

## BEHAVIOUR OF PRETILACHLOR UNDER GREEN MANURING IN PADDY FIELD AND ITS RESIDUES ESTIMATION IN GROUND WATER USING GC-MSTANDEM MASS SPECTROMETRY

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### ABSTRACT

Despite many management strategies, use of selective herbicides was found most effective in weed management (Asokaraja and Mohamed Ali, 1995). Pretilachlor share for weed control in paddy cultivation is more than 80% which can results in persistence of residue in soil, water and agricultural produce. But excess use of herbicides have several environmental constrains because of their residual effects. Along with regular monitoring of residual status of these herbicides, there should be some practices in crop cultivation pattern like introduction of *Sesbania* as green manuring which can reduce the residual effect of these chemicals by adding organic carbon and increasing microbial activities and maintain soil health for long time (Duhan *et al.* 2014). Keeping in view, behavior of pretilachlor in soil under non-green and green manuring conditions at experimental fields and residue status in soil, under-ground water, grains, and straw in samples collected at farmer's field from major paddy-wheat growing regions of Haryana, India were analyzed. To support the persistence of pretilachlor residues in underground water it's leaching, adsorption and desorption behavior were also studied using state of art analytical technique GCMS-Tandem Mass Spectrometry.

### Methodology

#### Sample collection:

The paddy crop (variety HKR-47) was grown in kharif season in 2012 and 2013 at Regional Research Station Karnal on a field where long-term herbicide trial is under operation from 1999. *Sesbania* crop was grown after harvest of wheat crop before transplanting rice for incorporation as green manuring. The crop was grown by puddled transplanting in plots of size 16.8 m x 17m in randomized block design with three replicates. Periodic soil sampling was done on 0 (after 2 hrs of application), 3, 7, 15, 30, 45, 60 days after application of pretilachlor.

To know the residual status at farmer's field, the soil, paddy grain and straw samples were taken at harvest from major paddy-wheat growing regions of Haryana, India. The water samples from the tube-wells at farmers' fields were taken after 45 days of application of herbicide.

#### Extraction and clean up:

A representative 20 gm soil, grains and 10 gm straw samples were taken in 250 ml conical flask and added 50 ml of acetone to the samples. The samples were shaken over rotary shaker for one hour. The contents were decanted in separate conical flask by passing over a bed of anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to about 10 ml over rotary evaporator at 35°C. The samples were partitioned with hexane: ethyl acetate (9:1) thrice by taking 50, 30 and 20 ml after adding 50 ml saturated brine solution. The organic phases were collected by passing over Na<sub>2</sub>SO<sub>4</sub> in a separate bottle. The organic phase was concentrated over rotary evaporator to 10 ml at 35°C. No further clean-up was required for soil samples as the samples were clear and containing no color. But the grain and straw samples were further cleaned-up by column containing 10 gm alumina and 0.5 gm

charcoal sandwiched between Na<sub>2</sub>SO<sub>4</sub> layer above and below. 100 ml of hexane: ethyl acetate (9:1) was used as eluent for each sample. The extracts were collected by passing over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated up to dryness over rotavapour and the final volume was made to 5-10 ml by n-hexane and analyzed over GCMS/MS Triple Quadrupole.

A representative 50 ml water sample was taken in 250 ml separating funnel, and added 2 gm of NaCl to it. It was then partitioned with hexane: ethyl acetate (9:1) thrice by taking 50, 30 and 20 ml. The organic phases were collected by passing over Na<sub>2</sub>SO<sub>4</sub>. The organic phase was concentrated over rotary evaporator up-to dryness at 35°C. No further clean-up was required as the samples were clear and containing no color. The final volume was made to 5-10 ml by n-hexane and analyzed over GCMS/MS Triple Quadrupole.

**GCMS/MS operating parameters:**

GCMS/MS Agilent 7890 A was used for the residue analysis of pretilachlor in water from tube-well. The operating parameters were: Injection port: 280°C. Column: HP-5, column (30 m x 0.32 mm i.d. x 0.25 µm film thickness) of 5% diphenyl / 95% dimethyl polysiloxane. Oven temperature ramping was: 70°C (2 min) → at 25°C min<sup>-1</sup> → 150°C (0 min) → at 15°C min<sup>-1</sup> → 200°C (0 min) → at 8°C min<sup>-1</sup> → 280°C (2 min). Detector: Mass 7000 GCMS/MS Triple Quadrupole; detector parameters were: Source temperature = 230°C; Emission current = 35 µA; Energy = - 70 eV; Repeller voltage = 11 V; Ion body = 12 V; Extractor = - 7.2 V; Ion focus = - 7.4 V; Quadrupole One (MS<sup>1</sup>) Temperature = 150°C; Quadrupole Two (MS<sup>2</sup>) Temperature = 150°C. Gas flow rates Helium (carrier gas) = 1 ml/min. (through column) and 2.25 ml/min. (collision flow/quench flow). Nitrogen (collision cell) = 1.15 ml/min. Other parameters: Split ratio = 1: 10; Vacuum (high pressure) = 2.23X10<sup>-05</sup> Torr; Rough Vacuum = 1.51X10<sup>+02</sup> Torr; Injection volume = 2 µL.

