Research Article

# Determination of Nitrogen Transformation of Fipronil 5 % w/v SC in Loamy Sand Soil under laboratory condition

Tentu Nageswara Rao[1]*, Aalaysam Vijayalakshmi[2], SNVS Murthy[3], S Seshamma[4] and Kari Apparao[1]

[1]Department of Chemistry, Krishna University, Machilipatnam, AP, India
[2]Department of Marine Living Resources, Andhra University, Visakhapatnam, A.P, India
[3]Department of Chemistry, DLR PG College, G. Mamidada, AP India
[4]Department of Bio Chemistry, AN University, Guntur, AP, India

**Abstract**

The test item fipronil 5% w/v SC was applied in a loamy sand soil and incubated over a period of 28 days for nitrogen transformation test at concentrations of 1.78 mg/kg soil dry weight and 8.9 mg/kg soil dry weight. The concentrations tested were based on one and ten times the maximum recommended field application rates of 350 g a.i/ha and 1750 g a.i/ha of fipronil 5% w/v SC, respectively. Control consists of soil treated with equivalent quantity of distilled water. The soil was covered with perforated lids to ensure aerobic conditions was thoroughly mixed and then incubated at 20±2ºC in the dark on the day of treatment. Samples were collected on day 0, 7, 14, 28 days after application and analyzed for nitrate content i.e., NO-3-N. Nitrogen turnover was determined based on changes in the content of nitrate-N (NO-3-N) in the test soil. The deviation in soil nitrate-nitrogen content determined at 28 days after application of the test item to soil compared to the control was 2.54% and 3.42 % for the single and ten times test concentrations, respectively. There is no significant variation between the treatment groups and control sample.

The rate of nitrate (NO-3-N) formation between 14 and 28 days after application of the test item to soil deviate from control by 3.56 % and 5.52 % for 1.78 and 8.9 mg/kg soil dry weight, respectively. Deviations in nitrate (NO-3-N) levels and nitrate (NO-3-N) formation rates in soil treated with up to and including 8.9 mg/kg of test item/kg soil dry weight were less than ±25%, compared to control indicating no significant effect occurred in nitrogen transformation.

**Keywords:** Nitrogen Transformation, Loamy sand soil, HPLC, LOQ, Fipronil 5% w/v

***Correspondence**
Author: Tentu Nageswara Rao
Email: tnraochemistry@gmail.com

## Introduction

Soil microorganisms are very vital for the breakdown and transformation of organic be counted and its mineralization. Transformation of nitrogen and carbon takes place in all fertile soils. Despite the fact that the microbial groups liable for those processes vary from soil to soil, the pathways alterations are essentially the same. long-time period interference with those biochemical procedures could doubtlessly affect the nutrient cycling for that reason altering the capability the soil. The impact of chemical compounds on the soil microbial community needs to be assessed if products are implemented to soil or if an publicity of soil probably.

Living organisms each plant life and animals, represent a vital factor of soil. The pioneering investigations of some of early microbiologists confirmed for the first time that the soil become not an insert static fabric but a medium pulsating with life. The soil is now believed to be a dynamic or alternatively a dwelling gadget, containing a dynamic populace of organisms/microorganisms. Cultivated soil has incredibly greater populace of microorganisms than the fallow land, and the soils rich in natural remember include lots greater population than sandy and eroded soils [1].

Pesticides in soil undergo a spread of derivative, delivery, and adsorption/desorption processes depending at the chemical nature of the pesticide and soil properties. Insecticides have interaction with soil organisms and their metabolic activities and might modify the physiological and biochemical behavior of soil microbes. Microbial biomass is an essential indicator of microbial activities and presents direct assessment of the linkage between microbial sports and the nutrient alterations and other ecological strategies. Many recent studies display the unfavorable impacts of insecticides on soil microbial biomass or boom in respiration implies the enhanced boom of bacterial population. Some microbial corporations are able to using implemented pesticide as supply of energy and nutrients to multiply whereas the pesticide may be toxic to other Organisms [2, 3]. Likewise on occasion, application of pesticides reduces microbial variety however increases practical range of microbial communities even every now

and then show the tendency of reversible stimulatory/inhibitory outcomes on soil microorganisms [4]. Insecticides application can also inhibit or kill sure organization of microorganisms and outnumber different companies by means of releasing them from the competition.

