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Residues of pendimethalin, oxyfluorfen, quizalofop-ethyl and imazethapyr in groundnut and their persistence in soil

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ABSTRACT

A field experiment was conducted at Junagadh Agricultural University, Junagadh on medium black calcareous soil during kharif seasons of 2013 and 2014 to determine the residues of pendimethalin, oxyfluorfen, quizalofop-ethyl and imazethapyr herbicides in the soil and groundnut plant. The Analysis of QuEChERS procedure using GC-MS and LC-MS/MS analytical methods with average recoveries ranged from 85.6 to 95.5 per cent at three spiking levels (0.10, 0.25 and 0.50 µg g^{-1}) revealed that the residue of pendimethalin, oxyfluorfen and quizalofop-ethyl persisted both in soil and plant upto 30 days after harvest of the crop. Afterwards, the residues were detected below the detection limits (BDL). Persistence of imazethapyr residues in soil were detected below the limit of quantification however, in plant it persists to the levels of 0.0016, 0.0015 and 0.015 µg g^{-1} at 30, 60 DAS and at harvest of groundnut, respectively, which were higher than the lowest detectable limit (0.01 µg g^{-1}). Residues of all four herbicides in kernel were detected far below the tolerance limits approved by Food Safety and Standards Authority of India and European Union standards and hence, risks associated with dietary exposure of these herbicides is considered safe for human consumption point of view. Being short duration nature of persistence in soil and plant, these herbicides were also safe for the environment as well as for rotational crops. However, a pre-harvest interval (PHI) between last application and harvest for these herbicides should be 40 to 90 days.

***Keywords*:** Groundnut, imazethapyr, oxyfluorfen, pendimethalin, persistence, quizalofop-ethyl, residues

Among all the chemicals used as herbicides for the control of weeds has become imminent especially in the irrigated and assured rainfed agriculture for a wide variety of reasons like non availability of working force, high labour cost, high choice of application of herbicides, quick weed control and unfavorable climatic conditions for timely cultural weeding operations *etc*. Pre-emergence herbicides *viz*., pendimethalin (Kumar *et al.*, 2013) and oxyfluorfen (Ramalingam *et al.*, 2013) and post-emergence herbicides *viz*., imazethapyr (Kalhapure, 2013) and quizalofop-ethyl (Samant *et al.*, 2014) are recommended in groundnut for controlling weeds, higher crop yield and elevated income in different parts of the country. After application of these herbicides to soil, undergoes decomposition and a part may be taken by plants accumulating in the edible parts, which are found to be toxic in nature. The residual activity of herbicides depends upon the soil type, soil moisture and temperature (Dharumarajan *et al.*, 2008). Application of recommended dose of herbicides may not pose serious problem for environmental pollution (Adachi *et al.*, 2007). However, when used with repeated and higher dosage of herbicides, some unintended negative impacts are persistence in soil, pollution of ground water, toxic residues in food (contamination), feed and fodder and adverse effect on non-target organisms may be exists.

In order to ensure consumer safety, the USA has set up MRL of pendimethalin and imazethapyr as 0.1 and 0.1 mg kg^{-1}, respectively in peanut kernel (FAS International MRL Database 2013). In European Union (EU), MRL has also been established for pendimethalin (0.1 mg kg^{-1}) and oxyfluorfen and quizalofop-*p*-ethyl (0.1 mg kg^{-1}) in peanut kernel (EU Database on MRLs 2013). In India, the MRL for oxyfluorfen (0.05 mg kg^{-1}) and imazethapyr (0.1 mg kg^{-1}) has been established only in peanut oil (Food Safety and Standards Authority of India, 2011). Peanut is extensively used as a cooking medium, especially in central and western India and the raw peanut consumption has recently increased due to attention as a functional food for good health. Taking note of the points highlighted above a field experiment was conducted to determine the persistence of herbicides residue in the soil and groundnut.

