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Mobility of nano-formulated chlorpyrifos in sandy loam soil: A comparative study with conventional chlorpyrifos

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Abstract
The mobility of nano-formulated chlorpyrifos was analyzed in sandy loam soil, under laboratory conditions. During this analysis, nano-formulated chlorpyrifos mobility was contrasted with conventional chlorpyrifos. The leachates and soil samples at different depths of soil column were monitored over the time. The residues of chlorpyrifos in leachates and soil were estimated by high pressure liquid chromatography (HPLC). The results indicated a noticeable decrease in the mobility of nano-formulated chlorpyrifos compared to the conventional form, where the nano-formulated chlorpyrifos was detected only up to 10-15 cm of depth while the conventional chlorpyrifos was detected up to the depth of 25-30 cm. This result emphasizes the potential advantages of nano-formulations in reducing the spread of pesticides outside the intended areas.

Keywords: Soil mobility, chlorpyrifos, nano-formulation, environment

1. Introduction
Pesticides are frequently used in intensive agricultural operations in an effort to increase the crop yields. However, there are instances where increased yield is accompanied by the presence and persistence of pesticide residues in the soil and water. A significant part of the ecosystem, i.e. soil serves as a sink for most of the pesticides used in agriculture (Lucas *et al.*, 2019) [1]. In terms of pollutants storage through soil organic carbon, it functions as a filter, buffer, and has degradation potential (Rasool *et al.*, 2022) [2]. One of the most crucial factors influencing the pesticides behavior in the environment is the rate at which they degrade in the soil. Excessive use of pesticides in farming may have a negative impact on the environment and, in turn, human health. Since pesticide leaching compromises the quality of subterranean water, it is a serious environmental hazard (Carpio *et al.*, 2021) [3]. Many conventional formulations in India such as organophosphates are approved for application on a range of crops. A such conventional formulation is Lethal 20 EC (Chlorpyrifos 20% EC). This insecticide, have been found effective in controlling some insect pests such as termites etc. The broad-spectrum insecticide chlorpyrifos (O, O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothionate) in this ready-mix formulation works as a cholinesterase inhibitor. Since it works well against both sucking and eating insects, it is frequently used to manage pests in different types of crops. It sorbs firmly to soil particles and has a low water solubility. It is quickly degraded to produce TCP, the main metabolite, which is persistent and somewhat mobile in soil (Huang *et al.*, 2021) [4]. In order to minimize the environmental negative impacts of chlorpyrifos, the chlorpyrifos containing biopolymeric nano-formulations were synthesized by using chitosan and guargum biopolymers, crosslinked by using a green crosslinking agent i.e. citric acid.
In present study, an experiment was conducted under laboratory conditions to determine the leaching capability of both pesticides in sandy loam soil.

2. Materials and Methods
2.1 Chemicals and Reagents
The Chlorpyrifos (> 98% purity) used for preparation of calibration curve was procured from Sigma Aldrich. The conventional formulation of chlorpyrifos i.e. 20 % EC chlorpyrifos under the name Lethal 20 EC, was procured from local market. The nano-formulated chlorpyrifos used was the same as reported in previous paper (Maan *et al.*, 2023) [5]. Acetonitrile of HPLC grade was used as the solvent, Sodium Chloride used during extraction process was procured

from HiMedia Laboratories Pvt. Ltd. Sodium sulphate used was procured from Central Drug House Pvt. Ltd.

2.2 Preparation of standard Solution
A standard stock solution of chlorpyrifos (technical grade) was prepared in acetonitrile with 1 mg/ml concentration of chlorpyrifos. The standard solutions required for preparation of calibration curve (1.00, 0.50, 0.25, 0.12, 0.06 and 0.03 µg/ml) were prepared by serial dilutions of stock solution using acetonitrile as a solvent. All standard solutions were stored at 4 °C.

2.3 Instruments
Analysis of chlorpyrifos was carried out on high performance liquid chromatography, HPLC (Thermo Scientific Vanquish HPLC model) manufactured by Thermo Fisher Scientific company and having Chromeleon 7.3.0 software.

During HPLC analysis C_{18} column (3 × 150 mm and 3 µm) was used as a stationary phase and column temperature was maintained at 24 °C. The injected sample volume was 15 µl. The mobile phase composition was acetonitrile: water (90: 10, v/v). The flow rate of the mobile phase was 0.3 ml/min. The detection wavelength for the chlorpyrifos pesticide was selected as 230 nm.