An insecticide is a substance used to kill insects. They consist of ovicides and larvicides used in opposition to insect eggs and larvae, respectively. Pesticides are used in agriculture, remedy, industry and by means of clients. Pesticides are claimed to be a chief factor behind the growth in agricultural, medicinal drug, enterprise and by using clients. Insecticides are claimed to be a major thing in the back of the boom in agricultural 20th century's productivity. Nearly all insecticides have the capacity to significantly alter ecosystems; many are toxic to human beings; a few pay attention along the meals chain. Fipronil is phenylprayazole insecticide that became registered to be used in 1996. It is a nervous system disruptor powerful on contact or ingestion. Fipronil is regularly used to deal with rice seeds, and can be discovered in numerous tick and lice manage medications for pets.

Fipronil is a Phenylpyrazole insecticide, with toxic to insects by means of touch or ingestion and is widely utilized in agriculture. The half-lifestyles of fipronil at special soil water content material and temperatures are 122 to 128 days. The microbial biomass in clay loam soil elevated with insecticide (Fipronil) treatment at some stage in the primary 10 days of incubation, but declined from day 14 onward was mentioned. However, in sandy loam soil, the biomass decreased with an growth of insecticide awareness on day 1, but multiplied thereafter. Specially, several studies have been accomplished on concerns referring to microbial degradation of insecticides.

The maintenance of soil fertility depends on the size and hobby of soil microbial biomass, that's of fundamental importance within the biological cycles of virtually all important plant nutrients [5]. Susceptible acid insecticides might be usually anionic and less probable to be absorbed to soil. Microbial breakdown is the breakdown of chemical substances by means of microorganism together with fungi and bacteria [6]. The degradation of soil microorganism at the benzene ring of the insecticide hydrolysis product become reported [11]. Elements inclusive of soil temperature, humidity, pH, and natural content material affecting the degradation of insecticide in soil have additionally been pronounced [7-10]. Microbial degradation of fipronil in soil microorganism is an essential element for the complete degradation of fipronil inside the field.

Microbial breakdown tends to increase when:

- Temperature are warm
- Soil pH is favorable
- Soil moisture and oxygen are adequate
- Soil fertility is good

## Experimental

### *Materials*

- Laboratory balance, Sartorious Mechatronics India Private Limited, Bangalore, India
- Hot Air Oven, supplied by Universal engineering Co.
- pH meter, Supplied by Eutech Instruments Private Limited, Singapore
- Test sieve (2 mm), supplied by Jayant Scientific Ind.
- Sonicator (Ultra), supplied by Fast clean
- Rotary Evaporator, supplied by Heidolph LR
- Distilled Water Unit, supplied by Stone-fin.
- Digital Hygro Thermometer, supplied by TFA Germany
- Centrifuge, supplied by Eltek
- UV/Vis Spectrophotometer, Model UV-1700, Shimadzu
- HPLC, Model UV-1700, Prominence, Shimadzu

### *Standards, Reagents and samples*

The analytical standard of fipronil (97.5%), was obtained from Sigma Aldrich. Acetonitrile (HPLC Grade), Ammonium Acetate, Ammonia, Sodium Hydroxide were purchased from Rankem, New Delhi, Analytical grade regeants, Copper Sulfate penta hydrate, Potassium Dichromate, Sodium sulfide, Sodium Thiosulfate Pentahydrate, Potassium sulfate, Hydrogen Peroxide, Calcium Carbonate, Potassium Nitrate, Chloroform, Ferrous Sulfate, Perchloric acid, Ferroin indicator, Phosphoric acid, Silver sulfate, Potassium hydroxide, Ethanol, Chromo tropic acid,

Dextrose anhydrous and Phosphoric acid were supplied from Merck Limited and fipronil 5% w/v SC Brand name is Stemer, was purchased from local market.

***Experimental Procedure***

Loamy sand soil was collected from a non agricultural field with the sampling depth of 0-20 cm. For at least four years prior to test initiation, no pesticides had been used on the soil. No organic or mineral fertilizers had been applied to the soils for two years to study initiation, respectively [12].

***Preparation of soil***

Prior to the initiation of the study, the stored soil collected from the field was air dried and sieved through a mesh of particle size 2 mm. After determining moisture content and Maximum Water Holding Capacity (MWHC) of test soil, moisture content of soil was adjusted to 22.5 % which was 50% of MWHC with distilled water. For the nitrogen transformation test, powdered Lucerne meal was added to the soil at the rate of 5 g/kg of soil dry weight and the soil was thoroughly mixed and kept in dark at 20±2°C for pre-incubation. Each soil sample contained approximately 2000 g test soil on dry weight basis for the nitrogen turnover. Pre-incubation was carried out as bulk samples for all the three test systems at 20±2°C in the dark.

