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# Dissipation kinetics and distribution of fipronil and its toxic metabolites in Banana, cv. Nendran (AAB)

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**Abstract**
In the study under taken to analyze the dissipation, metabolism and persistence of fipronil in banana, cv. Nendran (AAB), in red loam soils (AEU 8-southern laterites) of Trivandrum, Kerala, India, with treatments as, absolute control (No application of fipronil), recommended practice of soil application of 30 mg a.i. of fipronil per plant per application, applied 3 times on 0, 60 and 150 days of planting and double dose of fipronil, in samples *viz*., leaves, fingers bunches and flower bud, central core of pseudo-stem and corm sampled and analyzed for residue at definite time intervals revealed that QuEChERS method can be conveniently applied for extracting the residue from various parts of banana plant and has resulted in satisfactory values for validation parameters.
Residue of fipronil and their toxic metabolites in the 1st, 2nd and 3rd leaves of banana on penultimate day of completion of pre-bunching application was found to be below the detectable levels (BDL) throughout the period of sampling and this may be attributed to low absorption, very fast metabolism of very low levels of absorbed molecule and mobility. However, on 40th day the fipronil was detected in the 4th leaf to the extent of 0.034 µg $g^{-1}$ and was not detectable (i.e., BDL) on 50th day indicating safety from residue of harvested produce for consumption especially at recommended dose. Sample matrices of blossom bud, flower bract alone, bunch on 15th day of emergence, bunch on 30th day of emergence, peel, bunch on harvest, pseudo stem and corm were below detectable level of fipronil and their metabolites and even with an additional application of treatment on the day of bunching also did not register any detectable level of fipronil and their metabolites.

**Keywords:** Fipronil, agrochemicals, pesticide, dissipation, residue, banana

## Introduction
Fipronil, a systemic insecticide belonging to Phenylpyrazole group, granular form of which is recommended as a substitute for two banned insecticides for the control of banana rhizome weevil (KAU, 2015) [6] in Kerala. It is a broad-spectrum insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system. Fipronil contamination of ground water was lesser (Singh *et., al* 2015) [8], however was found to persist in sandy loam and clay loam soils for several days (USEPA, 1996, Zhu *et al.*2004) [9, 10] and suggested to be affecting non target organisms especially those inhabiting soil and aquatic habitats (Bonmatin *et al.* 2015) [3]. The safety, absorption, translocation and dissipation of fipronil both in soils as and plants need to be studied as nendran variety of banana cultivation is widely practiced in red loam soils in Kerala. Since almost all parts of the crop are consumed, the studies on residue status and disappearance pattern of this chemical need to be evaluated. Hence a study on the absorption, translocation and persistence of fipronil in different parts of banana plant when applied in the rhizosphere of banana grown in the red soil was under taken.

## Materials and Methods
A field experiment designed as per randomized block design (RBD) was undertaken in the red soils, Kaolinitic isohyperthermic, typic kandiustults (GOK, 2007) [4] at Instructional Farm of College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India.

Soils of the experimental plots analyzed as per standard procedures was moderately acidic with a pH of 5.7, electrical conductivity of 0.4 $dSm^{-1}$, having medium organic carbon content of 1.5 percent, with high available P and K (196.1, 358.4 kg $ha^{-1}$ respectively). HCl extractable essential micronutrients *viz*., Fe, Zn, Mn, Cu were in the sufficiency range. However, the sandy loam soil (with silt and clay content were 8.7 and 19.5 percent respectively was deficient in secondary nutrients *viz*., Ca, Mg and micronutrient B) were cultivated and managed as per package of practices and recommendations for crops, KAU, 2011 [5] except for the study treatments as $T_1$- Absolute control (No application fipronil), $T_2$- Recommended practices ($RP_f$) of 30 mg a.i. of fipronil per plant, applied thrice *viz*., on 0, 60 and 150 days of planting and $T_3$– Double dose of $RP_f$ (i.e., $RP_f$ x 2), applied as per above schedule of $T_2$.

**Chemicals and reagents**

Certified reference standards of fipronil (purity 98.4% w/w) procured from, Sigma Aldrich, Switzerland, Fipronil desulfinyl, (purity 98.5% w/w), Fipronil sulfide (purity 98% w/w/) and Fipronil sulfone (purity 99.7% w/w) obtained from Bayer crop science, Germany were used as analytical standards. The solvents and other reagents *viz*., acetone, ammonia solution, dichloromethane (HPLC grade), methanol, acetonitrile (LC-MS/MS grade), magnesium sulphate (anhydrous), primary Secondary Amine, Sodium Chloride (AR grade), Sodium Sulphate (anhydrous), Calcium Chloride anhydrous (AR grade), Florosil - chromatography grade) were either of HPLC / LC-MS/MS or AR grade was used for residue analysis. Sodium sulphate, sodium chloride and magnesium sulphate were activated prior to use. All equipment and instruments were calibrated to meet performance criteria. Commercially available granular form of Fipronil (Regent 0.3G) formulation marketed by Bayer crop science, India was used for soil application in the experimental plot.