2.4 Experiment
The leaching experiment was carried out under the laboratory conditions. The procedure adopted during experiment was similar to the procedure as adopted by Rani *et al.*, 2014 [6]. The sandy loam soil used was gathered without any prior pesticide application history from the Research Farm at CCS Haryana Agricultural University in Hisar. The soil was allowed to air dry in the shade, then sieved using a 2-mm sieve. The pertinent soil values were as follows: pH 7.6, organic carbon 0.67 percent, and EC 2 dSm^{-1}. Plexiglass columns (90× 2.2 cm internal diameter) were used for the soil column experiment. Each column was sequentially filled with soil up to the height of 40 cm, as shown Fig.1. Before packing, the cotton plug was kept at the distal end of the column to allow only the passage of leachates. The both type of formulations of chlorpyrifos were added to the top 5 g of the soil to maintain the dose of 50 µg/g i.e. 0.25 mg in 5 g of soil. For fortification of conventional chlorpyrifos, the conventional formulation was diluted in distilled water and 2.5 ml of 100 ppm was simultaneously applied to the top 5 g of soil in the column carrying the active ingredient equal to 0.25 mg. Similarly, the 10.6 mg powdered form of nano-formulated chlorpyrifos was also added to the top 5 g of soil in another column. As described in our previous paper (Maan *et al.*, 2023) [5], the active ingredient present per 10 mg of nano-formulated chlorpyrifos was 0.235 mg. So, to keep the similar dose in both cases, 10.6 mg of chlorpyrifos loaded sample of nano-formulations was added into the top 5 g of soil in soil columns for performing soil mobility of chlorpyrifos. One column packed with soil was kept as a control to which no pesticide was added. After application of conventional and nano-formulated chlorpyrifos, the columns were irrigated with 35 ml of water daily (equivalent to 300 mm rain). When the water addition was finished after 6 days, the soil columns were left to drain for 36 h. Three leachate fractions were collected from each treatment. After completion of soil mobility test, all the intact soil cores were taken out of the plexiglass column and were sliced into sections/pieces of 5 cm each (0-5,5-10,10-15,15- 20,20-25). All these pieces were air-dried and grinded.

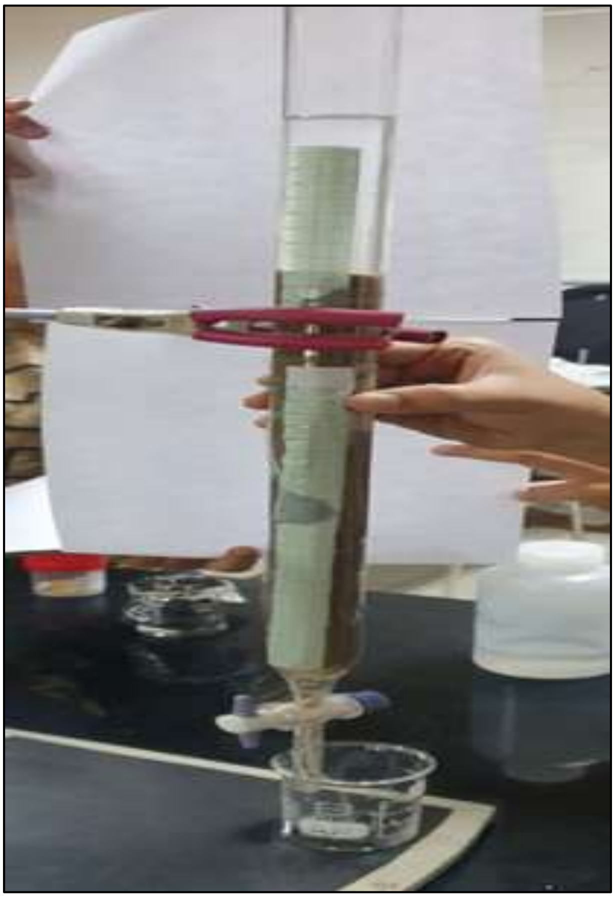

Fig 1: Soil Column experiment

2.5 Extraction and Clean up
Chlorpyrifos residues from soil and leachates were processed by QuChERS method (Lahotey 2011) [7]. During this method, 15 g of the ground, sieved and dry representative subsoil was added to the 30 ml mixture of Acetonitrile: Water (30: 30). 3 g of NaCl was added into the filtrate of above mixture and shaken for at least 15 minutes. The acetonitrile layer was taken out of the two separated layers, added with 9 g of sodium sulphate (Na_2SO_4) and filtered. Again, the 10 ml of filtrate was added with 0.4 g primary secondary amines (PSA) + 1.14 g magnesium sulphae ($MgSO_4$). The PSA was used to remove sugars or fatty acid metabolite (if present) and $MgSO_4$ was used to remove the water traces. The finally obtained filtrate was concentrated upto 3ml for analysis by HPLC. Similarly, leachate samples were also processed by QuChERS method for analysis by HPLC.