***Validation of analytical method with potassium nitrate***

The analytical method for nitrate analysis in soil was validated by using potassium nitrate solution. Stock solution of nitrate was prepared by dissolving 652 mg of potassium nitrate in 1L of deionised water. The concentration of stock solution of NO3--N was 400 mg/l. An aliquot of 1.25 ml of stock solution of Nitrate was transferred into a 10 ml standard flask and placed in a cold water bath (temp < 10oC), added 3 ml of 0.1% chromotropic acid prepared in conc.H2SO4 drop by drop, swirled and left undisturbed for about 5 minutes. As soon as addition of chromotropic acid, yellow color was developed. The solution was made up to the mark with deionised water. The concentration of prepared nitrate derivative of chromotropic acid was 50 mg/L. From the 50 mg/l solution of nitro derivative, calibration solution 10, 5, 3, 2, 1, 0.5, 0.1 mg/l solutions were prepared with distilled water and analyzed for the absorbance under UV-Visible Spectrophotometer and the absorbance at 420 nm was noted down. Slope, intercept and correlation co-efficient were determined. The details details were presented in **Table 1.** Accuracy of the analytical method was checked by fortifying known quantities nitrate at 0.3 mg/kg, 5.0 mg/kg in 10 g of test soil. The percentage of recovery found was 104.56 and 101.89 at low and high levels, respectively.

**Table 1** Linearity with potassium nitrate solutions

| S. No. | Concentration in mg/L | Absorbance |
|---|---|---|
| 1 | 0.10 | 0.023 |
| 2 | 0.50 | 0.126 |
| 3 | 1.00 | 0.254 |
| 4 | 2.00 | 0.530 |
| 5 | 3.00 | 0.768 |
| 6 | 5.00 | 1.296 |
| 7 | 10.00 | 2.684 |
| 8 | Slope | 0.27 |
| 9 | Intercept | -0.0160 |
| 10 | CC | 0.9998 |

***Application of test item***

Both treatment solutions of fipronil 5% w/v SC were prepared by dissolving 0.2028 g of test item into a 100 ml volumetric flask. 1 ml of Acetonitrile was added to the flask and sonicated to dissolve the test item and flask was made up to the mark with distilled water and shaken well to homogenise the contents and coded as T2. 10 ml of T2 was pipetted out in a 50 ml volumetric flask made up to the mark with distilled water which was coded as T1. 25 ml of T1 solution was used to treat soil (T1) meant for 0.1.78 mg/kg of soil dry weight. 5ml of T1 was used for dose verification by HPLC. 24.995 ml of T2 solution was used to treat soil (T2) meant for 8.9 mg/kg of soil dry weight. 5 ml of T2 solution was used for dose verification by HPLC.

Control soil consisted of soil treated with 5 ml of distilled water. After treatment, soil in test containers was thoroughly mixed. Each treatment group contained approximately 2450 g of soil on dry weight basis for the nitrogen transformation test. Test systems were incubated as bulk samples for each treatment and control.

***Chromatographic separation parameters***

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (PhenomenexLuna-C18) Column temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and HPLC water (65:35 (v/v)). The flow- rate used was kept at 1.0 mL/min. A detector wavelength was 275 nm. The retention time of fipronil about 5.6 min. The slope intercept method was used for this analysis.

***Validation of analytical method for fipronil analysis***

Analytical method for fipronil analysis was validated in terms of specificity, linearity and recovery is tested in distilled water [13]. The linear solutions of concentrations 10, 5, 1, 0.5, 0.1 and 0.01 µg/ml were prepared with Acetonitrile and were injected into HPLC instrument and checked for the instruments response (peak area) at each concentration. The details were given in the **Table 2**. A graph was plotted between peak area and concentration in µg/ml. A calibration curve showed in **Figure 1**. The instrument response was found linear in the range 0.01 µg/ml and 10.0 µg/ml. The slope, intercept and correlation coefficient were calculated and they are 4775, 11.33 and 1.0000, respectively. Recovery (assay accuracy) of the method in distilled water was checked at two levels. One was at 0.1 µg/ml and another was at 0.01 µg/ml. Percentage of recovery found was 90.68, 94.52 % at low and high levels, respectively.

