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Terminal residues of propaquizafop in cotton seed, lint and soil

ANIL DUHAN* AND SAMUNDER SINGH

Agrochemicals Residues Testing Laboratory, Department of Agronomy, CCS Haryana Agricultural University, Hisar- 125 004

**Email : a.duhan@rediffmail.com*

ABSTRACT: A supervised field trial was conducted in order to assess the terminal residues of herbicide propaquizafop in cotton lint, seed and soil. Propaquizafop formulation 10 EC was applied @ 62.5 and 125 g/ha on 45 days after sowing (DAS). Seed, lint and soil samples were collected at harvest time and processed for residues extraction. After clean up, the residues were estimated using gas chromatography tandem mass spectrometry (GC-MS/MS) by developing a new multiple reaction monitoring programme (MRM). Method validation was achieved by performing recovery experiments at two fortification levels of 0.003 and 0.005 ìg/g. The average recoveries obtained from cotton lint, seed and soil samples were above 85 per cent. The limit of detection and limit of quantification (LOD and LOQ) were found to be 0.001 and 0.003 ìg/g, respectively. It was observed that residues of propaquizafop in cotton seed and lint were below detectable level of 0.003 ìg/g. In soil, 0.005 and 0.007 ìg/g of propaquizafop residues were observed at single and double dose applications, respectively.

Key words : Cotton, GC-MS/MS, lint, propaquizafop, residues, seed, soil

India is second largest producer of cotton in the world. It is considered as major cash crop of Haryana with annual production of about 2.5 million bales from 6.39 lakh ha in 2015. There is a great scope to increase productivity as weeds are the main pest significantly denting the cotton productivity and reduce cotton yield by 38-50 per cent (Singh, 2014). Major weeds infesting the crop in northern India includes *Portulacastrum* (Santhi), *E. colona* (Sawank), *C. rotundus* (Motha), *D. arvensis* (Kondhra), *D. aegptium* (Makra), *Digitaria sanguinalis* (Jhernia), *Tribulus terrestris* (Bhakri), *Amaranthus spinosus* (Chaulai), *Launaea asplenifolia* (Jangli gobhi), *Cynodon dactylon* (Doob), *Sorghum halepense* (Baru), *Leucas aspera* (gumma).

Manual weeding is less efficient in cotton due to frequent rains and irrigations. Because of hot and dry conditions at plantations, limited area is covered under pre plant incorporated (PPI) or pre emergence (PRE) herbicides which provide weed free growing period to crop only before the onset of rains or first irrigation. In these situations role of post-emergence (POE) herbicides become important for effective weed management in later stages. But there is very limited choice in efficient application of POE herbicides. Propaquizafop, 2-isopropylidenamino-oxyethyl (*R*)-2-[4-(6-chlorchinoxalin-2-yloxy) phenoxy] propionate belong to aryloxyphenoxypropionate, group is a systemic post emergence herbicide and widely used for the control of grassy weeds in cotton and other crops (Singh, 2014). Propaquizafop shows low acute toxicity via the oral and dermal routes, as well as the inhalation. Propaquizafop and its principal metabolite quizalofop are stable under sterile soil conditions and therefore it can be concluded that the aerobic metabolism of propaquizafop is due to biological activity. The rate of hydrolysis of propaquizafop is pH dependent and got increased under alkaline soil

conditions. But its application in later stage (about 45 to 50 DAS) can retains its residues in soil or plant matrix. Persistence of residues in crop produce can impose hazardous health effects to living organisms and their persistence in soil can impose phyto toxic effects in succeeding crops. Therefore, the present study was aimed at trace level estimation of propaquizafop residues in cotton lint, seed and soil at harvest time in agro climatic conditions of Hisar, Haryana by developing a standard sample processing method along with a new MRM programming over for GCMS/MS.