MATERIALS AND METHODS

A field experiment was carried out at the Junagadh Agricultural University, Junagadh (Gujarat) on medium black calcareous soil during two consecutive *kharif* seasons of 2013 and 2014. The soil was clayey in texture and slightly alkaline in reaction (pH 8.0 and EC 0.34 dS m^{-1}), low in available nitrogen (236.5 kg N ha^{-1}), while medium in available phosphorus (22.9 kg P_2O_5 ha^{-1}) and potash (241.7 kg K_2O ha^{-1}). Ten treatments comprising of weed management practices *viz*., pendimethalin 0.9 kg ha^{-1} PE *fb* IC & HW at 40-45 DAS (T_1), oxyfluorfen 0.18 kg ha^{-1}PE *fb* IC & HW at 40-45 DAS (T_2),

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quizalofop-ethyl 40 g ha^{-1}POE at 25-30 DAS *fb* IC and HW at 40-45 DAS (T_3), imazethapyr 75 g ha^{-1} POE at 20-25 DAS *fb* IC and HW at 40-45 DAS (T_4), pendimethalin 0.9 kg ha^{-1} PE *fb* quizalofop-ethyl 40 g ha^{-1} POE at 20-25 DAS (T_5), pendimethalin 0.9 kg ha^{-1} PE *fb* imazethapyr 75 g ha^{-1} POE at 25-30 DAS (T_6), oxyfluorfen 0.18 kg ha^{-1} PE *fb* quizalofop-ethyl 40 g ha^{-1} POE at 25-30 DAS (T_7), oxyfluorfen 0.18 kg ha^{-1} PE *fb* imazethapyr 75 g ha^{-1} POE at 25-30 DAS (T_8), weed free (T_9) and unweeded control (T_{10}) were evaluated in randomized block design replicated thrice. The groundnut (cv. GG-20) was grown with standard package of practices. Herbicides were applied as per treatments using manually operated knapsack sprayer fitted with flat fan nozzle using spray volume of 500 L ha^{-1}. Species wise weed density(m^{-2} area) were recorded at 30 DAS, 60 DAS and at harvest of the crop.

Samples collected for soil (at 30 DAS, 60 DAS, harvest and 30 days after harvest) and plant (at 30 DAS, 60 DAS and harvest) were analysed with the help of QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure in Gas Chromatography Mass Spectrometry (GC-MS) and for imazethapyr in Liquid Chromatography Mass Spectrometry/Tandem Mass Spectrometry (LC-MS/MS) described by Sharma (2007). The results were interpreted through accuracy and validation of the analytical methods in terms of average recoveries of all the four herbicides ranged between 85.6 to 95.5 per cent at three spiking levels of 0.10, 0.25 and 0.50 µg g^{-1} (Table 1).

Table 1: GC-MS and LC-MS/MS herbicide recoveries in soil, green plant and peanut kernel

Specifics	GC-MS						LC-MS/MS					
	Pendimethalin			Oxyfluorfen			Quizalofop-ethyl			Imazethapyr		
Soil samples												
Concentration levels (mg kg^{-1})	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50
Recovery (mg kg^{-1})	0.084	0.234	0.485	0.092	0.225	0.478	0.089	0.241	0.507	0.093	0.239	0.472
Mean % recovery		91.5			92.5			95.6			94.3	
Plant / kernel samples												
Concentration levels (mg kg^{-1})	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50
Recovery (mg kg^{-1})	0.081	0.235	0.471	0.089	0.221	0.481	0.092	0.241	0.481	0.096	0.237	0.487
Mean % recovery		89.0			85.1			94.8			96.1	

Method and level of quantification

The reference standards of pendimethalin, oxyfluorfen, quizalofop-ethyl and imazethapyr were used for quantification, recovery and determination of retention time of the herbicide.

Extraction and procedure

For extraction of herbicides from soil, the samples were analysed as per the QuEChERS procedure suggested by Anastassiades *et al.* (2003) in which the soil was first extracted with acetonitrile followed by clean-up using dispersive solid phase extraction (d-SPE) with Primary Secondary Amine (PSA). The extraction was later partitioned with solvent cyclohexane. Salts like anhydrous $MgSO_4$ or NaCl was added (Pizzutti *et al.*, 2009) in order to increase the recovery of the pesticides and analyzed with the procedure as depicted in Fig. 1 (A and B) in GC-MS/LC-MS/MS with the given column specifications and parameters in table 2.