**GCMS/MS Programming:**

The instrument was tuned properly before injection of standard samples (1ppm) of pretilachlor, butachlor and anilofos. All the tuning parameters were found in adequate range. For confirmation and quantification a programming was developed in Multiple Reaction Monitoring (MRM) Mode on the basis of m/z ratio and collision energies used in SCAN and PI monitoring as per the programming detail given below:

Compound	Precursor Ion	Product Ion	Collision
Pretilachlor	262	202	10
	262	162	10
	262	147	10
	262	132	10

Retention time for pretilachlor was observed to be 18.55 minutes. Limit of detection (LOD) and limit of quantification (LOQ) for three herbicides were found 1.0 and 3.0 ppb, respectively.

**Calibration Details – Linearity Check**

A calibration curve was plotted for concentration of the standard injected versus area observed and the curve was found linear up to the lowest concentration range 0.001 µg/ml.

**Results**

It was observed in controlled experiment under green manuring, that the residues of pretilachlor, dissipated at faster rate than under non-green manuring conditions with half life of pretilachlor as 7.3 days and 8.9 days. There were no residues in paddy grains and straw collected from such field.

In shallow tube-well water, it was observed that 4 out of 21 sites were having pretilachlor residues ranging between 0.21 – 0.81 µg/ml (above MRL - 0.1 µg/ml). Eight sites were having residues below MRL. Other have residue below detectable level. Residues of pretilachlor at 150 different locations were also tested in tube-well water having water table greater than 50 feet or more. No

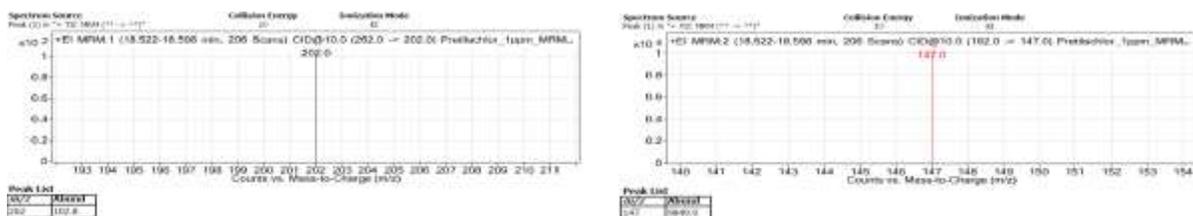
residues of these four major herbicides were observed above MRL. This suggest moderated to low leaching potential of pretilachlor upto 20-30 feet under natural environmental conditions where water recycling is more. Further the sites where underground water have pretilachlor residues have sandy textured soil (near river Yamuna), in which leaching is more prominent than the other heavy textured soil.

Pretilachlor residues were found in harvest time soil samples within range 0.010 to 0.05 µg/gm in 15 out of 125 sites at farmers' field. Rice seeds were found to contain residues within range 0.11 to 0.052 µg/gm (below MRL- 0.1 µg/gm in rice) at 10 out of 125 sites. Four sites were found to contain residues between 0.010 to 0.293 µg/gm in straw samples. This may be attributed toward use of 'briefcase pesticides'. As in the sample collection region, there is heavy use of low grade local made cheap herbicides, the manufacturers of which used to make some herbicide this year and some other herbicide on next year (briefcase pesticides) by purchasing technical from big manufacturers to which they formulate according to themselves keeping in mind the cheap manufacturing cost so that such herbicides can easily be promoted in the market. No one knows about what formulation they are using to make their product cheap. Pretilachlor accumulation and detoxification via glutathione-S-transferase (GST) in paddy plant is heavily decided by a safener-fenclorim (Luciano *et al.* 2003). Both, pretilachlor and safener work in antagonistic way. If the quantity of safener is not accurate, the chances of accumulation of pretilachlor in paddy grain and straw may increase. Here the formulation of branded products works whereas the formulation of 'briefcase pesticides' fails and results in residues accumulation.