Cotton crop was grown in *kharif,* 2015 following recommended agricultural practices at Research Area of Department of Agronomy, CCS Haryana Agricultural University Hisar. Propaquizafop 10 EC formulation under trade name of 'Agil' and technical grade (with purity of 92 %) was procured from Makhteshim Agan India Pvt. Ltd New Delhi. The formulation was applied @ 62.5 and 125 g/ha as single and double dose respectively on 45 DAS. Cotton lint, seed and soil samples were taken at first picking so as to evaluate the maximum possibility of residues persistence in these commodities. Solvents like acetone, dichloromethane, hexane and other chemicals were procured from Merck, Darmstadt, Germany. All the solvents were glass distilled before use so as to remove traces of non volatile impurities, water and acids like acetic acid.

Extraction : After manual de-linting, the cotton seeds were dried at 40°C for five days and crushed in mixer grinder. Soil samples were dried under shade, crushed and sieved before use to remove the debris. The representative sample of cotton lint (10 g), crushed seed (50 g) and soil (50 g) were separately taken in a 1l Erlenmeyer flask. The residues were extracted with 200 ml mixture of acetone: water (7:3) by shaking over a mechanical shaker for one h. The extract was collected in a separate flask. The residual material was re extracted with 100 ml of acetone: water mixture and combined extract was filtered under suction through Whatman Number 40 filter paper.

Clean up cotton seed and lint : Before clean up, the extract obtained from seed extraction, was partitioned with 50 ml hexane using a separatory funnel so as to remove the oil content from the extract. Organic (Hexane) phase was discarded. The aqueous phase thus retained from seed and the extract obtained from lint and soil extraction were partitioned thrice with 300 ml of dichloromethane (150, 100 and 50 ml). The lower dichloromethane layer was collected in a separate flask each time after passing through a bed of anhydrous sodium sulfate. The organic dichloromethane layer was evaporated to dryness over a rotary vacuum evaporator (Heidolph make) at 40°C and the residues were reconstituted in 5 ml methanol. The quaternary ammonium amine ion exchange solid phase extraction columns (Supelco) were used for clean up of the extract thus obtained from cotton seed and lint. The columns were pre washed with 20 ml mixture of methanol. The extract obtained after partitioning was introduced into the column and eluted with 20 ml methanol. The eluant was collected and evaporated to dryness over a rotary vacuum evaporator at 40°C and the residues were reconstituted in 5 ml methanol for analysis over GC-MS/MS.

The extract from soil samples obtained after partitioning was introduced into C 18 solid phase column which was pre conditioned with 6 ml of methanol and water (1:1). The eluant was collected and separated twice with 25 ml of

dichloromethane in a separating funnel and collected in a separate flask each time after passing through a bed of anhydrous sodium sulfate. The combined dichloromethane layer was evaporated to dryness over a rotary vacuum evaporator at 40°C and the residues were reconstituted in 5 ml methanol for analysis using GC-MS/MS.

Chromatographic analysis : Analysis of the propaquizafop was carried out in Agrochemicals Residues Testing Laboratory, Department of Agronomy, CCS Haryana Agricultural University, Hisar using GC-MS/MS (Agilent 7890A series with 7000 GCMS/MS detector). The instrument was tuned properly before injecting standards of propaquizafop. The operating parameters were: injection port temperature: 280°C. Column: HP-5 (30 m x 0.32 mm i.d. x 0.25 ìm film thickness). Oven temperature ramping was: 70°C (2 min hold), then increased at 25°C/min to 150°C (0 min hold), then increased at 15°C/min to 200°C (0 min hold), then increased at 8°C/min to 280°C (10 min hold). Detector parameters were: source temperature, 230°C; emission current, 35 ìA; energy, – 70 eV; repeller voltage, 11 V; ion body, 12 V; extractor, –7.2 V; ion focus, –7.4 V; quadrupole one (MS^1) temperature, 150°C; quadrupole two (MS^2) temperature, 150°C. Gas flow rates: helium (carrier gas), 1 ml/min though column and 2.25 ml/min as collision flow/ quench flow, nitrogen (collision cell), 1.15 ml/ min. Other parameters: split: pulsed splitless; vacuum (high pressure), 2.23x10–5 torr; rough vacuum, 1.51x102 torr; injection volume, 2 ìl. Under these instrumental conditions, the retention time of propaquizafop was observed to be 39.03 min. The confirmation and quantification of propaquizafop was achieved by developing a programme in SCAN, product ion (PI) and finally in multiple reactions monitoring (MRM). The preliminary information for parent ion and other fragmented ions of propaquizafop was provided in SCAN analysis. Parent ion peak at m/z 442.6 confirmed the molecular mass of propaquizafop to be 443.6. Various other peaks at m/z 370.7, 298.2, 242.6, 91.2, 191.8, 135.9, 100.4 and 56.5 represented different fragmented ions of propaquizafop. PI and MRM analysis are normally concerned with quantitative estimation of a compound. In PI, one or more precursor ions are selected and fragmented over a wide range by giving different collision energies. Characteristic ions with relatively high intensity and strong anti-turbulence were selected as monitoring and quantitative ions along with the collision energies best suited for this action. The parent ion at m/z 442.6 (high intensity) in SCAN was selected as precursor ion in this study for PI analysis and fragmented with different collision energies of 5, 10, 20 and 30. The best fragmentation of precursor ion was obtained at collision energy 20. In MRM, the most abundant precursor ions at m/z 442.6 and 299 were fragmented to various product ions as m/z 255.1, 299 and 91 at collision energy 20 (Fig. 1).