Clean-up

The extracted plant sample supernatant was treated in a 15 mL centrifuge tube with 0.3 g (±0.1g) PSA and 0.9 (±0.1g) $MgSO_4$. Then 6 mL of mixture was taken into a centrifuge tube and vigorously vortexed for 30 seconds. Then it was centrifuged for 1minute at 3,500 rpm. Approximately 2 mL of supernatant was collected and evaporated to dryness. The dried residue was re-dissolved in 2 mL ethyl acetate and analysed for GC-MS or LC-MS/MS (Fig. 1-B). No specific clean-up procedure was adopted for soil residue analysis as the extraction procedure contains chemicals that help in clean-up steps simultaneously through shaking and centrifugation. Fats have limited solubility in acetonitrile and a certain fraction of it from matrix may got co-extracted which may interfered with the analysis of the target compounds. Hence, different clean-up strategies were adopted for residue analysis in seed using aliquots of the extract (1.0 mL upper layer of acetonitrile extract) without and with d-SPE clean-up. For d-SPE clean-up step, 1.0 mL extract was added to one tube containing 150 mg anhydrous $MgSO_4$ to remove water and effect of different sorbents like 50 mg C_{18} or 50 mg PSA or 50 mg florisil per mL of extract.

Estimation

One µL of reference standard solution and test sample were injected simultaneously. The peaks by their retention time were identified and the peak area was measured. The amount of residues of herbicide was calculated in µg g^{-1} or mg kg^{-1} with the formula shown in fig. 1 :

$$= \frac{\text{Peak area (sample)} \times \text{Conc.std. (ppm)} \times \mu\text{L std. injected} \times \text{Final volume of the sample (1 mL)}}{\text{Peak area (std.)} \times \text{weight of the sample (2 g)} \times \mu\text{L of sample injected}}$$

Take 10 g soil + 20 mL acetonitrile

↓

Shake vigrously for 1 minute

↓

Add 4 g $MgSO_4$ + 1 g NaCl

↓

Centrifuge at 3,300 rpm for 5 minutes

↓

Take 10 mL supernatant in 15 mL tube, containing 250 mg PSA and 1.5 g $MgSO_4$

↓

Sonicate for 1 minute

↓

Centrifuge for 10 minutes at 4,400 rpm

↓

Collect 4 mL supernatant

↓

Evaporate to near dryness at 40 °C

↓

Reconstitute residues with 1 mL cyclohexane & injected to GC-MS/LC-MS/MS

(A)

Chop the sample (1-2 kg) and homogenize in high volume homogenizer

↓

Weigh 15 ± 0.1 g sample into 50 mL centrifuge tube

↓

Add 30 ± 0.1 mL acetonitrile into the centrifuge tube

↓

Homogenize the sample at 15,000 rpm for3 minutes

↓

Add 3.0 g ± 0.1 g NaCl and shake well for 2 minutes

↓

Centrifuge for 3 minutes at 3,000 rpm to separate organic layer

↓

Take 15 mL of organic layer in test tube + 10 g sodium sulphate and shake well …… (i)

Dispersive solid phase clean-up

Weigh 0.3 ±0.01 g PSA sorbent and 0.9 ±0.01 g anhydrous $MgSO_4$ into 15 mL tube

↓

Transfer 6 mL organic layer from (i) to centrifuge tube, cap and vortex for 30 seconds

↓

Centrifuge for 5 minutes at 3,000 rpm

↓

↓	↓
Take 3 mL aliquot & evaporate to dryness	Take 2 mL aliquot & evaporate to dryness
↓	↓
Reconstitute with 1.5 mL n-hexane And filter for GC-MS	Reconstitute with 4 mL acetonitrile, centrifuge and filter for LC-MS/MS

(B)

Fig. 1 : QuEChERS procedures used for pesticide residue analysis in soil (A) and plant (B)

