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Investigation of dissipation kinetics and half-lives of fipronil and metalaxyl in soil

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Abstract
The environmental half-lives of pesticides are investigated in order to determine the amount of pesticide accumulation and environmental pollution. However, for the majority of active compounds, these data are often lacking. To address this, we examined experimental data on the half-lives of fipronil and mealtaxyl in sandy loam soil in GCMS. According to the dissipation kinetics data, metalaxyl concentrations declined significantly over a period of 50 days, but fipronil concentrations declined gradually. Fipronil's and metalaxyl's experimental DT50 values are 84 and 65 days, respectively. Factors such as soil type, temperature, and time that impact the half-life of metalaxyl and fipronil.

Keywords: Fipronil, metalaxtyl, half-life, GCMS, sandy loam soil

Introduction
Several pests are controlled with chemicals known as pesticides in order to control them. Nematicides, herbicides, insecticides, fungicides, rodenticides, molluscicides, and plant growth regulators are among them [1, 2]. Pesticide use has increased dramatically in recent years, harming the ecology, particularly by poisoning soil and water. The scientific community has been working hard to create novel approaches to reduce pesticide pollution [3]. There is a connection between the environmental harm that pesticides inflict and their persistence. As a pesticide breaks down, new compounds are produced that may or may not be more dangerous than the original substance. Pesticides frequently decompose into minerals, water, and carbon dioxide [4].

It is necessary to take environmental half-life into account when calculating whether pesticides tend to accumulate in the environment [5]. The length of time required to reduce a pesticide's concentration in half is known as the half-life (DT50) of the pesticide. Compound half-lives are frequently expressed as intervals of time. Only organic substances are subject to the half-life notion. Because of degradation or dissipation, pesticide concentrations in the environment drop. First-order kinetics is frequently used to estimate the transformation rate of pesticides, meaning that the transformation rate is independent of the original pesticide concentration [6, 7].

The bulk of pesticides used in agriculture are sunk by soil, an essential part of the ecosystem [10, 11, 12]. Pesticides go through a number of stages of breakdown and change once they get into the soil13. Numerous physicochemical elements, including as adsorption/desorption, mobility, plant absorption, volatilization, and breakdown, are necessary for these activities [12, 14]. Therefore, to understand the behaviour and effects of pesticide degradation in the environment, a thorough understanding of the physical and chemical characteristics of pesticides is required [3]. Chemical hydrolysis, photolysis, and microbial degradation are some of the mechanisms underlying the degradation and transformation processes [10, 13].

Synthetic pesticides, herbicides, and fungicides were a major factor in improving agricultural output quality and quantity as well as in controlling pests. A pesticide should ideally be harmless for non-target species yet deadly for the pests it targets. Sadly, this isn't the situation. Pesticide usage, both widespread and haphazard, has had disastrous effects on the environment, other living things, and humans. Fipronil is a relatively recent broad-spectrum pesticide belonging to the phenyl pyrazole class. To combat a variety of agricultural pests, it is used as a seed treatment, foliar, bait, or soil treatment [15, 16, 17].

In several nations, Fipronil is approved for usage in both agricultural and non-agricultural settings. It is frequently used on a variety of crops, including rice, cotton, maize, vegetables, and fruits, to control thrips, termites, and click beetles [15, 17]. It is also used to get rid of fire ants, ticks, and fleas. Nevertheless, aquatic species are extremely hazardous to Fipronil [18]. Fipronil works by obstructing the chloride channel that is controlled by γ-aminobutyric acid (GABA). Numerous factors led to an increase in the use of Fipronil, including its lower application rate than conventional pesticides, environmental safety, effectiveness against insecticide-resistant species such as pyrethroid, carbamate, and organophosphate insecticides, and recent restrictions on organophosphate insecticides and prohibitions on organochlorines [15, 18]. Fipronil's half-life in soil varies greatly, ranging from 3 days to 7 months, depending on the soil's composition, temperature, moisture content, sterilisation, and chemical formulation [18, 19, 20]. Temperature increases markedly enhanced the breakdown of Fipronil in sandy loam and soil rich in organic matter [21]. Additionally, the non-sterile clay loam soil had a half-life of 9.72 days at 25 °C, whereas the sterile soil had a half-life of 33.51 days [17]. The exposure media is another element that affects pesticide half-lives. In peanut seedlings, Fipronil disappeared more quickly (1 day half-life) than it did in soil (more than 1 month half-life) [22].

