Effect of Organic Amendment on Mobility Behavior of Flupyradifurone in Two Different Indian Soils

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Abstract
Flupyradifurone is a novel neonicotinoid insecticide, mainly used in okra in subtropical conditions for controlling whitefly and jassids. The present experiment was designed to generate information on the leaching behavior of flupyradifurone, 3-[(6-chloropyridin-3-yl)methyl-(2,2-difluoroethyl)amino]-2H-furan-5-one, under different rainfall conditions by using packed soil columns. Under the continuous flow conditions, a significant quantity of flupyradifurone, 67.76% and 50.61% were recovered at 0 to 5 cm soil depth in case of both clayey and sandy loam soil, respectively. A considerable amount of the residue was confined to 0 to 20 cm soil depth, with or without farmyard manure (FYM) amendment. Under varying water flow condition, distribution of the residue in the upper 0 to 5 cm soil depth got enhanced (> 90% recovery). Among the test soils, residues were detected from the leachate fraction of sandy soil (0.08 µg/mL) only. The study pointed out that leaching of flupyradifurone in sandy loam soil got decreased after using FYM. The leaching of flupyradifurone increased with the increasing amount of water (40 to 160 mL) and the residues continued to travel down to the lower depth. It can be concluded that the use of FYM may be a viable option for reducing the mobility of flupyradifurone in sandy loam soil.

Keyword Flupyradifurone · Farmyard manure (FYM) · Rainfall · Leaching

The primary challenge facing modern farming is to enhance food production quality and quantity while keeping away any destructive impact on the environmental or natural resources. The use of agrochemicals to eradicate pests and diseases is one of the main components in sustainable agriculture. However, the environmental impact of these chemicals is a major concern in recent times. Some of the pesticides used in farming have the potential to contaminate groundwater (Arias-Estévez et al. 2008). A proper dose of pesticides applied in crops encountered by insect pests, whereas the use of an excessive amount move across the ecosystem, polluting water, air, and soil, either directly through the deliberate application (e.g. agronomic practices) or unintended ways (e.g. spray drift, burial of container, equipment washing, waste disposal from production facilities or urban pollutions, leakage at pesticide dump sites etc.) (Sannino and Gianfreda 2001; Jeschke et al. 2011). Estimating the ability of pesticides for transformation in soil and movement into the deeper soil layers and ultimately into groundwater is essential for risk assessment (Das and Mukherjee 2011).

Flupyradifurone, 3-[(6-chloropyridin-3-yl)methyl-(2,2-difluoroethyl)amino]-2H-furan-5-one, a novel neonicotinoid compound, belonging to the butenolide class of insecticides, introduced by Bayer Crop Science under the trade name Sivanto™ is a “bee-friendly” product (Nauen et al. 2015). The compound shows excellent biological efficacy against a wide variety of pests such as aphids, hoppers, and whiteflies in cereal grains (except rice), seed root vegetables (except sugar beet), legumes (succulent or dried), cotton, corn vegetables, non-animal feed, leafy vegetables (except Brassica), and soybean seeds (Carleton 2014). Flupyradifurone engaged in excitatory neurotransmission, interacts with insect nicotinic acetylcholine receptors (nAChRs) (Ihara et al. 2017). The groundwater ubiquity score (GUS) of flupyradifurone is 3.74, indicating the high potential of this compound to percolate down to an aquifer and transport through runoff to surface water bodies (Singh et al. 2018).

In soil, flupyradifurone forms two major metabolites, Difluoroacetic acid (DFA) and 6-chloronicotinic acid (6-CAN)
According to the organic carbon coefficient ($K_{oc}$) values, potential high mobility of the primary metabolite, DFA ($K_{oc} = 68 \text{ mg L}^{-1}$) and medium mobility of 6-CAN ($K_{oc} = 88 \text{ mg L}^{-1}$) has been observed. Laboratory degradation study in aerobic soil found that DT$_{50}$ value of DFA is 61 days and this metabolite is also considered for the acute toxicity of fish (LC$_{50} > 10 \text{ mg L}^{-1}$) and invertebrate (EC$_{50} > 10 \text{ mg L}^{-1}$) (EFSA 2015).

