/on green pods. MRL's are intended primarily as a check that the pesticide was being used correctly through good agricultural practices (Modak *et al.*, 2023).

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There is need for analytical methods to determine pesticide residues to ensure the safe consumption Urkude *et al.*, 2019). Profenofos was detected in the pesticide residual concentration of green pigeonpea pods. Prior to their instrumental examination by GC-MS/MS, pesticide residues were removed and cleaned up using QuEChERS, that was the Quick, Easy, Cheap, Effective, Rugged, and Safe approach developed by Anastassiades *et al.* (2003).

MATERIALS AND METHODS

Field operations for raising the crop

The field experiment was carried out in the Nagpur area during the *kharif* season of 2019. Three concentrations (0.1, 0.125 and 0.150) in addition to a control were tested. Every treatment plot measured 3 meters by 3 meters and same was repeated three times. The importance of time of sowing and spacing was mentioned by Ram *et al.* (2023), Himadri *et al.* (2022) and Kaur and Kumar (2022). The experimental field was prepared by undertaking all necessary field operations. The tilling was prepared for sowing the crop of pigeonpea. Profenofos 50 EC was evaluated against *H. armigera* pod borer on pigeonpea at the concentration of 0.1, 0.125 and 0.150 per cent with one control. Total two sprays were applied, one at the 50 per cent flowering stage of the crop and the second 15 days thereafter. Five plants from the net plot were randomly chosen in accordance with the layout plan and tagged as an observational plant. Observing the waiting period, from five plants in each plot, total of 100 green pods were randomly collected for observation of bioefficacy and same green pod samples from this experimental field were subjected to residue studies.

Residue analysis of profenofos in/on green pods of pigeonpea

Residue analysis involved two steps, viz., sample preparation (extraction and clean-up) and estimation.

Step 1: Sample preparation

A novel extraction technique, QuEChERS method was used for extraction and cleanup of profenofos from green pods followed by analysis using GC-MS/MS.

a) Extraction

QuEChERS extraction technique first coined by Anastassiades *et al.* (2003) applied for initial partitioning followed by an extract clean-up using dispersive solid-phase extraction (d-SPE). Figure 1 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 sample 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.

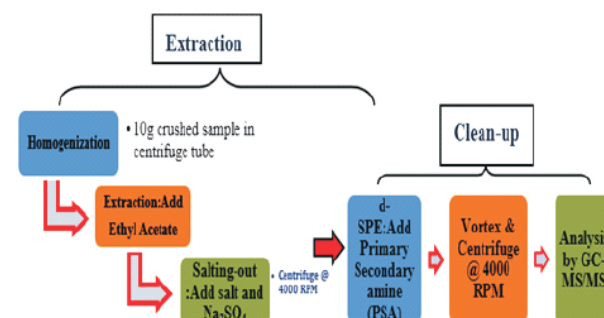


Figure 1. Scheme for QuEChERS Method

b) Sample extract cleanup

To reduce any interference, sample clean-up is done wherein the supernatant extract (1 ml) 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 0.2 µm sized pore PTFE membrane filter. 1µl extract was injected into the GC-MS/MS for profenofos analysis.

Step 2: Estimation

The analysis of profenofos residues in/on green pods of pigeonpea were determined using GC-MS/MS (Anonymous, 2007). The residues of profenofos 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 samples run under identical conditions. The data regarding GC-MS/MS analytical conditions are presented in detail in Table 1.

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°C hold 2.0 min 15°C min ⁻¹ to 160°C 3.0°C min ⁻¹ to 200°C hold 1.0 min 2°C min ⁻¹ to 230°C hold 1.0 min 8°C min ⁻¹ to 285°C hold 6.0 min
Carrier Gas	He 1.2 ml min ⁻¹
Carrier flow rate	1.2 ml min ⁻¹
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

Observations and calculations

From the chromatograms (Figure 2), residues of profenofos 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

$$\text{Residue } \frac{\mu\text{g}}{\text{g}} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Conc. of standard in } \mu\text{g ml}^{-1}}{\text{Weight of sample in g}} \times \text{Dilution Factor}$$

RESULTS AND DISCUSSION

In the present studies, a novel QuEChERS method was used for extraction and clean-up while estimation of residues was undertaken with the help of GC-MS/MS. QuEChERS method has a number of benefits over conventional extraction techniques. QuEChERS method requires low sample and solvent volumes, less time for sample preparation, has higher recovery rate, it is also more user-friendly and adheres to green chemistry principles. Hence, for extraction and cleanup of pesticide residue, the QuEChERS technique is the preferred tool because of its adaptability, efficiency and simplicity. Same QuEChERS method of extraction and clean-up of pesticide residues in grapes was reported by Banerjee *et al.* (2012) followed by analysis using GC-MS/MS.