**Table 2** Detector linearity with fipronil standard

| Concentration (mg/L) | Peak Area (mv-sec) |
|---|---|
| 0.05 | 241 |
| 0.1 | 559 |
| 0.5 | 2375 |
| 1 | 4724 |
| 5 | 23925 |
| 10 | 47752 |
| Slope | 4775 |
| Intercept | 11.33 |
| Correlation coefficient | 1.0000 |

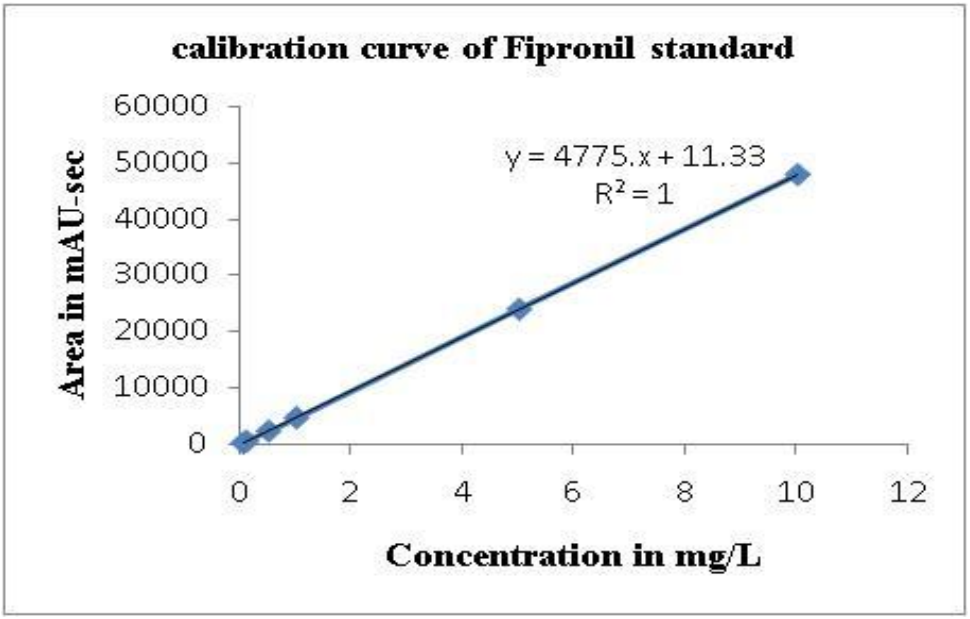


**Figure 1** Representative Calibration curve of fipronil standard

***Dose verification***

The solution meant for T1 and T2 were directly injected into HPLC following below Chromatographic separation parameters for dose verification. Dose verification details were presented in **Table 3**.The typical T1 and T2 dose chromatograms are showed **Figures 2** and **3**.

**Table 3** Dose verification Results

| Sample ID | Sample area | Slope | Intercept | Dilution Factor | Aliquote concentration (mg/L) | Recovered concentration (mg/L) | % of Recovery | Mean Recovery % |
|---|---|---|---|---|---|---|---|---|
| Standard 0.5 mg/L | 2348 | - | - | - | - | - | - | - |
| Control | - | - | - | - | - | - | - | - |
| T1R1 | 241 | 4775 | 11.33 | 1 | 0.053 | 0.048 | 90.75 | 90.35 |
| T1R2 | 239 | | | 1 | 0.053 | 0.048 | 89.96 | |
| T2R1 | 1183 | | | 1 | 0.267 | 0.245 | 91.90 | 92.01 |
| T2R2 | 1186 | | | 1 | 0.267 | 0.246 | 92.13 | |