In order to gain benefit of applied pesticides more efficiently, it is important to restrict their mobility in the soils. Organic amendments added in the soil may play a crucial role in reducing the leaching of contaminants as their combination with pesticides can significantly modify the fate (adsorption–desorption, degradation, mobility etc.) of the xenobiotics (Carpio et al. 2020). Addition of soil organic amendment leads to the enrichment of the active humified compounds such as humic acid (HA) and fulvic acid (FA) which plays a major role in anthropogenic activities such as supplying nutrients to the plants and microorganisms, enhancing soil fertility by improving soil buffering capacity. Owing to its high organic carbon content, the added amendment increases pesticide retention on soil particle, which in turn reduces the mobility of the pesticide and thereby prevents pesticide leaching to the groundwater (Das et al. 2015; Singh and Singh 2019).

Many studies have been conducted to investigate the effect of the organic amendment on pesticide movement in the soil. Different organic amendments (like manure-FYM, compost, sludge, crop residues and pyrolyzed biomass-biochar etc.) as a source of organic carbon are generally used for the immobilization of the pesticides in soil (Briceño et al. 2008; Larso et al. 2009). FYM contains humic substances (humic acids and fulvic acids) having different functional groups which may affect pesticide adsorption significantly during interaction with the compound (Mockeviciene et al. 2021). Under saturated flow condition, FYM has been found to reduce the leaching of fipronil in coarse-textured soil (Joshi et al. 2016). Application of organic amendment like FYM increases the organic carbon content of the soil and thus reducing the leaching losses of the chlorpyrifos (Srivastava et al. 2010).

Amendments are generally used to modify physical and chemical properties of soil which ultimately influence the ability of soil to adsorb contaminants. The proportion of contaminant to be adsorbed depends upon the affinity between the contaminant molecule and soil particle. Adsorption and leaching both are interconnected phenomena (Kumar et al. 2015; Singh and Singh 2019). Different interaction mechanisms are responsible for adsorption of soil contaminant by organic amendment. Partitioning/sorption of organic contaminant to organic amendment depends partially on the structure of humic material and partially on the chemical properties of the contaminant (Prosen et al. 2007).

Adsorption of organic contaminant on biochar takes place through different adsorption mechanism like electrostatic interaction, precipitation, H-bonding ion exchange reaction etc. (Abbas et al. 2018). On application of organic amendment to soil, it introduces solid organic matter along with dissolved organic matter (DOM) which is a combination of complex and dynamic compounds with varied molecular weights and chemical structures that can interact with organic contaminants in multiple ways, restrict or enhance their movement through the soil profile (Barriuso et al. 2011).

It is reported that sandy soils with a little amount of organic matter are most likely to be leached by mass flow, whereas clay soils (well-structured) are more likely to be leached by preferential flow (Das and Mukherjee 2011). Recent research has emphasized the significance of preferential flow on the mobility behavior of soil adsorbed pesticides. No such literature is available on the mobility behavior of flupyradifurone in soils under Indian subtropical conditions. Laboratory analysis of soil column might help to simplify the dynamic process of leaching (Sánchez et al. 2003). The objective of the present investigation is, therefore, to gain relative information on the leaching behavior of flupyradifurone in two diverse types of soil varying in physico-chemical properties (subtropical condition) under different rainfall circumstances. The effect of an amendment such as FYM on leaching behavior of flupyradifurone has been presented in this study. The findings of this research would help to further understand the ability of flupyradifurone to reach groundwater and aid to reduce ambiguity during the evaluation of environmental hazards by monitoring agencies (Singh et al. 2018).