Physico chemical properties of the soil under cotton crop show its loamy sand texture (Table 1). Linearity of calibration curve was studied using propaquizafop standard solutions within the range of 0.001 to 1 ìg/ml. Each run was repeated thrice and the detector response was measured in terms of peak areas. A calibration curve was prepared by plotting concentrations of propaquizafop in ìg on x axis against average peak area on y axis. The response was found to be linear with correlation coefficient (R^2) = 0.997 and regression equation as y= 51379x-1152. The limit of detection (LOD) and limit of quantification (LOQ) for propaquizafop was observed to be 0.001 and 0.003 ìg/g,

Table 1. Physico chemical characteristics of soil collected from experimental field

Soil depth (cm)	Clay (%)	Silt (%)	Sand (%)	Organic carbon (%)	Electric cond-uctivity (dS/m)	pH	Nitrogen (kg/ha)	Phosp-horus (kg/ha)	Potas-sium (kg/ha)
0-15	9.0	12.3	77.5	0.28	0.24	7.8	138	20	435
15-30	12.8	15.7	64.2	0.23	0.19	7.4	124	13.6	426

respectively. Recovery experiments were carried out to check the validity of method in crushed cotton seeds, lint and soil by fortifying the control samples of each matrix at 0.003 and 0.005 ìg/g levels in triplicate. The mean recoveries for each matrix ranged from 87.8 to 98.5 per cent with relative standard deviation (RSD) below 10 per cent (Table 2). Analysis of cotton lint and seed samples collected at harvest show residues of propaquizafop to below detectable level (BDL) of 0.003 ìg/g at recommended dose of 62.5 g/ha and double recommended dose of 125 g/ha. In soil samples, 0.005 and 0.007 ìg/g residues were observed at single and double dose applications, respectively. There were no residues in soil samples from control plots where herbicide was not applied.

Table 2. Per cent recovery of propaquizafop in cotton lint, seed and soil

Fortification level (ìg/g)	Lint	Seed	Soil
0.003	88.5[a]±3.1	90.1±2.2	87.8±1.7
0.005	92.3±1.8	98.5±3.5	91.0±4.3