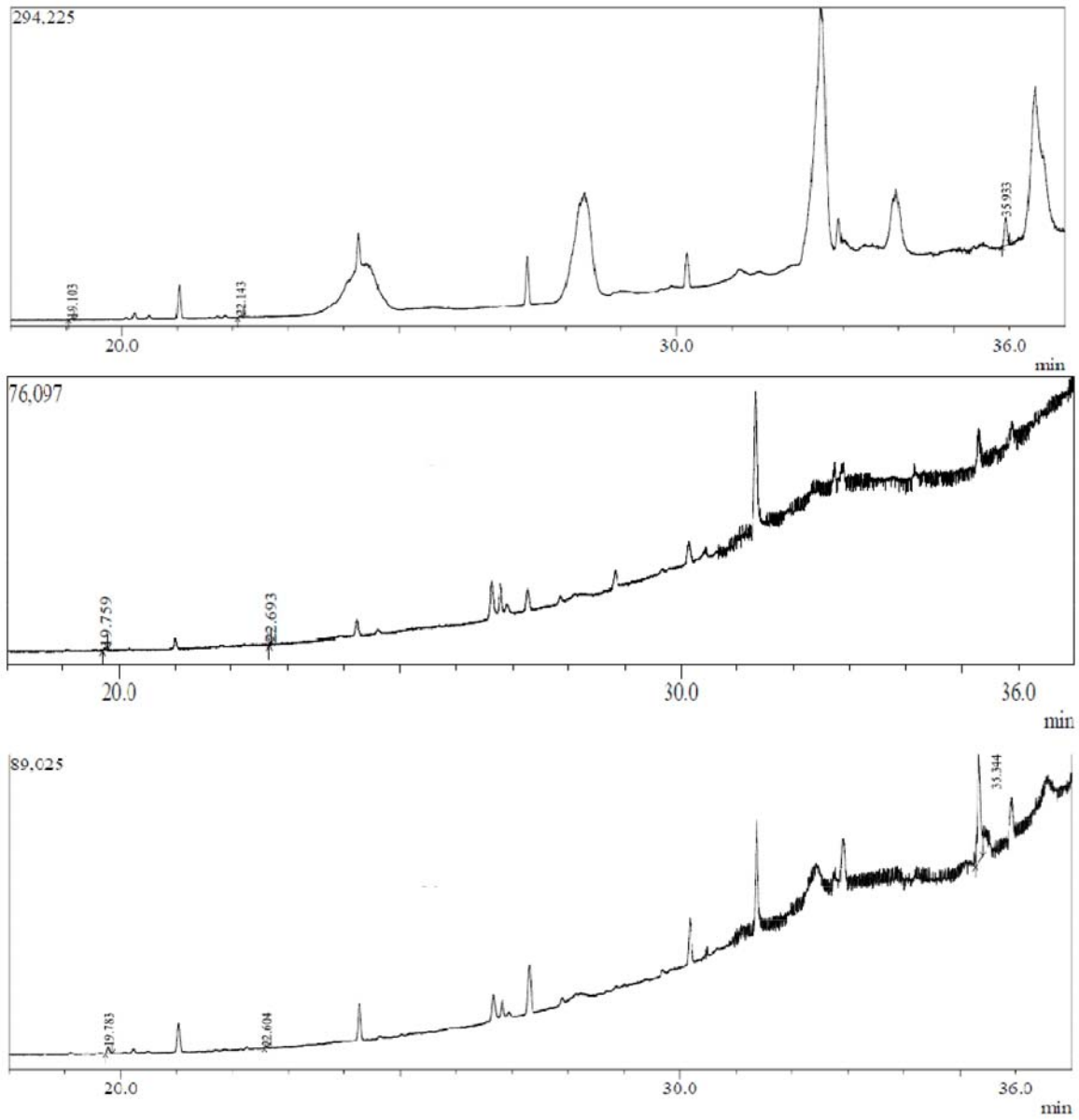

Fig. 2: Chromatogram of pendimethalin (T_1), oxyfluorfen (T_7) and quizalofop-ethyl (T_5) residues persists in soil at 30 DAS