In this studies residue of profenofos in green pods of pigeonpea were estimated. From the chromatograms (Figure 2), residues in green pods sprayed with 0.1, 0.125 and 0.15 per cent profenofos were found to be 0.204, 0.325 and 0.45 mg kg⁻¹ respectively, which were below the Maximum Residue Limit. The Codex Maximum Residue Limits (MRLs) for profenofos residues in/on several commodities ranged from 0.01 to 2 mg l⁻¹ (Anonymous, 2020). Data regarding the levels of profenofos residues in green pod samples collected from the field trial of *kharif* season are presented in the Table 2.

These results are corroborating with the findings of researchers, though there are slight variations in the doses or concentration used by them. Reddy *et al.* (2007) studied the dissipation of profenofos at 0.1% a.i. ha⁻¹ on chillies and recorded 0.36 mg kg⁻¹ initial deposits of profenofos which dissipated to 0.02 mg kg⁻¹ by 30 days amounting to the loss of 92.4 %. These levels were reduced to below detection limit after 15 days of application. Jain (2002) in his studies on assessment of residues of profenofos in/on pigeonpea

pods observed the deposits in the range of 3.0834-3.2865 and 5.0152 - 5.1787 ppm, these deposits dissipated by almost 100 per cent loss on 11th and 13th days after spraying the pigeonpea crop with profenofos active ingredient at the rate of 1000 and 2000 ml ha⁻¹, respectively.

Present studies also focussed on impact of pesticide residues in international trade. Keeping in view the level of residues found due to treatments of 0.1, 0.125 and 0.15 per cent for profenofos 50 EC, the evaluated spray treatments of profenofos can be considered safe to the consumers. From the above studies it was also found that profenofos did not persist in/on pigeonpea crop for longer time, therefore there is no risk in serving green pod covers of pigeonpea to animals.

Studies have shown that a number of Indian consignments face rejection in international trade because of MRL exceeding in food products. Rejection often leads to loss of income for exporters. It is also reported by Goyal *et al.* (2017) that animal feed and chemicals present in the feed above MRLs provided to cattle act as barrier to diary exports. Since the residue problem in food products is mainly due to indiscriminate use of pesticides, the spray concentration 0.1, 0.125 and 0.15 per cent profenofos will not pose any adverse economic impact on exports from India.

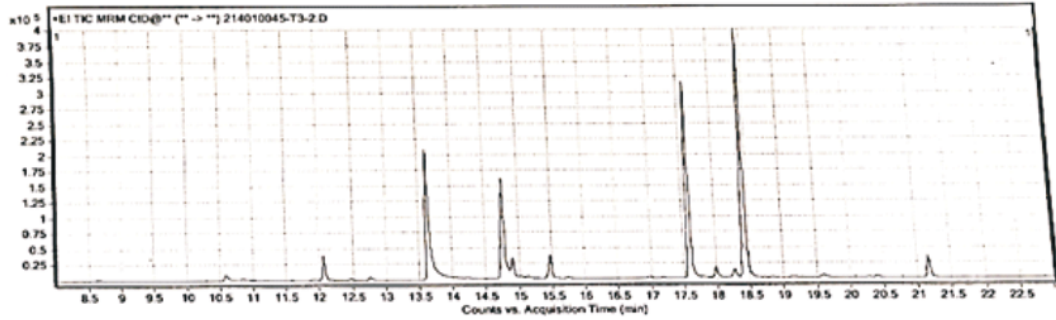
Indiscriminate use of pesticides has been linked with adverse effects on human health and environment reported by Jabir *et al.* (2014). In the present studies profenofos residues in green pods were found to be 0.204, 0.325 and 0.45 mg kg⁻¹ respectively, which were below the Codex MRLs indicating that profenofos pesticide with appropriate concentrations was used judiciously as good agricultural practice and not likely to have adverse effect on human health and environment.