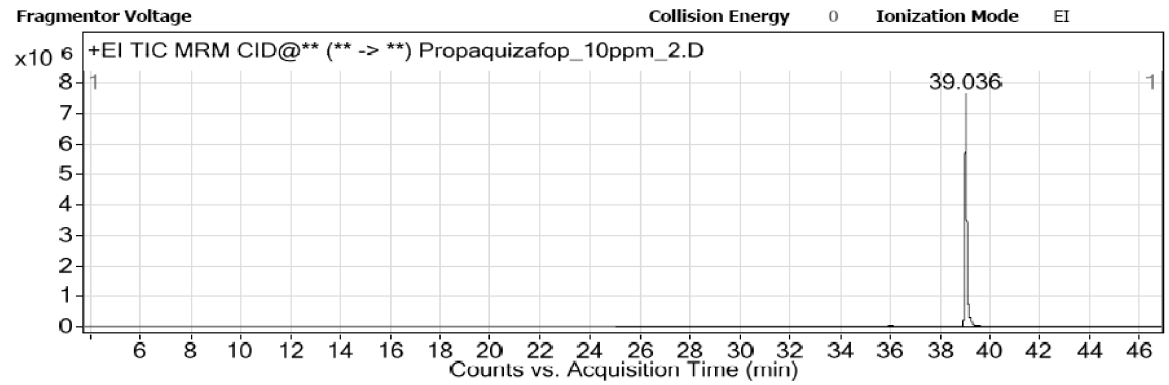
[a] Average of three replicates

Table 3. Residues of propaquizafop (ìg/g) in cotton lint, seed and soil

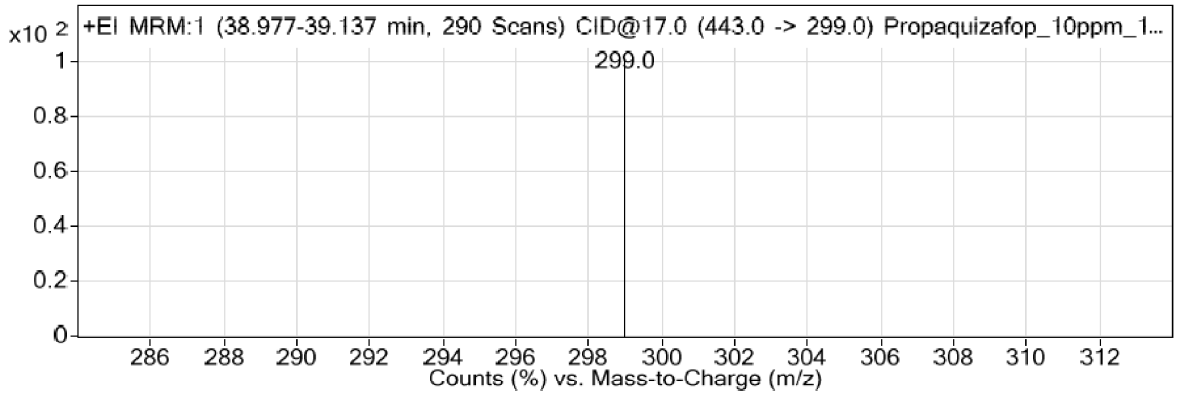
Parameters	Residues* (ìg/g)		
	Control	Single dose 62.5 (g/ha)	Double dose 125 (g/ha)
Lint	BDL	BDL	BDL
Seed	BDL	BDL	BDL
Soil	BDL	0.005±3.8	0.007±2.5

The rate of degradation of propaquizafop in soil is affected by a number of factors which include differential soil texture, pH, soil moisture, microbial activities and temperature conditions. Propaquizafop is an ester variant of the active substance quizalofop P. Degradation of propaquizafop proceeded rapidly via biological hydrolysis of the ester group (sterile soils showed no degradation), to form the main metabolite quizalofop which dissipates *via* several oxidative steps yielding quizalofop phenol, hydroxyl quizalofop, hydroxy quinoxaline, hydroxy quizalofop phenol and dihydroxy quinoxaline along with other several minor unidentified polar metabolites.