Materials and Methods

Sampling sites were located in CoochBehar, West Bengal (Latitude: 26°24′14.4″N; Longitude: 89°22′58.8″E), and ICAR-Indian Agricultural Research Institute, Delhi (Latitude: 29°39′26″N; Longitude: 78°07′24″E), India. The required soils for the experiment were collected manually from plough layers (depths between 0 to 15 cm) having no history of previous flupyradifurone application. The soil samples were spread on aluminium sheet and moisture was allowed to evaporate under natural room conditions for 4–5 days for air-drying. It was then ground, transferred via a mesh sieve of 2 mm size and stored in the plastic containers. Physico-chemical characteristics of the soils have been measured by using conventional techniques (Table 1). The pH of the soils was measured by taking 1:2.5 soil to water proportion, using the Control Dynamics pH meter (Model APX 175 E/C) equipped with calomel glass electrodes (Jackson 1967). Sand, silt, and clay fractions of soil were analysed.
by Bouyoucos hydrometer technique (Jackson 1967) and Walkley and Black process was used to measure organic carbon content of the test soils (Black 1965). FYM (Organic amendment) utilized for this experiment was procured from the Agronomy Division of ICAR-IARI, New Delhi [pH: 6.8; Organic carbon(%) 22.8; C:H:N(%) 24.1:2.6:9.7].

The analytical standard (99.4% purity) for the experiment was obtained as a gratis from M/s Bayer Crop Science, India. Primary metabolite, Difluoroacetic acid (DFA) was procured from Sigma Aldrich (mp, -1°C; bp, 133°C). Glass distillation of organic solvents such as dichloromethane, hexane, methanol, and acetone has been done. Sodium sulfate (Na2SO4) was washed with acetone and then activated 110°C for 4 h before use. HPLC grade acetonitrile was obtained from Merck India Ltd. De-gassing and filtration of acetonitrile was also carried out before use (Mate et al. 2014). Chemical structure of flupyradifurone has been shown in Fig. 1.

10 mg of flupyradifurone (analytical grade 99.4%) was correctly weighed and dissolved in 10 mL of HPLC grade acetonitrile in a volumetric flask to get a stock solution of 1000 µg mL⁻¹ (ppm). Working standards of 10 µg mL⁻¹, 20 µg mL⁻¹, 50 µg mL⁻¹ and 100 µg mL⁻¹ were prepared from standard stock solution by serial dilution with HPLC grade acetonitrile. At first, Flupyradifurone (analytical grade 99.4%) was fortified in 2 g of soil at 20 µg level and after fortification, the soil was blended with the help of a glass rod, so that the solvents get evaporated completely. The columns used in the experiment were made of polyethylene tubes (50 cm long and 2.1 cm thickness) and experimentation was carried out in triplicate. Column loading was done up to a height of 25 cm with 220 g of soil and to achieve uniformity in column packing; soil compaction was done by applying equal force. Leachate fractions were collected through the small holes created at the lower end of the column. Afterwards, the soil columns were allowed to equilibrate and uniform moisture thorough capillary action was maintained by placing the lower end of the columns in a jar of artificial rain (0.01 M CaCl₂ solution). At the top of the column, 2 g of spiked soil (containing 20 µg flupyradifurone) was spread homogeneously for proper distribution of the analyte and leaching initiated.

In continuous flow conditions, water (0.01 M CaCl₂ solution) was allowed to leach at the flow rate of 0.33 mL min⁻¹. Under this flow condition, 400 mL of water (0.01 M CaCl₂ solution) simulating 1160 mm of rainfall passed through the column continuously without allowing the column to dry at any stage. Whereas in discontinuous flow condition, water has been added in different amounts and allowed to percolate through the soil, without allowing the column to get dry. When there was a minimum amount of water in the top layer of the soil, the next set of water was added. A total of five water sets; 160, 80, 60, and 40 mL simulating rainfall of 460.22, 233.10, 102.81 and 58.94 mm were used to leach both the soil columns i.e. sandy loam soil (Delhi soil) and clay soil (West Bengal soil). The behavior of the flupyradifurone in both clayey and sandy loam soil amended with organic manure (FYM@2.5% level) was also studied under continuous flow condition; two treatments were followed for this study, (i) soil treated with flupyradifurone without amendment (T1) and (ii) soil treated with flupyradifurone amended with FYM (T2).