RESULTS AND DISCUSSION

Residues in soil and plant

Under field conditions, pendimethalin residues in soil were persisted up to 30 days after harvest (DAH) of the crop but at 30 DAS, the pendimethalin residues in soil ranged from 0.03070 to 0.03291 µg g^{-1}, which was higher than the lowest detection limit (0.01 µg g^{-1}). In plant, the residues of pendimethalin persisted up to 30 DAS (Fig. 3) to the level of 0.0376 µg g^{-1}, which was above the lowest detectable limit (0.01 µg g^{-1}). Afterwards, the pendimethalin residue in soil and green plant were detected below the minimum limit of quantification. Just like pendimethalin, the residue of oxyfluorfen at 0.18 kg ha^{-1} PE applied rate persists in soil up to 30 days after harvest of the crop. The persistence values of oxyfluorfen residue in soil (Fig. 2) and plant (Fig. 3) were detected to the tune of 0.0421 µg g^{-1} and 0.0608 µg g^{-1}, respectively which were well above the lowest limit of quantification (0.015 µg g^{-1}). Afterwards, the oxyfluorfen residue in soil and green plant were detected below the lowest residue limit (Table 3).

Persistence of quizalofop-ethyl in soil (Fig. 2) and plant (Fig. 3) actively recorded above the lowest limit