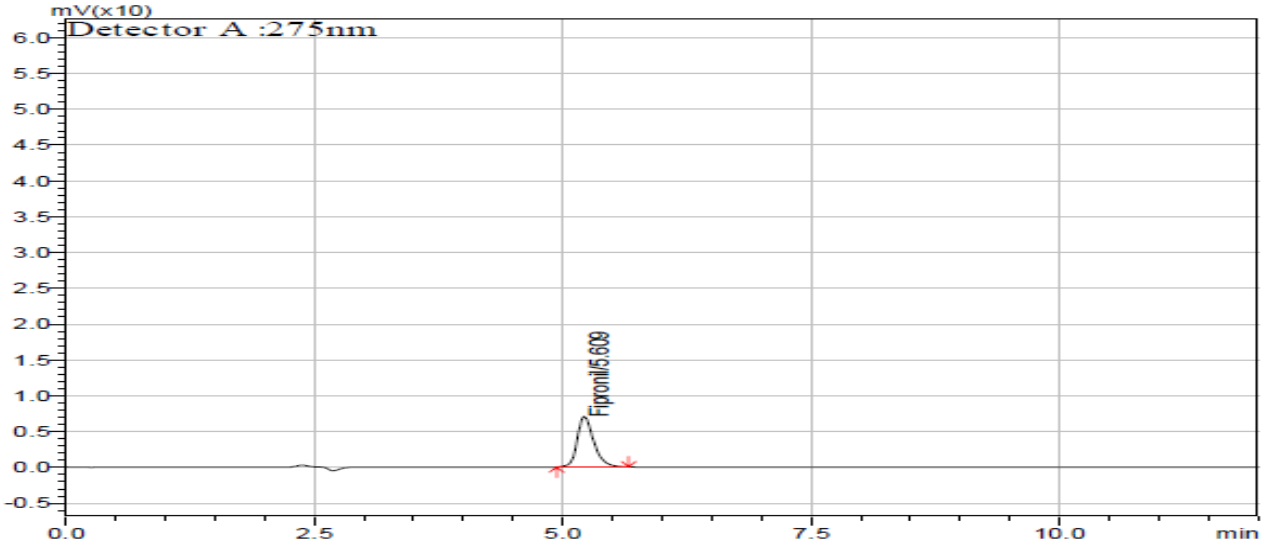


**Figure 2** Representative Chromatogram T1 Dose verification sample

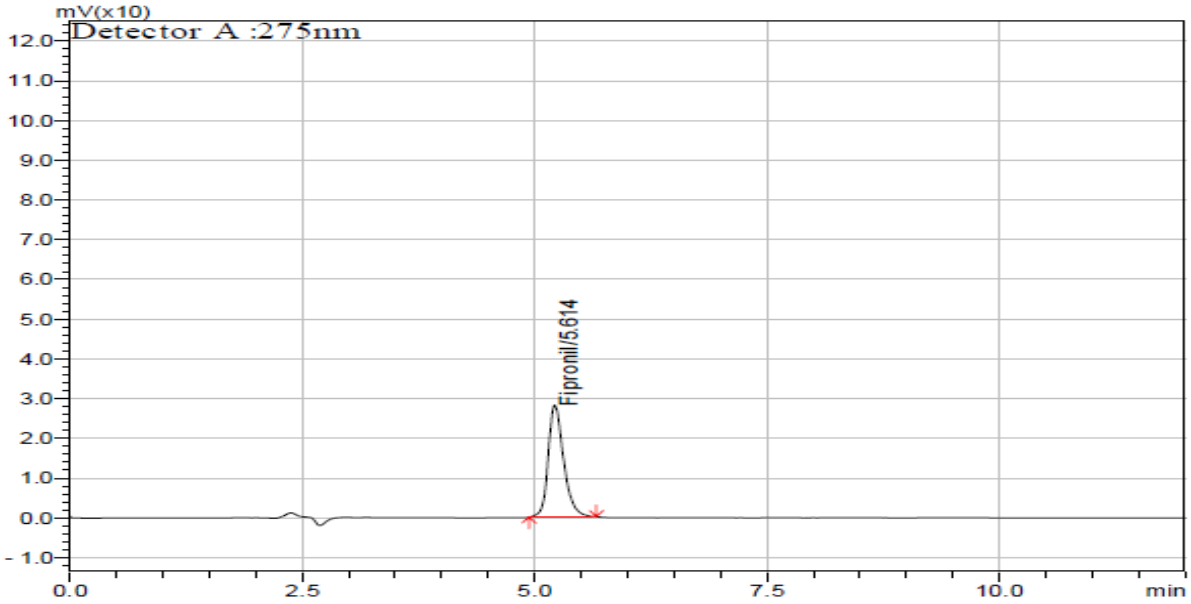


**Figure 3** Representative Chromatogram T2 Dose verification sample

***Sampling occasions and measurements***

Samples were taken at the following occasions after the application of test item and following the incubation in the dark at 20±2ºC. At each occasion, soil in the test systems was thoroughly mixed. Moisture was adjusted to 50 % of MWHC once in seven days and maintained the same throughout incubation period of the experiment. Day 0 (within 2 hours after application of test item), Day 7, Day l4 and Day 28. At each sampling occasion, the soil was thoroughly mixed and an aliquot was taken from the corresponding test system and following parameters were determined. 10 g of representative soil sample per treatment was weighed for dry weight determination /one replication. 20 g of representative soil sample per treatment was weighed for pH measurement/one replication. 10 g of representative soil sample in triplicate from each treatment for Nitrogen turnover. 10 g of representative soil sample per treatment to determine moisture content of soil/one replication. Occasion wise pH and moisture content were measured and the details were presented in **Table 4** and **Table 5** respectively.

**Table 4** pH Values

| **Sample ID** | **pH Measurement during Nitrogen Transformation at** | | | |
|---|---|---|---|---|
| | **Day 0 (25.0° C)** | **Day 7 (25.2° C)** | **Day 14 (25.2° C)** | **Day 28 (25.4° C)** |
| Control (Distilled water) | 5.87 | 5.89 | 5.89 | 5.78 |
| T1 (1.78mg/kg soil dry weight on active basis) | 5.82 | 5.74 | 5.76 | 5.75 |
| T2 (8.9 mg/kg soil dry weight on active basis) | 5.76 | 5.78 | 5.69 | 5.74 |

**Table 5** Moisture content Values

| **Sample ID** | **Moisture content (%) at** | | | |
|---|---|---|---|---|
| | **Day 0** | **Day 7** | **Day 14** | **Day 28** |
| Control (Distilled water) | 20.91 | 19.52 | 19.45 | 19.93 |
| T1- (1.78mg /kg soil dry weight on active basis) | 18.61 | 20.42 | 20.32 | 19.62 |
| T2 -(8.9mg/kg soil dry weight on active basis) | 19.53 | 18.68 | 18.52 | 19.41 |