The columns were left over for 24 h after the leaching process was finished; the soil cores having different depths were cut horizontally into 5 cm units. The average weight of each soil core was 44 g. The soil was air-dried over 72 h. In a stoppered conical flask (250 mL), soil samples were stored and each flask was supplemented with 10 g sodium sulfate anhydrous (Na₂SO₄, nH₂O) and 100 mL acetone. The samples were then shaken for 30 min on a horizontal shaker. The acetone fraction was transferred to a beaker (250 mL) and extraction of soil was performed similarly. Three extractions were conducted preceded by filtration of acetone fractions, which was then evaporated to dryness at room temperature and redissolved in 5 mL acetonitrile. Leachate fractions were collected from each column, filtered and diluted with 2% saturated NaCl solution (100 mL) and extracted by Dichloromethane (3 times with 30 mL DCM) through liquid–liquid partitioning (Singh et al. 2018). The DCM layers have been
collected, dried upon anhydrous sodium sulfate, concentrated at room temperature, redissolved in 5 mL of acetonitrile and, quantified by HPLC (Mate et al. 2014).

Estimation of the residue of flupyradifurone was done by using high performance liquid chromatography (HPLC) system (Shimadzu) equipped with a computer-operated dual pump (L-7100), an autosampler (L-72000), PDA detector and Lichrospher RP-18 column (length- 300 mm). Conditions for analysis were: a mixture of water and acetonitrile (30:70, v/v) as the mobile phase at a flow rate of 0.5 mL min$^{-1}$ at a wavelength of 280 nm with an injection volume of 10 µL and the total run time of 10.00 min. Under these conditions, the retention time for flupyradifurone was 5.98 min. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the instrument for flupyradifurone were 0.005 µg mL$^{-1}$ and 0.05 µg mL$^{-1}$ respectively. The representative HPLC profile depicting well-resolved peak of flupyradifurone and its metabolite [Difluoroacetic acid (DFA), 6.58 min Rt] in the wet soil and leachate is presented in Fig. 2.

The data were processed using three-way analysis of variance (ANOVA) to test differences among the treatment means. A post hoc mean separation test ($p < 0.05$) was further conducted using Tukey’s honestly significant difference (HSD) test using SAS 9.3 (SAS Institute, Cary, North Carolina, USA). The values followed by a similar uppercase letter within a column are not significantly different at $p < 0.05$ level of significance.

**Results and Discussion**

Adsorption of pesticides in soils is highly affected by the clay content and organic carbon content, as demonstrated by the low recovery of the test compound in sandy loam soil relative to clayey soil (Singh et al. 2018). Soil acts as a filter and buffer when pesticides are applied in the field, reducing the concentration of the pesticides in soil by degradation and sorption processes (Brown et al. 1995). Chemical composition and content of soil organic matter are considered to have a major impact on pesticide adsorption. The partition distribution coefficient ($K_{oc}$) of a pesticide is typically determined based on the organic carbon partition coefficient ($K_{OC}$) and organic carbon fraction present in the soil (Kodešová et al. 2011). The structural moiety of neonicotinoids has been found to undergo crosslinking reaction with functional groups of organic amendment resulting in reduced mobility of the compound in the soil profile (Rodríguez-Liébana et al. 2018). Flupyradifurone, a basic compound belonging to the neonicotinoid family, was adsorbed to the surface sites of the soil colloid by electrostatic interactions, ion-exchange reactions, or surface complexation (Khalid et al. 2020). However, no study on the exact interaction mechanism between flupyradifurone and the organic amendment has been conducted to date.