**Instrumentation**

The cleaned extracts were analysed on an Ultra Performance Liquid Chromatography equipped with Triple Quadrupole Mass Spectrometer (Sciex- API 3200). The samples as well as standards were injected into the equipment for spectral matching and quantification of residues.

**LC-MS System**

The ACQUITY (Waters, USA) UPLC system was used for chromatographic separation with a column (100mm x 2.1 mm, 5 micron particle size) maintained at 40 °C. Elution was done using two eluents (solvent mixtures), *viz.*

**A:** 10 per cent methanol in water + 0.1 per cent formic acid + 5 mM ammonium acetate

**B:** 10 per cent water in methanol + 0.1 per cent formic acid + 5mM ammonium acetate

The optimized gradient elution for flow rate of the solvent system with a flow rate of 0.75 mL/ min was obtained with 80 percent flow from reservoir A* and 20 percentage from B. The gradient elution of the was monitored for 8 minutes at differential flow rates, for A being 50, 30, 10, 20, and 80 percent at 1, 2, 4, 6 and 8 minutes of injection. The effluent from LC was then introduced into triple quadrupole, API 3200 (ABSciex, USA) MS/MS system. System contains ion source gas 1 (at 50 psi), ion source gas 2 (at 40 psi) and curtain gas (at 30 psi) with ion source temperature of 550°C and ion spray voltage source of -5000 V. The residues were quantified in MS/MS system. For each analyte, two selective reaction monitoring (SRM) transitions were taken.

**Table 1:** LC-MS/MS parameters and selection of SRM for quantitative and qualitative ions for fipronil and its metabolites in analyte matrix.

| Instrument parameter | Molecule | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fipronil desulfinyl | | | Fipronil sulfide | | | Fipronil | | Fipronil sulfone | |
| Retention time (minutes) | 3.08 | | | 3.28 | | | 3.17 | | 3.43 | |
| | Quan | Qual | | Quan | Qual | | Quan | Qual | Quan | Qual |
| Q1-Precursor ion | 386.9 | | 389 | 434.9 | | 421 | 434.9 | 419.00 | 451 | |
| Q3 Product ion | 281.9 | 350.9 | 352.8 | 330 | 250 | 384.9 | 330 | 261.90 | 414.8 | 281.9 |
| DP (Volt) | −35 | −35 | −36 | −36 | −36 | −37 | −36 | -30.00 | −29 | −29 |
| EP (Volt) | −5 | −5 | −6 | −6 | −6 | −6 | −6 | -9.00 | −5 | −5 |
| CEP (Volt) | −26 | −26 | −26 | −23 | −23 | −25 | −23 | -38.00 | −24 | −24 |
| CE Volt | −43 | −26 | −26 | −23 | −36 | −19 | −23 | -17.00 | −23 | −37 |
| CXP- (Volt) | −6 | −6 | −6 | −6 | −6 | −7 | −6 | -6.00 | −7 | −6 |

Quan- Quantitative;Qual-Qualitative; Q1-Precursor ion; Q3- Product ion; DP-declustering potential;
CE-collision energy; CXP-collision cell exit potential; EP-entrance potential; CEP-collision cell entrance potential

$$\text{Pesticide residues in the sample were calculated in } (\mu g\ g^{-1}) = \frac{\text{Peak area of sample} \times \text{Concentration of standard injected} \times \text{Dilution factor}}{\text{Peak area of standard}}$$

Laboratory experiments were carried out to ascertain the accuracy, relative standard deviation (RSD value), linearity and limit of quantitation (LOQ) of the methods followed for estimation *viz*., QuEChERS (Anastassiades, 2007) [1] and to ascertain the method to be followed for extraction and purification of residues from the field samples.