Table 2. Residues of profenofos 50 EC in green pods of pigeonpea

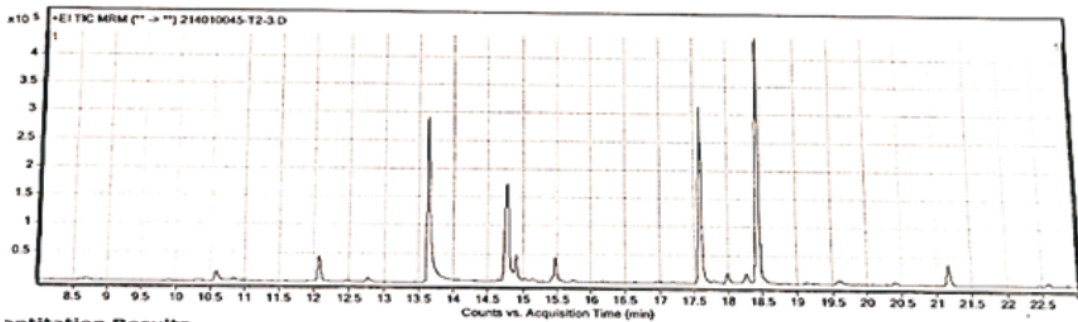
Sample component	RPL	Residue level in ppm			
		Spray concentration of profenofos (% a.i.)			
		0.100	0.125	0.150	Control
Green pods	i	0.226	0.312	0.466	BDL
	ii	0.206	0.326	0.463	BDL
	iii	0.179	0.336	0.421	BDL
	mean	0.204	0.325	0.45	BDL
	(±SD)	(0.0236)	(0.0121)	(0.0251)	

BDL = Below detectable limit 0.01 ppm

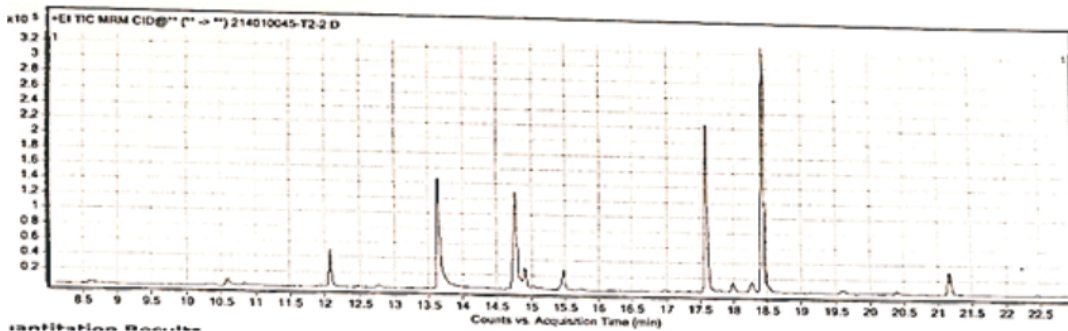
RPL = Replication

**T1X (0.1%)**

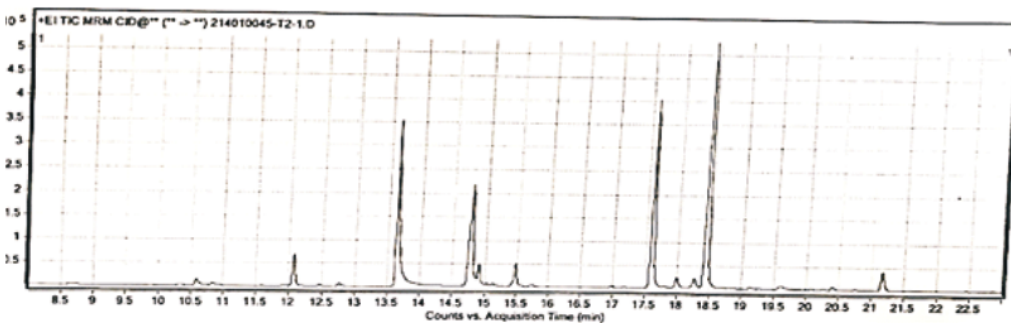
RT	Area	Transition	Results
12.076	88672	337.0- >267.0	0.226 mg kg ⁻¹

**T1Y (0.1%)**

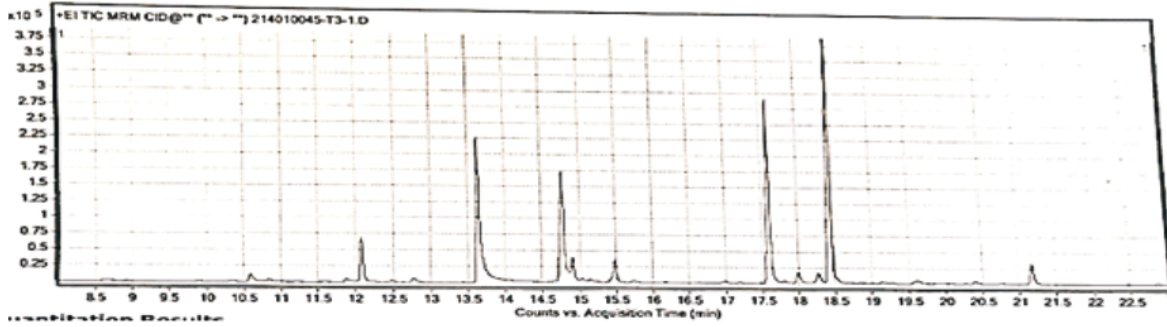
RT	Area	Transition	Results
12.076	80928	337.0- >267.0-	0.206 mg kg ⁻¹