Flupyradifurone was distributed through the column when it was leached continuously with 400 mL of water (simulating 1160 mm of rainfall). In both the soils, namely, clayey, and sandy loam, 67.76% (13.5 µg g$^{-1}$) and 50.61% (10.1 µg g$^{-1}$) of flupyradifurone have recovered in 0 to 5 cm soil depth (without using FYM) (Table 2). The variation in the amount of flupyradifurone residues in different depth of both the soil types is attributed to the difference in per cent composition of clay and organic carbon content of the soils. The residues of flupyradifurone in the leachate of the clayey soil were below the quantifiable limit (<0.05 µg mL$^{-1}$) due to the high clay content whereas 0.08 µg mL$^{-1}$ residues were recorded in the leachate of sandy loam soil. In the clayey soil columns, with no FYM amendment, flupyradifurone moved down up to the depth of 15–20 cm recording 0.73 µg g$^{-1}$ residues and beyond 20 cm depth of soil column residues were below the quantifiable limit (<0.05 µg g$^{-1}$). While sandy loam soil has a low leaching potential, flupyradifurone still leached up to a depth of 25 cm in sandy loam soil column. According to the GUS score index, flupyradifurone is a member of a high mobility group compound (Gustafson 1989). In the present experiment, the results indicate that mobility of flupyradifurone is higher in unamended sandy loam soil as compared to unamended clayey soil, as more adsorption of flupyradifurone takes place in clayey soil which decreases the mobility potential of the compound and limits the compound to leach into the water table. High organic carbon-containing soils displayed greater sensitivity towards the sorption of any organic compound (Singh et al. 2018). Clayey soil used in the experiment had higher organic carbon content (92%) and clay content (28.6%) as compared to sandy loam soil (0.31% organic carbon content and 10.4% clay content).

In the initial 0–5 cm depth, 14.53 and 12.66 µg g$^{-1}$ residues of flupyradifurone were detected in FYM amended soil column of clayey and sandy loam soil, respectively, while in

**Fig. 2** HPLC chromatogram of Flupyradifurone. *Retention time (Rt) of Difluoroacetic acid (DFA)- 6.58 min
following 5–10 cm depth, 1.30 and 1.17 μg g⁻¹ residues were recorded for both the soil types. Furthermore, in 10–15 cm depth, residues of flupyradifurone in FYM treated clayey soil column was below quantifiable level (< 0.05 μg g⁻¹), whereas in sandy loam soil it was 1.02 μg g⁻¹. In contrast to unamended soil columns, the residues of flupyradifurone in 20–25 cm soil depth was below quantifiable level (< 0.05 μg g⁻¹) in case of clayey soil and 0.08 μg g⁻¹ in case of sandy loam soil (Fig. 3). This indicates that only 0.38% of the test compound is detected in 20–25 cm depth of sandy loam soil. The retention of flupyradifurone in the upper soil layer and restricted mobility in the presence of FYM could be beneficial aspects for plants (Table 2). The results reported are similar as in the experiment by Fenoll et al. (2010), where the use of organic amendment reduced the mobility of the studied pesticide. Joshi et al. (2019) reported that use of organic amendments (cereal straw and fresh cow dung slurry) increases soil organic carbon content and thereby facilitates degradation of the compound which ultimately reduces the chance of groundwater contamination due to leaching. In clay soil, integration with biochar decreased the leaching potentiality of the compound whereas no noteworthy result was found in the case of loamy soil (Larsbo et al. 2013). In case of silty loam soil, there is a higher possibility of leaching than in sandy loam soil, and it has been proved productive to minimize or prolong the leaching of a compound by addition of vermicompost (Fernandez-Bayo et al. 2015).

The leaching behavior of the flupyradifurone in clayey soil was compared to that in sandy loam soil through packed columns under varying flow conditions (Das and Mukherjee 2011). Discontinuous flow condition simulates intermittent rainfall. Downward movement and recovery of flupyradifurone from both the soils namely clayey and sandy loam through packed soil column, on percolating 160, 80, 60, and 40 mL water imitating 460.22, 233.10, 102.81 and 58.94 mm of rainfall has been shown in Fig. 4. Under this condition,