The samples were cut into small pieces of 250 g per replicate and it was macerated in a blender. To the 10 g of the ground sample taken in 50 ml centrifuge tube, 20 ml of HPLC grade acetonitrile was added and kept at -20°C for 20 minutes. The sample was then homogenised (Heidolph Silent Crusher-M) at 14000 rpm for 3-4 min. Activated sodium chloride (4.5 g) was added to the homogenised sample and vortexed for 2 min on a rotospin and then centrifuged for 5 min at 2,500 rpm. An aliquot of 12 ml clear upper layer of the sample was transferred into a 50 ml centrifuge tube prefilled with 5 g pre-activated sodium sulphate and vortexed for 2 min for removing traces of moisture, if any. The extract was cleaned up by dispersive solid phase extraction (DSPE). From this, 8 ml of the upper layer was transferred in to a 15 ml centrifuge tube containing 0.125 g PSA, 0.8 g anhydrous magnesium sulphate, 0.05g end capped C18-octadecylsilyl and 0.025g graphitized carbon black. The mixture was again vortexed for 2 min and centrifuged for 5 min at 2,500 rpm. From the

cleaned supernatant liquid extract, 5ml was transferred to turbovap tube and evaporated to dryness at 40 °C and 7.5 psi nitrogen flow under a gentle stream of nitrogen using turbovap setup. The residue was then reconstituted in 2 ml of methanol and filtered through a 0.2-micron PVDF syringe filter (13mm) which was used for UPLC-MS/MS analysis.

## Results and Discussion

Mean of method validation parameters for fipronil and its metabolites with matrix match samples of banana leaves, pseudo-stem, bunch finger, flower and corm, collected from the specially maintained control plots plants at the respective stages of harvest for parameters *viz*., percentage recovery, relative standard deviation (RSD) value of fipronil desulfinyl, fipronil, fipronil sulfide, and fipronil sulfone obtained through QuEChERS method ranged from 80.0 to 119.9 percent, while the corresponding values for precision ranged from 0.4 to 12.9 percent, which were in the acceptable range. However, wide range of variation for Percentage recovery (Accuracy), RSD value (Precision) in different matrices have been observed (Table: 2). Dutta (2006) obtained a recovery of fipronil and its metabolites from cabbage samples which ranged from 80.84 to 88.3 +6.8%.

**Table 2:** Mean recoveries of fipronil and metabolites using QuEChERS from diverse plant parts of banana after fortification at 0.01, 0.02, 0.05 and 0.1 µg $g^{-1}$ levels of spiking, respectively.

| Sl. No | Plant part | | Fipronil Desulfinyl | Fipronil sulfide | Fipronil | Fipronil sulfone |
|---|---|---|---|---|---|---|
| 1 | Fingers of bunches | Accuracy | 83.9-101.3 | 93.6-105.8 | 80.9-113.2 | 92.9-101.9 |
| | | Precision | 2.8-12.9 | 5.5-12.8 | 4.1-12.5 | 2.2-12.8 |
| 2 | Leaves | Accuracy | 80-97.1 | 94.8-112.9 | 99.6-119.9 | 90-114.7 |
| | | Precision | 3.9-12.8 | 3-13.3 | 2.3-14.7 | 1.8-12.4 |
| 3 | Pseudo stem of banana | Accuracy | 83.3-105.9 | 94.8-112.9 | 80.6-114.7 | 82.7-107.3 |
| | | Precision | 1.1-16.2 | 0.4-17.1 | 2-13.9 | 1.5-16.1 |
| 4 | Flower bud of banana | Accuracy | 82.5-106.9 | 88.7-115.3 | 80-100.3 | 93.4-115.2 |
| | | Precision | 0.7-12.9 | 0.5-13.4 | 0.4-12.9 | 0.5-13.1 |
| 5 | Corm of banana | Accuracy | 89-108.4 | 83.4-116.6 | 81-117.1 | 80.2-108.6 |
| | | Precision | 5.1-12.6 | 6.1-12.5 | 2.5-14.8 | 5.8-15.3 |

Beevi *et al.* (2014) [2] contented that, the recovery of pesticides in LC-MS/MS ranged between 70-120 percent may be treated as satisfactory on obtaining for values in that range for all the 26 compounds including fipronil (71.13 percent), when tested at the respective LOQ. The Linearity and Limit of Quantitation (LOQ) for recovery of residue from different parts of banana were was 0.01 to 0.1 µg $g^{-1}$ and 0.01 µg $g^{-1}$ respectively.

Samples of 1st, 2nd, 3rd and 4th leaves of banana collected for residue analysis of fipronil and its metabolites at two different levels of application in soil *viz*., normally recommended dose and its double rate are were found to be below detectable limit (BDL) even on day the 50 after application and the same for 4th leaves of banana are depicted in the table-3.