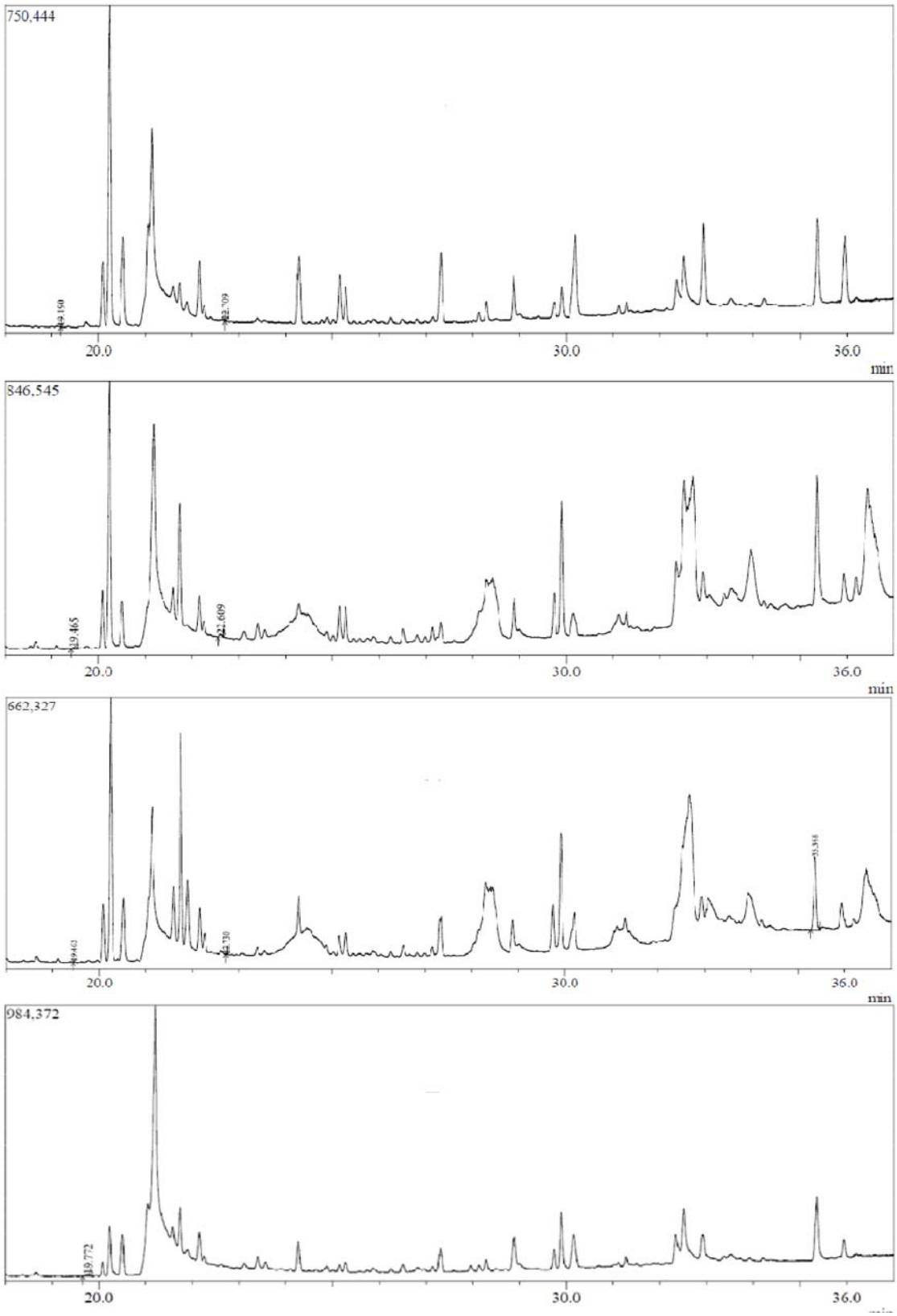

Fig. 3: Chromatogram of pendimethalin (T_1), oxyfluorfen (T_7) and quizalofop-ethyl (T_3) residues persists in green plant at 30 DAS

