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Insecticide Pyriproxyfen and its Metabolites' Ultimate Fate and Toxicological Effects on the Soil Ecosystem

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Abstract

Pyriproxyfen (PYR), an insecticide with high insect pest specificity and low mammalian toxicity. The environment may be exposed to the creation of roughly 10 metabolites as a result of its breakdown. According to reports, some of the metabolites have particularly poisonous and movable properties. Serious worry may be raised by their ability to contaminate the environment and cause poisoning. The information on how various metabolites are formed in soil, their ultimate fate, and their toxicological repercussions is scarce in the literature that is currently available. By studying the dissipation behaviour of various metabolites in soil under sub-tropical agro-climatic conditions in north India, we were able to study the metabolic pathway of PYR. PYR was applied to the soil in field settings at (T1) 100 and (T2) 200 g a.i. /ha. Periodically, samples were taken, prepared, and examined using GC-MS tandem mass spectrometry. Six metabolites were created during the decomposition process: 4-OH-PYR, POP, POPA, 4-OH-POPA, PYPA, and PYPAC. On the first day of PYR application, the majority of metabolites manifested fairly early and reached their peak concentration. However, despite having varying half-lives that ranged from 2.6 to 30 days, their leftovers lingered for more than 30 days. The results of the toxicological analysis showed that the soil enzymes sucrose, catalase, urease, and dehydrogenase were extremely sensitive to the metabolites C, E, and F. Adult honeybees are not adversely affected by PYR. Additional research is required to stop metabolites' adverse effects on unintended organisms and the environment because of their persistent behaviour and toxicological effects.

Keywords: Enzyme activity; Pyriproxyfen; Metabolites; Persistence Soil; Toxicity to Honey Bees

Introduction

Application of pesticides to protect crops is a crucial component of contemporary agriculture. Annual crop losses from insect pests and plant diseases are estimated to be between 15% and 20%. Due to their long-lasting new method of action, lower application dose, good efficacy, and least toxicity towards non-target organisms, synthetic pesticides play a vital role in modern agriculture. Their non-selectivity toward severe pest resistance is another factor in their popularity, especially in light of the fact that most insect pests are evolving resistance to traditional pesticides, which are failing to stop the spread of secondary pests and diseases. Despite the advantages of using insecticides. Despite the advantages of using pesticides to increase food safety, their excessive and careless use may cause serious environmental and health problems [1].

A significant portion of the insecticides used globally find their final resting place in soil. Pesticides are introduced to the soil after application through spray drift, rain washing plant surfaces, etc. Plants may absorb pesticides from the soil or they may breakdown into different chemical forms (metabolites). Pesticide dispersion and metabolism in the environment are greatly influenced by a number of physico-chemical characteristics of soil, including moisture content, organic carbon content, texture, ion exchange capabilities, pH, temperature, microbial activity, and light exposure. But sometimes degraded products may turn out to be more harmful than their parent (WHO, 2006). Investigating the environmental destiny of pesticides and their degradation products or metabolites is crucial in order to reach the genuine risk assessment [2, 3].

Materials and Method

Chemicals, reagents and standards

Sumitomo Chemicals Ltd. India provided PYR with the technical grade analytical standard. Eight metabolites—A, B, C, E, F, G, H, and I—were created in the current investigation and put to the test for purity in the lab using the techniques recommended by Liu et al (2019). The synthetic metabolites' purity varied from 95.5 to 98.7%. Metabolite D was not examined since GC-MS/MS had trouble detecting it. PYR formulation was acquired from a neighbourhood market. The Merck Company provided all of the chemicals used in sample preparation and analysis (Darmstadt, Germany). In 100 mL of acetonitrile, stock solutions (1000 g/mL) of PYR and its metabolites were created. By using the proper dilution method, serial dilutions of different concentrations from 0.0001 to 1.0 g/mL were created from stock solution [4, 5].

Sample preparation

With a few minor adjustments, the matrix solid phase dispersion (MSPD) approach as recommended by Kumari et al. (2008) was used to extract the soil samples. A representative 20 g soil sample was collected and combined with 10 g silica gel, 0.3 g charcoal, and two layers (2–3 cm) of anhydrous sodium sulphate in a glass Petri dish before being packed in glass columns. Ethyl acetate: hexane (7:3, v/v) in 150 mL was used to elute the analyte from the columns. Separate eluents were gathered and placed in 250 mL glass reagent vials. The extracts were first reduced in volume by rotary evaporation (Heidolph) and then blown to dryness (Crescent Scientific India) before being dissolved in 2 mL acetonitrile. The samples were filtered using a disposable Millipore 0.22 m. Before residue analysis, place polypropylene syringes nylon syringe filter [6].