3. Results and Discussion
3.1 Estimation by HPLC
The HPLC chromatogram (Fig. 2) the peak at 8.4 min was corresponding to chlorpyrifos while the remaining peaks corresponded to the solvent.

The standard curve was also prepared before the analysis of samples, as shown in Fig. 3. During this standard curve preparation, peak areas were recorded for chlorpyrifos standards at different concentrations as discussed in Table 1. Regression coefficients explained the linearity of curves with value of 0.99939 i.e. very close to 1. The straight line equation obtained was $y = 1.408x + 0.0089$.

Table 1: Peak areas corresponding to different concentrations of chlorpyrifos

Concentration (ppm)	Peak Areas (mAu*min)
1.0	1.4194
0.5	0.7086
0.25	0.3639
0.12	0.1716
0.06	0.1139
0.03	0.0377

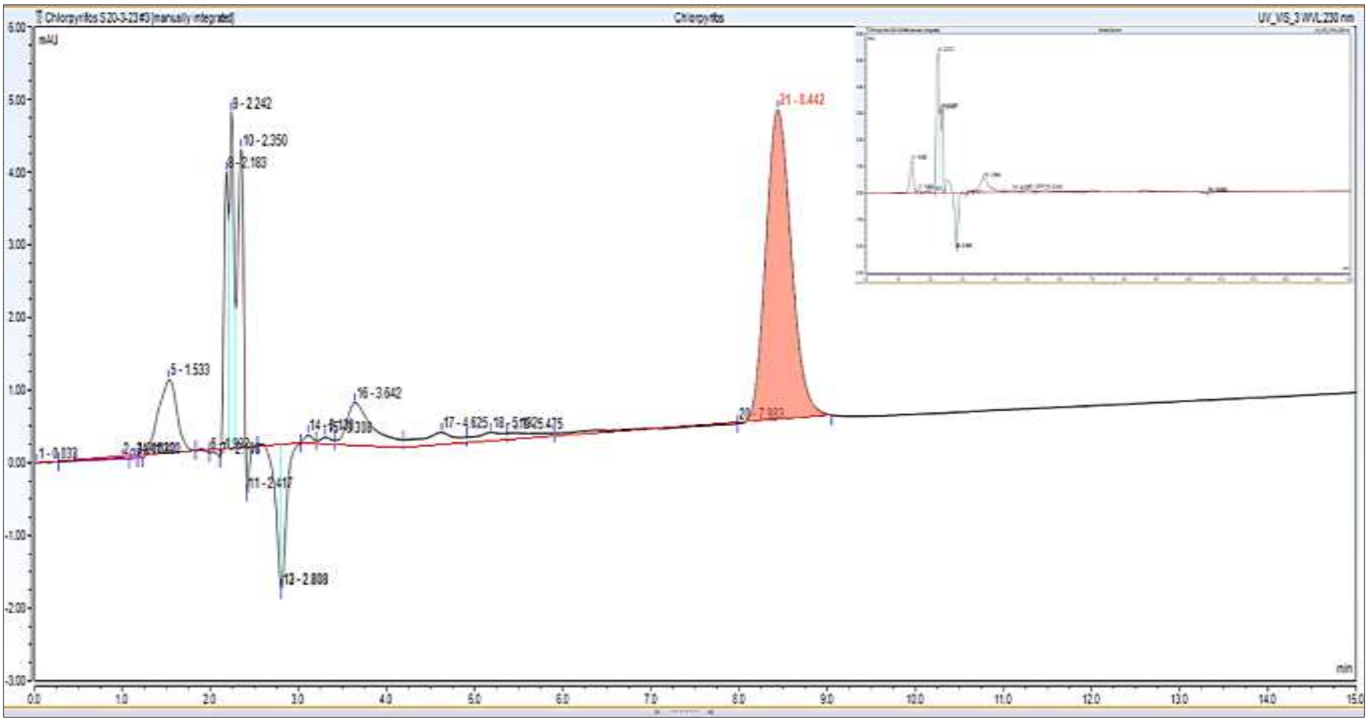

Fig 2: Chromatogram of chlorpyrifos

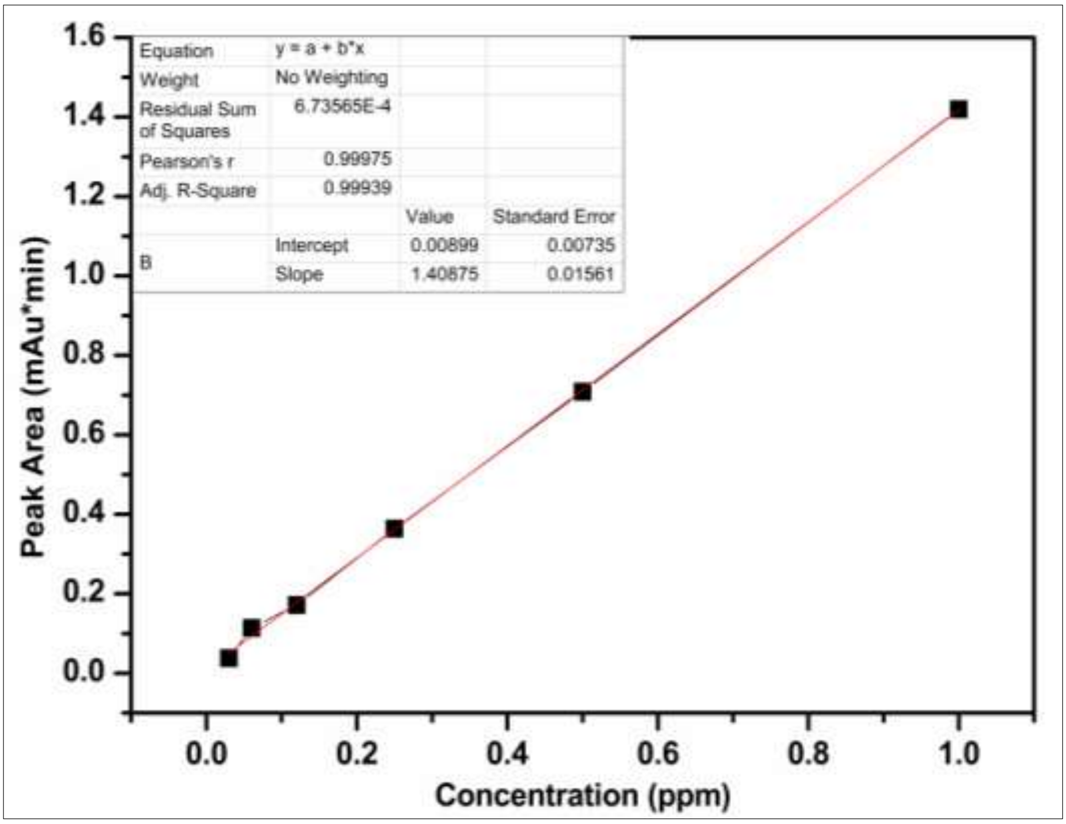

Fig 3: Standard curve of chlorpyrifos using HPLC

LOD stands for the limit of detection i.e. the lowest amount of a substance which can be distinguished from the absence of that substance while the LOQ stands for limit of quantification i.e. the smallest possible concentration of analyte into the test sample which can be determined with repeatability and accuracy (Jannetto, 2017) [8].

The LOD and LOQ for HPLC were determined by using the method based on standard deviation of the response and slope. The standard deviation of response and slope were calculated by regression analysis in MS excel.

$$\text{LOD} = \frac{3.3 \times \text{Standard deviation of response}}{\text{Standard deviation of slope}} \quad (1)$$

$$\text{LOQ} = \frac{10 \times \text{Standard deviation of reponse}}{\text{Standard deviation of slope}} \quad (2)$$

The standard deviation of response obtained from regression statistics was 0.00628 and the value of slope was 1.40402. The obtained values of LOD and LOQ were 0.015 and 0.045 ppm.