**Table 3:** Residue of fipronil and its metabolites in 4th leaf of banana, µg $g^{-1}$

| Treatment and molecule | Time interval in days (No of days after completion of treatment dosing on150th day after planting) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before 0th ** | ***0th | 1st | 3rd | 5th | 7th | 10th | 15th | 20th | 25th | 30th | 40th | 50th |
| $T_1$ control* | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| $T_2$- (POP) Fipronil desulfinyl$^{\alpha}$- | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| Fipronil sulfide$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| Fipronil$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| Fipronil sulfone$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| $T_2$-Total Fipronil* | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| $T_3$-(2 x POP) Fipronil desulfinyl$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| Fipronil sulfide$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| Fipronil$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | **0.034** | BDL |
| Fipronil sulfone$^{\alpha}$ | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| $T_3$-Total Fipronil* | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | **0.034** | BDL |

Foot note: *mean of total fipronil were BDL; **-150th day before treatment imposition;
*** - 2 hours after 3rd application and BDL-below detectable limit; $^{\alpha}$Fipronil and its metabolites; POP: - Package of practices Recommendations: Crops, KAU

Mortensen *et al* (2015) [7] too suggested that fipronil cannot be classified as systemic insecticide, though there are reports which suggested that fipronil is taken up by the root and translocated into the plant (Bonmatin *et al*., 2015) [3]. However, no toxic metabolite of fipronil was detected in any of the leaf samples, indicating a faster metabolism and dissipation of fipronil in banana, both at normal and double doses, thereby ensuring safety from its toxic residues in leaf samples. The results are in agreement with the findings of Dutta *et al.*, (2008), in cabbage where fipronil got dissipated with a half-life of 7.5-7.6 days and suggested that the fipronil applied cabbage is safe for consumption, only when it is soil incorporated.

Application of fipronil even at double doze did not leave any residues in first 3 banana leaves, indicating that there is no effective symplastic translocation of fipronil or its toxic metabolites to these leaves when applied to the rhizosphere soil. This may also be attributed to the nature of the crop having height 2 meters. At double the recommended dose of application fipronil was present in the fourth leaf (table: 4) only on 40th day of application (0.034 ppm) and the same was BDL by 50th day.

**Additional soil application of treatments after bunch emergence**

Banana plants maintained to study the residue and persistence of fipronil after application on 0th, 60th and 150th day were subjected to an additional dose of fipronil (in $T_2$ and $T_3$ treatment), respectively during bunch emergence. The residues present in various plant parts are presented in Table 4. Even after additional application of treatments just after bunch emergence, no residue of fipronil and their metabolites were present in various harvested parts of banana plant.

**Table 4:** Residue of fipronil and their metabolites in banana at harvest due to additional application of treatment at bunching.

| Treatment and molecule | Mean residue (µg g$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Flower bud* | Peel alone | Fingers of Bunch on Harvest | Pseudo stem | Corm |
| $T_1$ Control | BDL | BDL | BDL | BDL | BDL |
| $T_2$- 30 mg a.i. fipronil /plant | BDL | BDL | BDL | BDL | BDL |
| $T_3$- 60 mg a.i. fipronil/plant | BDL | BDL | BDL | BDL | BDL |

***** Harvested 3 days after complete emergence of fruit forming fingers

**Pseudo-stem injection at five times the recommended dose on bunch emergence stage**

The residue of fipronil in bunches following application at five times the recommended dose applied as injection in the pseudo stem at the time of bunch emergence using special syringe are presented in the table-5. It is obvious that all residue would have dissipated to BDL in the sample on 15th and 30th day of emergence of bunch and hence residues of fipronil and its metabolites were not detected in the samples. The residue of fipronil and its metabolites were not detected in flower bud, flower bract alone, bunch pulp and in the peel.

**Table 5:** Effect of application of five times the recommended dose as pseudo stem injection at bunch emergence stage on residue levels in flower bud and bunch.

| Residue of fipronil, µg g$^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|
| Treatment and molecule | flower bud | Flower bract alone | Bunch pulp alone (on 15th day of emergence) | Bunch (on 30th day of emergence) | Peel | Bunch pulp alone |
| $T_1$ control | BDL | BDL | BDL | BDL | BDL | BDL |
| $T_2$- fipronil | BDL | BDL | BDL | BDL | BDL | BDL |

**Conclusion**

Insecticide fipronil formulation 0.3 GR application to soil has not led to the residue accumulation of fipronil and its toxic metabolites *viz.*, fipronil desulfinyl, fipronil sulfide and fipronil sulfone and they were not translocated into 1st, 2nd and 3rd leaves, male flower bund and bunches of bananas at various intervals of sampling till 50th days after application of 3 doses of insecticides as basal, 60 days and 150 days after planting. Even at double the recommended dose application of fipronil insecticides did not result in residues in leaves and were below the detection limit in all samples collected from 1st three leaves during the different sampling days. In the fourth leaf, 0.034 µg g$^{-1}$ of residue of fipronil molecule was noted only on 40th day indicating a very low and insignificant level of translocation of this chemical into the foliage when applied in soil and not found in the flower bud and bunches even at double the recommended dose of application.

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