Metalaxyl (methyl-N-(2,6-dimethylphenyl)-N-(2-methoxyacetyl)-DL-alaninate) is a systemic acylanilide fungicide that is used for managing plant diseases caused by oomycete pathogens such Phytophthora, Pythium, and downy mildew. Ciba-Geigy Corporation introduced it in 1977, and it has been in commercial usage since 1979 [23]. Metalaxyl's biological method of action is to suppress fungal RNA production [24, 25]. Metalaxyl has been shown to translocate in the xylem with a low lipophilic characteristic (logKow value of 1.6) [26, 27], implying that it can slowly penetrate the leaf cuticle and quickly travel into the plant. However, adding a surfactant can improve Metalaxyl's penetrating ability [28]. Once inside the plant, Metalaxyl degrades and accumulates before eventually dissipating systemically by moving upward with the transpiration stream [29]. Metalaxyl can be applied in a number of ways, including trunk painting, foliar spraying, and soil soaking. Along with other detrimental effects on the environment, the durian industry's extensive use of Metalaxyl to suppress P. palmivora has resulted in the emergence of isolates that are resistant to the drug. With a half-life spanning from 0.4 to 6.0 days, the dissipation patterns of Metalaxyl in grape, pepper, potato, tomato, cucumber, and Swiss chard plants were investigated [30, 31, 32, 33, 34]. Nevertheless, no evidence of Metalaxyl breakdown or dissipation in durian has been found.

The aim of this study was to determine the T1/2 of two pesticides, Metalaxyl and Fipronil, and ascertain how well they dissipated in sandy loam soil.

Materials and Methods

Two pesticides, viz. Fipronil and Metalaxyl of ≥ 98% purity, were obtained from Sigma Aldrich. Acetonitrile and methanol (GC-MS grade), and certified standard chemicals (MS grade, ≥ 99.9% purity) like, formic acid, acetic acid, ammonium acetate and ammonium formate were also purchased from Merck. The commercial formulations of the selected pesticides, Fipronil (FIPROX) and Metalaxyl (Matrix) were obtained from local market of Dantiwada.

Composite soil samples were taken in the field at a depth of 0-15 cm to determine the various physical and chemical properties of the soil at the SDAU experimental farm in Sardarkrushinagar. This farm has never used pesticides. The purpose of the samples was to determine the physico-chemical properties of the experimental sites. To find the percentage of sand, silt, and clay in the soil, the hydrometer method [35] was used. A pH metre was used to measure the electrical conductivity (EC) and soil pH in a suspension made of five grammes of soil and twenty-five millilitres of Milli-Q water. Wet-digestion Walkley and Black method was used to calculate the proportion of organic carbon in the soil and Ammonium Acetate method used for Cation Exchange Capacity of soil.

The following calculation was used to determine the necessary amount of trade formulation for each pot weighing 2 kg, containing 5 percent Fipronil and 35 percent Metalaxyl.

$$Rh = \frac{Ai \times At}{Ci} \times 100$$

Rh = Required quantity of trade formulation of pesticide (mL or g or ha^{-1})
Ai = Quantity of active ingredient to be applied (g)
At = Quantity of soil to be treated (g)
Ci = Concentration of active ingredient in the trade formulation.

Each pot was filled with the necessary amount of water, and the measured amount of the corresponding pesticide was thoroughly mixed and added to the pots.

Samples were processed on 0 (1 hour), 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 45 and 50 days after treatment and analyzed for the residues of Fipronil and Metalaxyl by an in-house developed method utilizing QuEChERS approach.