### Table 2: Effect of FYM under continuous flow pattern of water in clayey and sandy loam soil

| Depth of soil, D (cm) | Clayey soil (μg g⁻¹) | Sandy loam soil (μg g⁻¹) |
|-----------------------|----------------------|-------------------------|
|                       | T1 (Without FYM)    | T2 (With FYM)           | Mean (S × D)   | T1 (Without FYM) | T2 (With FYM) | Mean (S × D) |
| 0–5                   | 13.55 (67.76)       | 14.57 (72.86)           | 14.06A        | 10.12 (50.61)   | 12.65 (63.25) | 11.39A        |
| 5–10                  | 1.22 (6.11)         | 1.32 (6.58)             | 1.27B         | 2.1 (10.5)      | 1.17 (5.86)   | 1.64B         |
| 10–15                 | 1.15 (5.73)         | 0                       | 0.57C         | 1.29 (6.45)     | 1.02 (5.1)    | 1.16C         |
| 15–20                 | 0.73 (3.65)         | 0                       | 0.37D         | 1.04 (5.2)      | 0.55 (0.07)   | 0.79D         |
| 20–25                 | 0                    | 0                       | 0E            | 0.45 (2.23)     | 0.08 (0.38)   | 0.26E         |
| Mean (S × T)          | 3.33A                | 3.18B                   | 3             | 0.45 (2.23)     | 0.08 (0.38)   | 0.26E         |
| Mean (S)              | 3.25                 | 3.05                    | 3.09          |
| Residues in leachate (μg g⁻¹) | ND                 | ND                      | 0.08          | ND              |

*ND Not detected, Figures in parenthesis shows F-fractionation, T treatments (T1, T2), Mean values followed by a similar uppercase letter within a column are not significantly different at p < 0.05 level of significance*
flupyradifurone was relatively less mobile and residues were below the detectable limit in the leachate fraction of sandy loam soil. Upon leaching of the soil column with 40 mL of water, flupyradifurone was identified in 0–5 cm depth of soil column (Table 3). Residues of flupyradifurone leached to lower depths of the column on the addition of more quantity of water. When leaching was done with 60 to 80 mL water, residues of flupyradifurone were detected up to a depth of 10 to 15 cm. However, greater than 90% of the recovered compound was restricted in 0 to 5 cm depth of soil column. Leaching potential of flupyradifurone in clayey soil decreased as compared to sandy loam soil under differential water flow conditions. In general, analysis of soil cores for varying water flow expressed that on an increasing amount of water from 40 to 160 mL, residues get distributed, and mobility of the compound slowly gets increased and downward movement of the residues takes place from top to bottom of the soil column. Literature reports that for most of the pesticides, the fraction of the compound used stays in the top layers of the soil and is distributed in the soil column with the addition of water (Nauen et al. 2015). Similar results were observed in case of flupyradifurone. Therefore, the significant factors attributed to a higher leaching of pesticides in soils are high water solubility, low pesticide adsorption, sandy texture of soil and also immediate rainfall after pesticide spray (Tiryaki and Temur 2010; Das and Mukherjee 2011).

The present findings conclude that the downward movement of flupyradifurone in soil columns was higher in case of sandy loam soil as compared to clayey soil under Indian subtropical condition. Based on this analysis, it can be stated that flupyradifurone may leach down faster in sandy loam soil than in clayey soil but barely reach the water table. The leaching of flupyradifurone increases with the increasing amount of water and the residues continue to travel down to the lower depth. Therefore, flupyradifurone can travel downwards to a higher soil profile depth in sandy loam soil having higher porosity under high rainfall conditions and thereby maybe a possible source of groundwater contamination. In particular, the introduction of the low-cost organic amendment (FYM) could be a potential alternative for reducing mobility and thereby protecting the aquifers. The higher amount of organic carbon and clay content in case of clayey soil may account for reducing downward mobility of flupyradifurone in the soil column. The present findings may be used further for modelling the environmental fate of flupyradifurone under amended soil conditions and to optimize pesticide dose in combination with organic amendments as an attempt to prevent the chance of groundwater pollution. However, comprehensive analysis under field conditions in an undisturbed column is recommended to gain better insight into flupyradifurone leaching in the environment and impact of organic amendment on mobility behavior of the pesticide through the soil profile.

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