**T1Z (0.1%)**

RT	Area	Transition	Results
12.076	70041	337.0- >267.0	0.179 mg kg ⁻¹

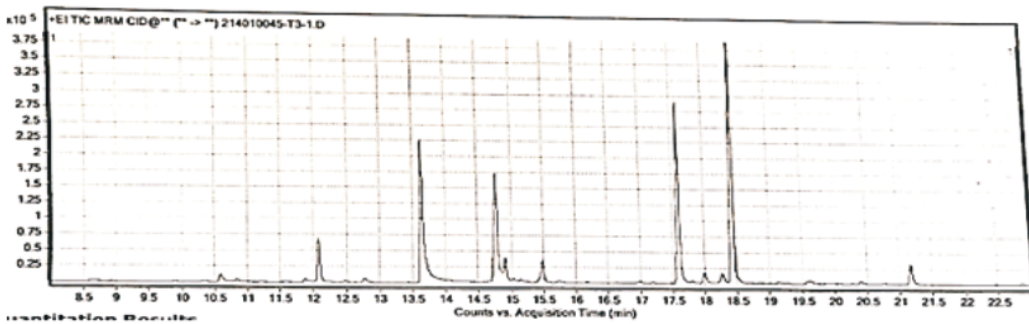
**T2X (0.125 %)**

RT	Area	Transition	Results
12.076	123285	337.0- >267.0	0.312 mg kg ⁻¹



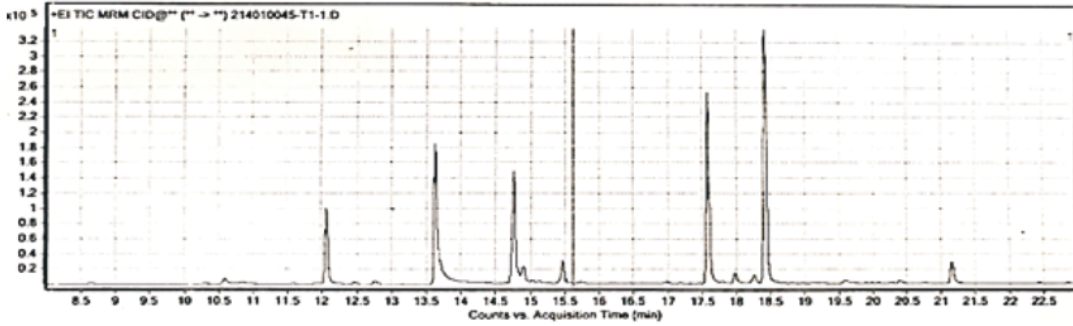
T2Y (0.125 %)

RT	Area	Transition	Results
12.076	128651	337.0- >267.0	0.326 mg kg ⁻¹



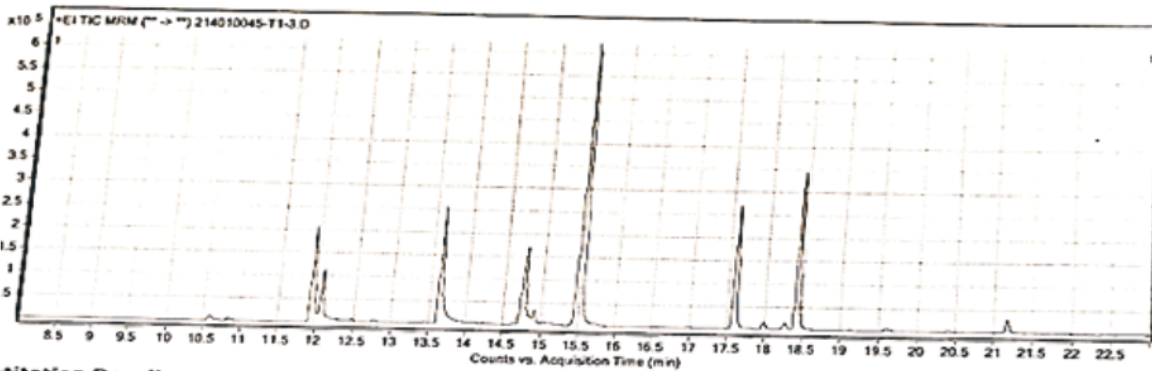
T2Z (0.125 %)