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Instrumentation for analysis

The sample was analysed using a GC-MS tandem mass spectrometry (Agilent 7890 A series with 7000 GCMS/MS detector). The injection port's temperature was kept at 280°C. The study was performed using an HP-5capillary column with dimensions of 30 m x 0.32 mm i.d. x 0.25 m film thickness. The ramping oven temperature was set to increase from 70°C (2 min hold) to 150°C (0 min hold), to 200°C (0 min hold), and then to 280°C (8 min hold) (2 min hold). Source temperature of the detector was 230°C; emission current was 35 A; energy was 70 eV; repeller voltage was 11 V; ion body was 12 V; extractor was 7.2 V; and ion focus was 7.4 V. The temperature of quadrupole one (MS1) and quadrupole two (MS2) were both kept at 150°C. The fuel was helium. At a flow rate of 1 mL/min through the column, helium served as the carrier gas. At flow rates of 2.25 and 1.15 mL/min, respectively, helium and nitrogen were used as the collision flow and quench flow in the collision cell. The high-pressure pump's vacuum was 2.23 10-5 torr, while the rough vacuum was kept at 1.51 102 torr. 2 l of injection volume was administered in a pulsed split-less mode. Over a m/z range of 100-500, MS spectra were collected. The instrument detection limit (IDL) was set at 1 g/L. By graphing the relationship between residue concentration and time, the kinetics of PYR and its metabolites were identified [7].

In order to find the equations for the best-fit curves, the maximum squares of the observed correlation coefficients were used. Table 1 provides information about various MRM programming parameters used during the investigation. In the Supplementary Material, chromatograms and mass spectra of PYR and its various metabolites have been supplied (S1).

To achieve a final concentration of 1 g/g, 100 g of dry soil were combined with the appropriate amount of PYR and its metabolites standard solutions in 250 mL beakers. Without using PYR or any of its metabolites, the control samples were collected in a similar manner. The beakers were covered with aluminium foil and maintained at 25 2 °C with 90% relative humidity. By spraying a calculated amount of water at 2-3 day intervals, the field capacity moisture level of 1/3 bar tension was maintained throughout the trial period. On days 0, 30, and 60, soil samples were collected. The measurement of sucrose activity followed Guan's (1986) description with a few minor adjustments. 15 mL of sugar and 5 mL of water were added to two grammes of soil. Five drops of toluene and two grammes of dirt were mixed with 15 mL of sucrose, 5 mL of phosphate buffer (pH 5.5), and 15 mL of sucrose. Following a shake, the substance was put in an incubator (MEMMERT GmbH+ Co. KG, Germany) for 24 hours at 370.1°C. Following filtration, 1.5 mL of salicylic acid was added to 0.5 mL of the filtrate, which was then heated in a water bath for 5 minutes at 100°C. Following the proper dilution, sucrose activity was evaluated colorimetrically at 508 nm. Using the Maxwell and Bateman (1967) method, the catalase activity was determined using 2.95 ml of 0.06 M phosphate buffer, 10% (w/v) H2O2, and 0.05 ml of enzyme extract. Using a UV-visible spectrophotometer, the decrease in absorbance was recorded at 240 nm [8, 9].

Discussion

The recommended administration of T1 dosage had no negative effects on honeybees, and a 31% mortality rate was seen in the first 10 days after feeding. However, the T2 dose produced a greater mortality of 40.2% in the analogue days count and was marginally more harmful than the T1 dose ($p>0.05$). In neither of the two application dosages, LC50 was attained (Fig. 5). PYR is a juvenile hormone analogue and acts as an insect growth regulator, with more acute toxicity at larval or pupal stages than at the adult stage, as predicted by its method of action. We focused on what would be more useful in terms of its toxicological effects after application in the field for insect pest management rather than studying the toxicological evidence of PYR during early development stages of honeybees. Previous research from Bitondi et al. 1998 and Zufelato et al. 2000 provides strong support for our findings about the toxicity to adult honeybees [10].

Conclusion

We investigated the ultimate destiny, toxicity, and various metabolites of the pesticide PYR that were produced during the process of soil degradation in north India's subtropical agroclimate. With a half-life between 3.7 and 4.5 days, PYR decomposed quickly, and on 45 DAA, essentially no residues remained. The observed half-life was shorter than several earlier studies from other parts of the world, which may have been caused by variations in climatic conditions and soil types. Six metabolites were produced in soil during degradation: 4-OH-PYR (A), POP (C), POPA (E), 4-OH-POPA (F), PYPA (G), and PYPAC (H). Two significant metabolites, however—5"-OH-PYR (I) and DPH-PYR (B)—were not found in our investigation. Their short lifespan is likely to be the cause of their disappearance.

Their brief appearance on a day between frequent sampling meant for persistence investigation may be the likely cause of their disappearance. The majority of metabolites appeared on the PYR application day and peaked in concentration on 1 DAA in soil, according to a study on how they dissipate. With the exception of 4-OH-PYR, all of the metabolites had half-lives that ranged from 2.6 to 30 days in soil. The eventual destiny of these metabolites and PYR in soil was also discovered to depend negatively on a number of environmental parameters, including light intensity, soil particle size, pH, cation-anion exchange capacity, moisture content, temperature, and microbial activity. However, the parent PYR and all of the metabolites pose a concern of extended persistence and environmental loading/contamination.

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Potential conflicts of interest

No conflict or competing interests in the publication of this paper.

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