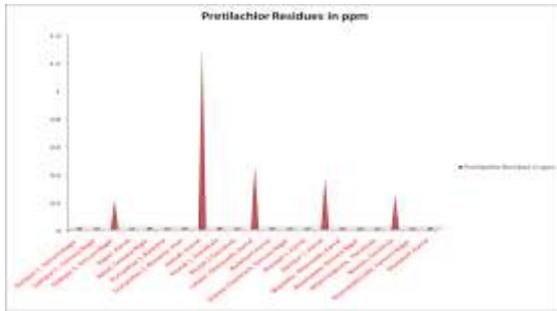
Low to moderate leaching potential of pretilachlor was observed at lower dose. But higher dose show good leaching in an experiment conducted under lab conditions so as to know the leaching behavior of pretilachlor. The results from adsorption/desorption behaviour of pretilachlor applied at different concentration from 10 to 50 µg revealed that the total amount of pretilachlor adsorbed decreased with increase in initial concentration from 10 to 50 µg, whereas amount of desorption increases with increase in concentration of application. This revealed the conclusion that pretilachlor have leaching behaviour. The amount of pretilachlor adsorbed in soil ranged from 39.76-45.76%, whereas desorption in soil was ranged from 46.79% to 60.35%.

## Conclusions

Presence of pretilachlor residues above MRL at 4 out of 21 sites in underground water, 15 out of 125 sites in soil, 10 out of 125 sites in rice seeds within range 0.11 to 0.052 µg/gm (below MRL- 0.1 µg/gm in rice) and four out of 125 sites in straw samples between 0.010 to 0.293 µg/gm is going to be an alarming situation for the policy makers before recommending weeds management strategies. Persistence of the residues may be due to indiscriminate use without following good agricultural practices by the farmers or may be due to higher ground water re-cycling through tube-well irrigation and heavy use of 'briefcase pesticides'.



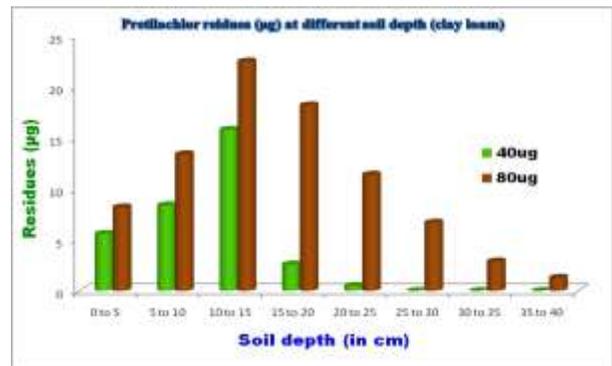
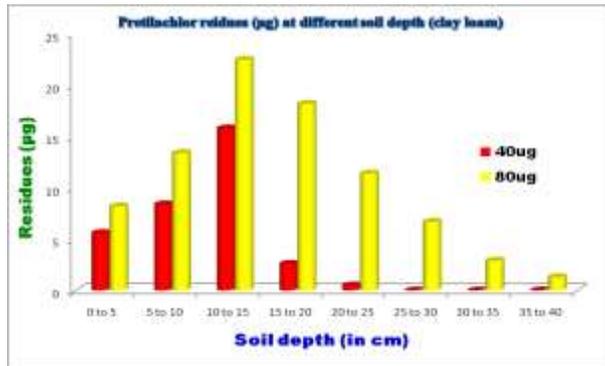
Figures 1 and 2: MRM analysis of pretilachlor.



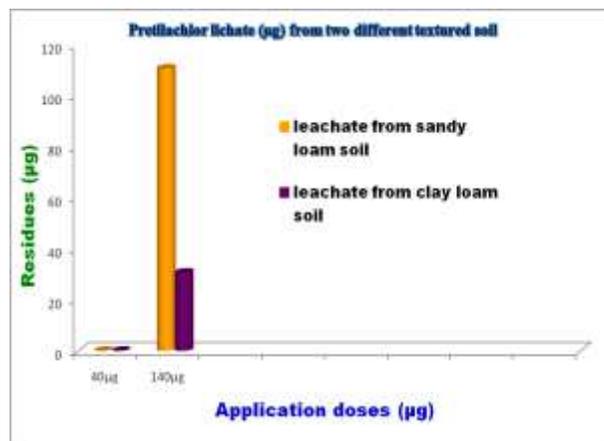
**Figure 3:** Pretilachlore residues in ground water from various sites at farmer's field.



**Figure 4:** dissipation pattern of pretilachlor under green and non-green manuring at farmer's field.



**Figure 5 and 6:-** leaching behavior of pretilachlor in two texturally different soils.



**Figure 7:-** leachate of pretilachlor collected from two texturally different soils.

## REFERENCES

1. Asokaraja N, Mohamed Ali A. 1995. Herbicide residue studies in rice eco-system. In VI Biennial Conference of Indian Society of Weed Science, Feb 9-10, 1995, Annamalai University, Annamalai Nagar, Tamil Nadu, pp 141.
2. Luciano S, Daniele DB and Costantino V. 2003. Persistence and detoxification of Pretilachlor and Fenclorim in rice (*Oryza sativa*). *Agronomie*, **23**: 147-151.