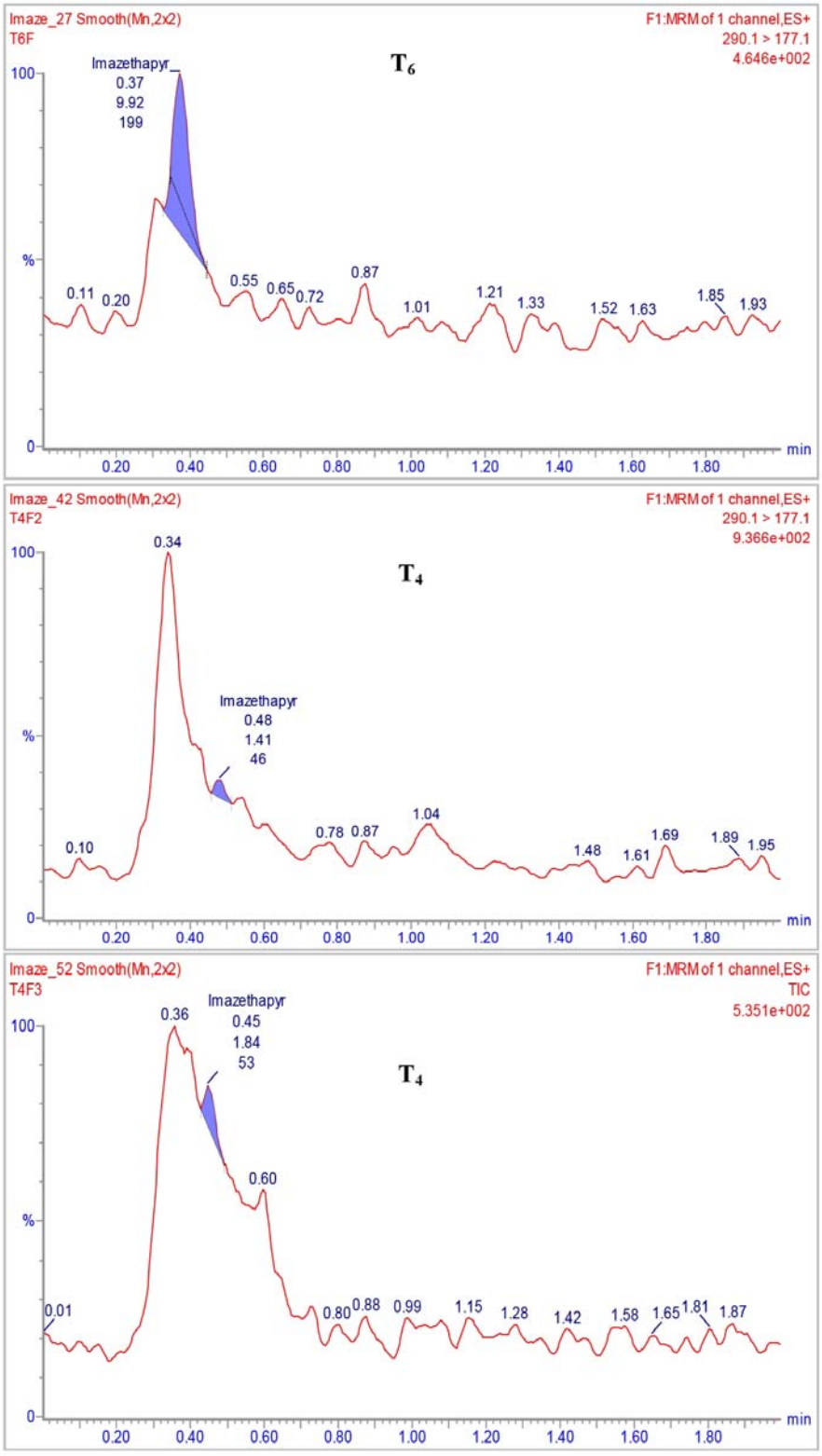

Fig. 4: Chromatogram of imazethapyr residues persists in green plant at 30 DAS (T_6), 60 DAS (T_4) and at harvest (T_4)

Table 2: Parameters and column specifications of GC-MS/LC-MS/MS analysis

Column specifications	GC-MS method	LC-MS/MS method
Detector	Mass detector	Mass detector
Liquid phase	Ethyl acetate	0.1 % Formic acid in water (30%) and Methanol (70%)
Stationary Phase	5% diphenyl 95% dimethylpolysiloxane	-
Stationary Phase film thickness	0.25 µm	-
Length	30 meter	50 mm
Diameter	0.25 mm	2.1 mm
Carrier gas (99.99% pure)	Helium gas	Nitrogen gas
Carrier gas flow rate	1.0 mL per minute	700 L per hour
Injector port temperature	280 °C	350 °C
Detector temperature	230 °C	120 °C
Retention time (Minutes)		
Pendimethalin	19.33	
Oxyfluorfen	22.77	
Quizalofop-ethyl	35.65	
Imazethapyr	0.6	

Table 3: Persistence of herbicides residues in soil, green plant and groundnut kernel

Herbicides	Lowest detectable limit (µg g^{-1})	In soil			In plant haulm^{-1}				In kernel
		30 DAS	60 DAS	At harvest	30 DAH	30 DAS	60 DAS	At harvest	
Pendimethalin 0.9 kg ha^{-1} PE	Soil or plant or kernel: 0.01	0.0307 to 0.0329 (0.0315)	BDL*	BDL	BDL	0.03195 to 0.04433 (0.0376)	BDL	BDL	BDL
Oxyfluorfen 0.18 kg ha^{-1} PE	Soil or plant or kernel: 0.015	0.0401 to 0.0438 (0.0421)	BDL	BDL	BDL	0.04321 to 0.08737 (0.0608)	BDL	BDL	BDL
Quizalofop-ethyl 40 g ha^{-1} POE	Soil or plant or kernel: 0.01	0.0941	BDL	BDL	BDL	0.10355	BDL	BDL	BDL
Imazethapyr 75 g ha^{-1} POE	Soil: 0.008Plant or kernel: 0.01	BDL	BDL	BDL	BDL	0.0015 to 0.0017 (0.0016)	0.0015 to 0.0015 (0.0015)	0.0152 to 0.0149 (0.0015)	BDL