Prior to sampling time of residue study of Fipronil and Metalaxyl, sampled soil were subjected to recovery study. Soil were spiked at 3 different levels i.e. 0.05, 0.25 and 0.5 $\mu g\ g^{-1}$.

The detection of Fipronil and Metalaxyl residue were verified using gas chromatography-mass spectrometry (GC-MS). The mass-to-charge ratio (m/z) and retention time (Rt) were used to validate the presence of Fipronil and Metalaxyl. After injection, the samples had been confirmed to be in electron ionisation (EI) mode. The m/z ratio of the total ions chromatograph (TIC) and the fragmentations of selective ions monitoring (SIM) were used to identify the pesticide. These were compared to fragmentations of various mass numbers that were generated using the metalexyl and Fipronil standards. Fipronil and Metalaxyl parent compounds were identified to have molecular masses of 437.1 (confirmed by m/z: 351, 367 at Rt 16.40 min) and 284.1 (confirmed by m/z: 237, 198 at Rt 11.9 min).

Results and Discussion

Linearity study

A linearity study was performed to determine the performance of MS detector of GCMSMS. For the linearity study, a graph of detector's response v/s concentration was plotted (Figure 1). To establish the linearity of Fipronil and Metalaxyl on MS Detector, equal volume of seven different concentrations of the mixture of Fipronil and Metalaxyl viz., 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm were injected and their corresponding responses were recorded. As per the data obtained in linearity study, Fipronil and Metalaxyl were found linear in the range of 0.01 to 1.0 ppm. The R^2 value obtained from the correlation equation was calculated by adopting positive linear correlation model (y = a + bx) were 0.9996 and 0.9770 for Fipronil and Metalaxyl, respectively.

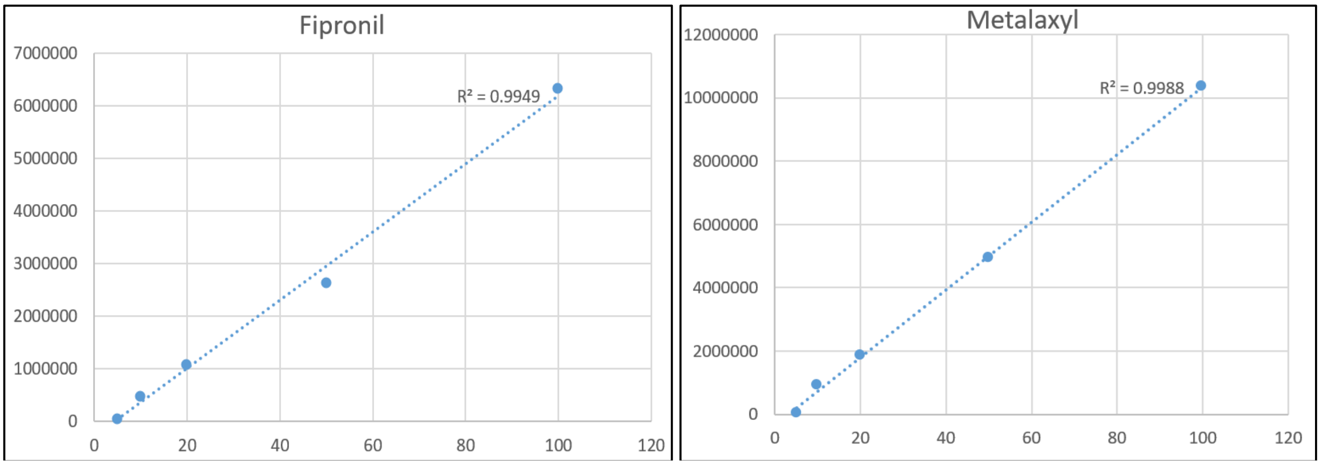


Fig 1: The linearity of Fipronil and Metalaxyl on MS Detector

LOD and LOQ

The final volume of the sample was considered as 1 g per ml for soil. The sample injected in GC-MS/MS was 10.0 µL for soil considering all these parameters the LOQ (S/N ratio >10) was worked out to be 0.05 ppm (0.05 µg/g) and LOD (Method Detection Limit-MDL) was 0.02 ppm i.e. 1/3 time of LOQ.