It has been noticed that prolonged half-life of propaquizafop at application of 100 g/ha could be due to reduced biological degradation of the herbicide at higher doses as the micro organisms, especially fungi (*Fusarium* spp.), were found to be critical factor influencing quizalafop depletion in soils (Sebiomo *et al.,* 2011, Zain *et al.,* 2013, Adhikary *et al.,* 2014, Ramparkash *et al.,* 2016). Sarkar and Majumdar (2013) reported that application of pre and post emergence herbicides in jute affected the total bacteria, actinomycetes and fungi population in soil initially, but the microbial population improved gradually and reached to normal level by harvest. The results in present study also revealed the enhanced microbial activities at harvest time



A



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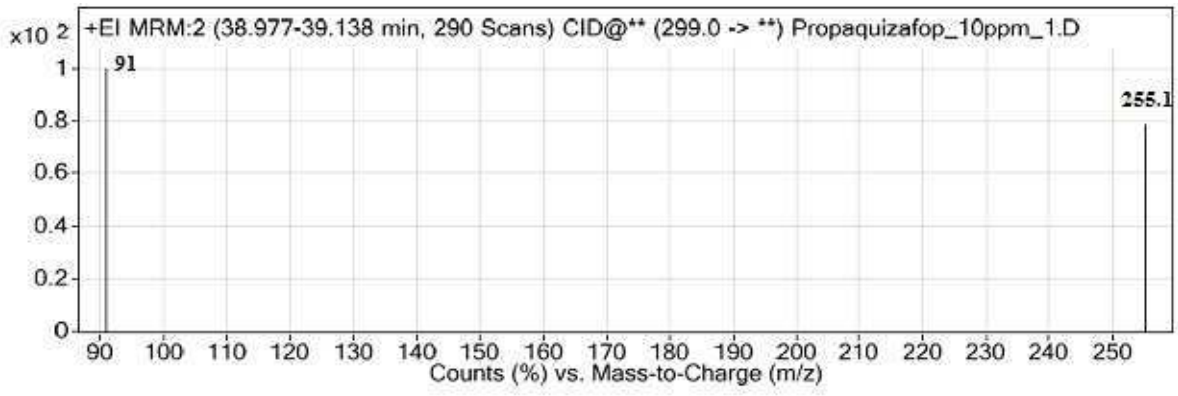

C

Fig. 1. GC-MS/MS standard SCAN chromatogram showing the retention time of propaquizafop; **(B and C)** mass spectra of precursor ion fragmentation at mass-to-charge (m/z) 442.6 giving products ions at m/z 299, 255.1 and 91.

resulted in degradation of propaquizafop residues to near detection limit of 0.003 ìg/g. Even though, there is no universally accepted classification of pesticide environmental persistence and propaquizafop can be categorized as slightly persistent in the soil by using classification based on the mean half-life of the pesticide in the soil (Jiye *et al.,* 2010).

CONCLUSION

From this study, it can be concluded that propaquizafop when applied at recommended and double the recommended dose as POE herbicide for control of grassy weeds did not adversely affects the cotton lint, seed and soil as residues do not persisted in any of the taste commodities.

REFERENCES

Adhikary, P., Shil, S. and. Patra, P. S. 2014. Effect of herbicides on soil microorganisms in transplanted chilli. *Glo. J. Bio. Agric. Health Sci.* **3** : 236-38

Jiye, Hu., Deng, Z., Chang, L. and Zhixin, Z. 2010. Simultaneous analysis of herbicide metribuzin and quizalofop-p-ethyl residues in potato and soil by GC-ECD. *Chromatographia*, **72** : 701-06.

Ramprakash, T., Madhavi, M. and Yakadri, M. 2016. Dissipation and persistence of propaquizafop in soil, plant and rhizomes in turmeric and its effect on soil properties. *Nature Environ. Poll. Tech.* **15** : 1217-20.

Sarkar, S. and Majumdar, B. 2013. Herbicidal effect on weed growth, crop yield and soil microbes in olitorius jute (*Corchorus olitorius* L.). *J. Tropic. Agric.* **51** : 23-29.

Sebiomo, A., Ogundero, V. W. and Bankole, S. A. 2011. Effects of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African J. Biotech.* **10** : 770-78.

Singh, S. 2014. Sustainable weed management in cotton. *Har. J. Agron.* **30** : 1-14.

Zain, N. M. M., Mohamad, R. B., Sijam, K., Morshed, M. M. and Awang, Y. 2013. Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. *African J. Micro. Res.* **7** : 367-74.

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