*Note: *BDL-below detectable limit*

of quantification (0.01 µg g^{-1}) up to 30 DAS to the levels of 0.0941 µg g^{-1} and 0.1036 µg g^{-1}, respectively. Afterwards, no residues of quizalofop-ethyl was detected in soil and plant above the lowest allowable determination limit as ascribed due to its rapid degradation by soil microbes. The retention time of oxyfluorfen and quizalofop-ethyl analytes were recorded up to 22.77 and 35.65 minutes, respectively on the GC-MS column of total ion chromatogram (TIC). No persistence value of imazethapyr residue in soil was detected above the limits of quantification (0.008 µg g^{-1}). However, in green plant and haulm mean imazethapyr residue levels of 0.00158, 0.00152 and 0.0151 µg g^{-1} were persisted at 30 DAS, 60 DAS and at harvest of the crop (110-118 days crop duration), respectively (Fig. 4) which were below the lowest detectable limit of 0.01 µg g^{-1}. However, due to its continuous persistence in the soil up to 30 DAH of the crop (although at BDL), there may be a chance of bioaccumulation in groundnut haulm through more plant uptake. Hence, may cause a threat to the dairy animals which feed peanut haulm as a fodder. However, the retention time of imazethapyr analyte was noted up to 0.6 minutes on the LC-MS/MS chromatogram. The results of present investigation are strongly supported with the findings of Babu *et al.* (2015) in groundnut and Sondhia (2015) in soybean.

Table 4: Effect of different herbicides on soil microbial population at harvest

Treatments	Microbial count at harvest (10^7 cfu g^{-1} soil)		
	2013	**2014**	**Pooled**
Pendimethalin 0.9 kg ha^{-1} PE *fb* IC and HW at 40-45 DAS	115.8	102.6	109.2
Oxyfluorfen 0.18 kg ha^{-1} PE *fb* IC and HW at 40-45 DAS	111.4	103.2	107.3
Quizalofop-ethyl 40 g ha^{-1} POE at 25-30 DAS *fb* IC & HW at 40-45 DAS	112.9	101.9	107.5
Imazethapyr 75 g ha^{-1} POE at 20-25 DAS *fb* IC and HW at 40-45 DAS	109.6	105.1	107.4
Pendimethalin 0.9 kg ha^{-1} PE *fb* quizalofop-ethyl 40 g ha^{-1} POE at 20-25 DAS	116.2	95.8	106.0
Pendimethalin 0.9 kg ha^{-1} PE *fb* imazethapyr 75 g ha^{-1} POE at 25-30 DAS	110.1	103.0	106.6
Oxyfluorfen 0.18 kg ha^{-1} PE *fb* quizalofop-ethyl 40 g ha^{-1} POE at 25-30 DAS	111.6	107.8	109.7
Oxyfluorfen 0.18 kg ha^{-1} PE *fb* imazethapyr 75 g ha^{-1} POE at 25-30 DAS	111.4	105.3	108.4
Weed free	126.0	115.6	120.8
Unweeded control	122.2	108.1	115.1
SEm (±)	**6.63**	**5.55**	**4.32**
LSD (0.05)	**NS**	**NS**	**NS**
C.V. %	**10.0**	**9.2**	**9.6**
Initial	**132.6**	**108.5**	**120.5**

Residues in peanut kernel

The results (Table 3) narrated that risk associated with dietary exposure of pendimethalin, oxyfluorfen, quizalofop-ethyl and imazethapyr herbicides in peanut kernel or oil was considered safe to the human being seeing that the residue levels of all herbicides did not exceed the tolerance limits prescribed by Food Safety and Standards Authority of India or European Union standards. Being less persistence nature in soil and plant, these herbicides were also safe for the environment and rotational crops. The herbicides tested under this field trial of peanut are already included in the list of "Registered Products under section 9(3) and Major Use of Pesticides under the Insecticide Act, 1968 for use in the country" (http://cibrc.nic.in/reg_products.doc and http://cibrc.nic.in/mup.htm). It indicated that the waiting period or pre-harvest interval (PHI) between last application and harvest for these herbicides should be 40 to 90 days as prescribed for various crops (Anon. 2016).

Effect on soil microbes

Application of either pre- or post-emergence herbicides to groundnut field did not cause significant influence on total microbial population at harvest (Table-4). However, numerically, the mean higher microbial population (120.8×10^7 cfu g^{-1}) was observed in the weed free, which was near to their initial status (120.5×10^7 cfu g^{-1}).

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