Recovery study

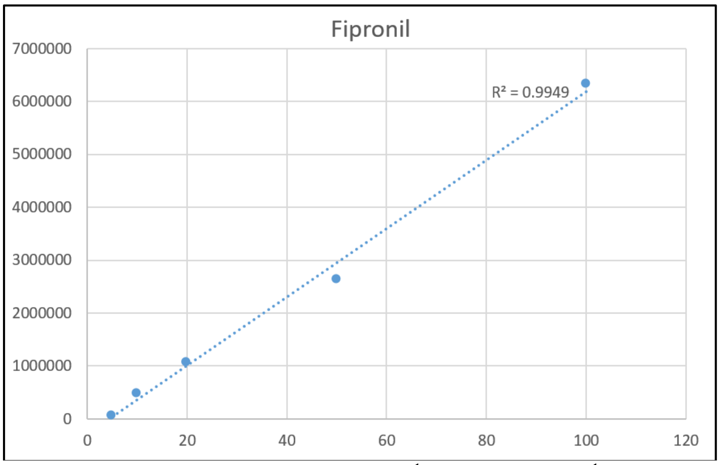
The results obtained from the recovery study revealed that mean recovery of Fipronil from soil ware 104.67, 99.07 and 98.47% at 0.05, 0.25 and 0.5 ppm spiking levels, respectively. percent RSDwr recorded for the corresponding for spiking levels was 4.87, 1.05 and 2.14%.

While the mean recovery of Metalaxyl was 94.00, 91.33 and 88.00%, at 0.05, 0.25 and 0.5 ppm spiking levels, respectively. The percent RSDwr recorded for the corresponding spiking levels was 13.58, 9.31 and 6.91%.

Dissipation of Fipronil and Metalaxyl

To assess the persistence of Fipronil and Metalaxyl, the pesticide was treated to the soil at lower @ 200 g a.i./ha and higher @ 600 g a.i./ha the recommended dose. The soils were sampled at 0 (2 hours after application) 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 45 and 50 days after application of pesticides. The result revealed a reduction in residues levels of all the two pesticides in soil with time.

At zero-days, the initial deposit of Fipronil was found to be 80.53 and 258.70 µg kg^{-1} with treatment Fipronil @ 200 g a.i. ha^{-1} and 600 g a.i. ha^{-1}, respectively. The corresponding residues levels of Fipronil on 3rd day were 80.14 and 254 µg kg^{-1}. The residue level further decline and reached 72.73 and 244.87 µg kg^{-1} on 10 days. And passed at near BDL level on 50 days at first treatment and alternative treatment have 178.13 µg kg^{-1} Fipronil residue level.



* mean value of Fipronil @ 200 g a.i. ha^{-1} and 600 g a.i. ha^{-1} used

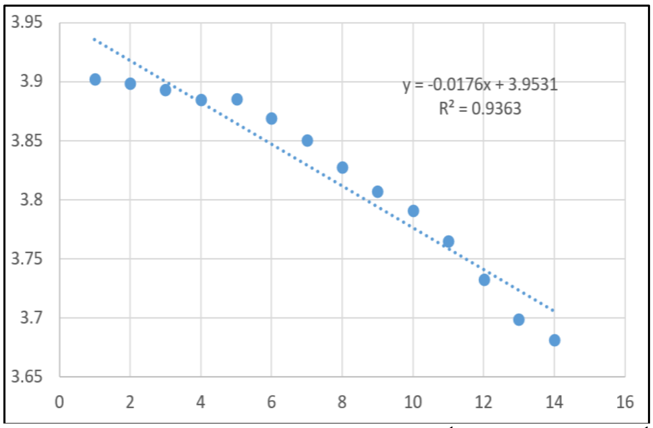
Fig 2: Residue level of Fipronil in soil*

The dissipation pattern of Fipronil in the soil is depicted as showing a slower rate with first-order kinetics. Regression equations were calculated y =-0.0038x + 3.9052 (R^2 = 0.9815) for first treatment and y =-0.0035x + 4.4216 (R^2 = 0.944) for second treatment. Based on the regression equation the average half-life (t1/2) of Fipronil was worked out to be 84 days.