RT	Area	Transition	Results
12.076	132741	337.0- >267.0	0.336 mg kg ⁻¹



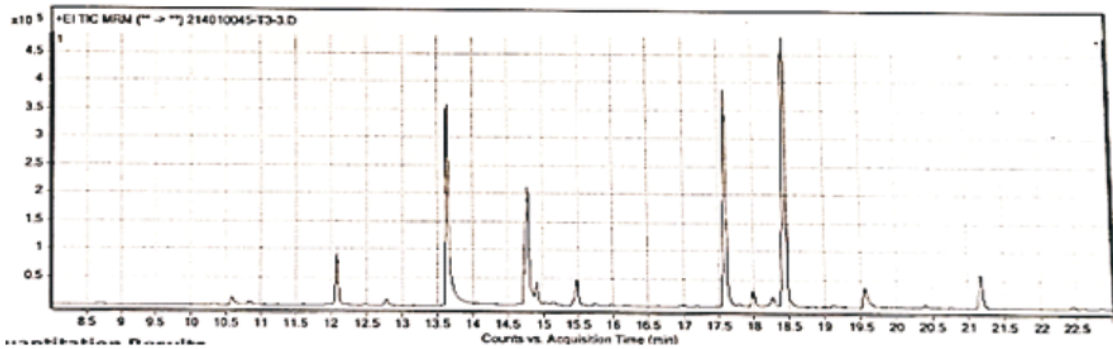
T3X (0.150 %)

RT	Area	Transition	Results
12.076	184781	337.0- >267.0	0.466 mg kg ⁻¹



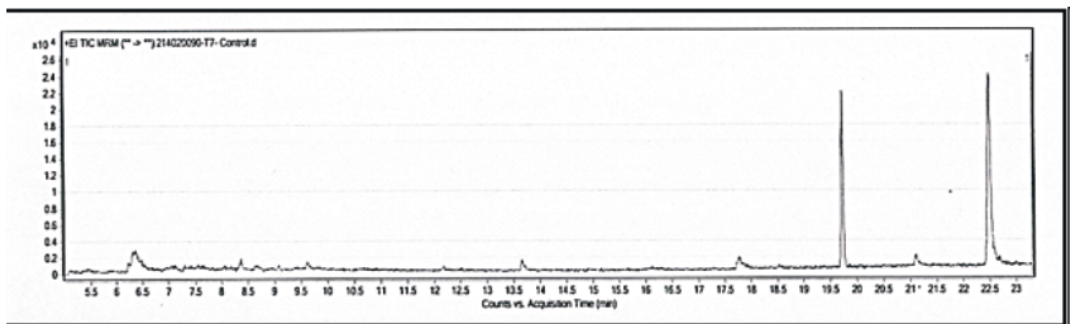
T3Y (0.150 %)

RT	Area	Transition	Results
12.076	183581	337.0- >267.0	0.463 mg kg ⁻¹



T3Z (0.150 %)

RT	Area	Transition	Results
12.076	166746	337.0- >267.0	0.421 mg kg ⁻¹



T4 Control

RT	Area	Transition	Results
12.076	65	337.0- >267.0	0.000 mg kg ⁻¹

Figure 2. Chromatograms of residues of profenofos 50 EC in green pods of pigeonpea

In present studies the observed values of profenofos 50 EC residues in green pod samples sprayed with 0.1, 0.125 and 0.150 per cent profenofos were found to be 0.204, 0.325 and 0.45 mg kg⁻¹ respectively, which were below the prescribed limit. So it is advocated to restrict the spray concentration of profenofos at 0.125 per cent to minimize the accumulation of residues in edible plant parts. From the above studies it was also found that profenofos did not persist in/on pigeonpea crop for longer time, therefore there is no risk in serving green pod covers of pigeonpea to animals. For extraction of pesticides, QuEChERS method, a novel analytical approach should be selected as, this method requires low sample and solvent volume, less time for sample preparation, higher recovery rate, user-friendly and in agreement of green chemistry principles. The waiting period should be respected. There is every reason to properly educate farmers for judicious use of pesticides with appropriate concentration of pesticide in the spray suspension.

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