3.2 Retention of Chlorpyrifos

The amount of chlorpyrifos was analysed into the leachates and the soil samples (5 cm slots) by using HPLC. The retention % was calculated with respect to the initial dose used during fortification. The equation for the calculation of % retention is as follows:

$$\%\ \text{Retention} = \frac{\text{Amount retention}}{\text{Amount added}} \times 100 \quad (3)$$

Chlorpyrifos retention percentages were calculated in several soil sections with dimensions of 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, and 35-40 cm. Table 2 & Fig. 4 displays the % retention findings. The highest retention was seen in the upper 5 cm of soil, or up to 61.24% and 67.43%, in soil samples fortified with nano-formulation and conventional formulation, respectively. The chlorpyrifos released from conventional formulation was found down to a depth of 25–30 cm, while the amount of chlorpyrifos released from nano-formulation has decreased below the LOQ (Limit of Quantification) of the HPLC even after 15-20 cm of depth. In the eluent fractions, no residues of chlorpyrifos were found. The findings showed reduced soil mobility of chlorpyrifos released from the nano-formulation. Therefore, it appears that the nano-formulated chlorpyrifos seems safer for groundwater than the conventional formulation.

Table 2: Soil mobility of chlorpyrifos released from nano-formulation and conventional formulation

Soil Depth (cm)	% Retention (Conventional)	% Retention (Nano-formulation)
0-5	67.43	61.24
5-10	8.56	9.58
10-15	5.20	1.08
15-20	3.14	<LOQ
20-25	1.02	
25-30	0.73	
30-35	<LOQ	
35-40	-	
Total Recovered	86.08	

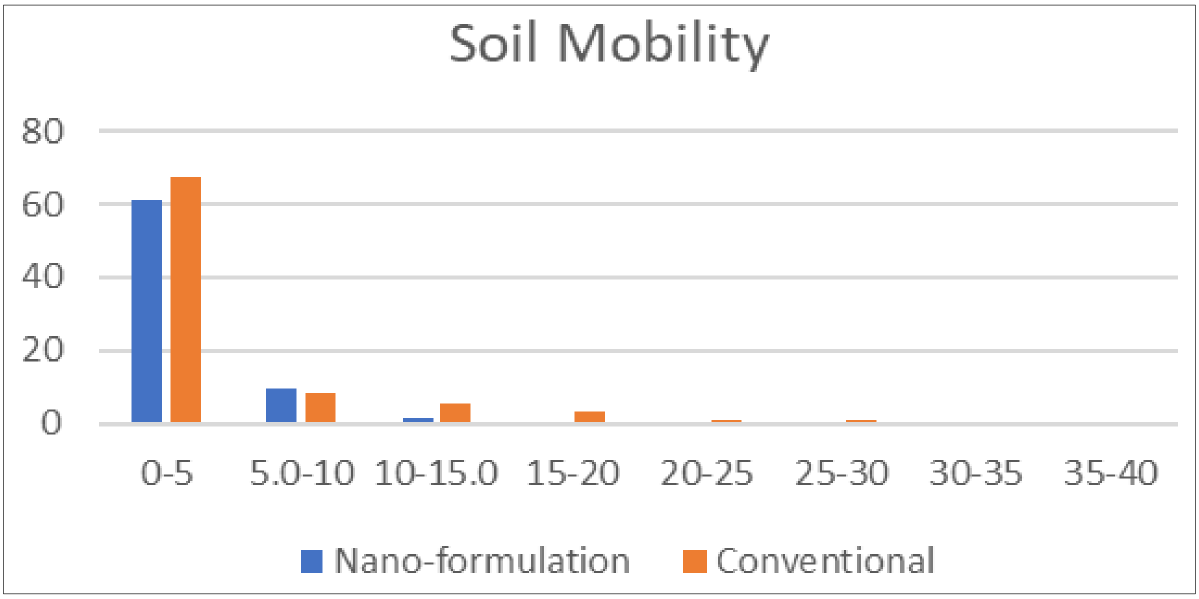

Fig 4: Percent retention of chlorpyrifos

4. Conclusions

The soil mobility of chlorpyrifos was found to be decreased after using the nano-formulated chlorpyrifos as compare to the conventional chlorpyrifos which indicates lower risks of contamination in surrounding ecosystems. Due to its restricted mobility, the pesticide may remain closer to the intended treatment region, where it will be most effective against the target pests and less likely to be exposed elsewhere. Hence, the application of nano-formulated chlorpyrifos seem to be safer for ground water than the conventional formulation.

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