In general, the pesticide type, soil type, sterilisation, temperature, pH, dose, application type, interval between treatments, and environmental factors all affect the DT50 of the pesticide in soil [36]. In laboratory conditions, Fipronil had a half-life of 132 days. The laboratory and field studies of Ying and kook revealed that environmental factors such as solar exposure, soil pH, moisture content, and microbial activity impacted Fipronil breakdown [38] Furthermore, the half-life of Fipronil in soil decreased from around three months to forty-three days when the temperature was raised from 4 to 30 °C [37]. According to comparable research by Ying and kook (2001), Fipronil and its metabolites remained in loam soil and decomposed slowly, with an average half-life of 188 days. The same phenomena have been observed in our study.

Metalaxyl application @ 200 g a.i. ha^{-1} and @ 600 g a.i. ha^{-1} ecorded initial deposits of 79.82 µg kg^{-1} and 258.18 µg kg^{-1} which declined to 78.30 µg kg^{-1} and 252.43 µg kg^{-1} within 72 hours. Thereafter the loss of residues was gradual and reached 74.05 and 238.36 µg Kg^{-1} on 10 days after application, respectively. At 35 days of the following application, the residue level was BDL for the treatment @ 200 g a.i. ha^{-1} and 155.08 µg Kg^{-1} for the treatment @ 600 g a.i. ha^{-1}.

The dissipation followed first order kinetics which is depicted in Figure 2 The regression equation for dissipation of Metalaxyl application @ 200 g a.i. ha^{-1} was calculated as y =-0.0044x + 3.9109 with half-life (t1/2) of 68.40 day and R2 value 0.9895. Whereas regression equation for dissipation of Metalaxyl application @ 600 g a.i. ha^{-1} was calculated as y =-0.0046x + 4.4252 with half-life (t1/2) of 65.43 day and R2 value 0.94.



* mean value of Metalaxyl @ 200 g a.i. ha^{-1} and 600 g a.i. ha^{-1} used

Fig 3: Residue level of Metalaxyl in soil*

Premasis Sukul and Michael Spiteller (2001) studied the biodegradation of Metalaxyl in six different Indian soils with varying soil properties under laboratory conditions. They observed that the half-life of Metalaxyl varies from 36 days to 502 days. And they suggest that neither mineralization nor volatilization is a major route of Metalaxyl dissipation [39]. In clay loam soils with natural microbiota, Marucchini and Zadra, found half-lives of 43 and 25 days for Metalaxyl and Metalaxyl-M residues, respectively [40]. The native microbiota and soil type have a major influence on the Metalaxyl half-lives in soil. Further research is necessary to demonstrate how soil qualities, such as biological, physico-chemical, and environmental factors, affect dissipation efficiency.

Conclusion

In sandy loam soil, this study provides an extensive evaluation of the environmental half-lives and dissipation kinetics of metalaxyl and fipronil at two application rates (200 g a.i. ha-1 and 600 g a.i. ha-1). The findings show that metalaxyl breaks down more rapidly, with half-lives of 65.43 days at 600 g a.i. ha-1 and 68.40 days at 200 g a.i. ha-1.These degradation patterns followed a pronounced linear line, as indicated by high R^2 values (0.9895 and 0.94). Fipronil, on the other hand, degraded more slowly, with an estimated average half-life of 84 days. With corresponding regression equations y =-0.0038x + 3.9052 (R2 = 0.9815) for the first treatment and y =-0.0035x + 4.4216 (R2 = 0.944) for the second treatment, the dissipation of fipronil also followed first-order kinetics. These results demonstrate how application rates have a major impact on pesticide persistence in soil, with higher application rates decreasing pesticides degradation. Fipronil's slower rate of dissipation highlights issues over its accumulation potential and long-term effects on the environment.

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