***Nitrogen turnover***

Soil nitrification was determined measuring the NO3--N content of aqueous soil extracts. The concentrations of NO3--N in the soil were then calculated from the measured values. 10 g soil in triplicate from each treatment was added 100 ml of deionised water in a 250 ml of beaker. The samples were shaken for 10 minutes. Soil suspensions were centrifuged at 3000 RPM for five minutes and decanted the supernatant solution followed by passing through charcoal. 1g of CaCO3 was added to the extracts to avoid loss of nitrate due to high acidity. pH was checked and adjusted to 4.5 by adding few drops of 10% acetic acid. All the extracts were added 5 ml of 1N Ag2SO4 solution to precipitise chloride ions and shaken well followed by filtering through whatman filter paper. Finally made upto 100 ml with distilled water. 50 ml of chloride free extract was transferred into round bottom flask and evaporated to lower volume (<5 ml) at 70oC. Extract was transferred into 10 ml standard flask carefully and kept the flask in cold water bath (<10oC) for five minutes followed by addition of 3 ml of 0.1% chromotropic acid prepared in conc.H2SO4. Finally contents were made upto mark with distilled water and analysed under UV-Visible Spectrophotometer. Absorbance at 420 nm was recorded. Nitrate content in soil aliquot was determined with slope intercept curve obtained from linearity of Potassium Nitrate solutions.

The concentration of NO3--N in the soil was expressed as mg of NO3--N per kg soil dry weight. The results of the nitrogen turnover were presented in **Tables 6-9**.

***Formula of Calculation***

Nitrate content = milligram of nitrate/Soil DW x 1000

Rate of formation of nitrate formation (mg nitrate/kg soil dry weight/day):

The rate was calculated for each time interval and treatment as follows:

Rate of nitrate formation = [(mg nitrate/kg soil dry weight on sampling day ‘a’) – (mg nitrate/kg soil dry weight on previous sampling day)] ‘a’ days

Where ‘a’ = 7, 14, 28, days.

**Table 6** Summary of No3--N in mg/kg soil dry weight and the deviation from the control

| Days | 0 | 7 | 14 | 28 |
|---|---|---|---|---|
| **Control (Distilled water)** | | | | |
| NO3- - N | 86.68 | 87.65 | 87.85 | 87.00 |
| T1 (1.78 mg/kg of soil dry weight on active basis) | | | | |
| NO3- - N | 84.42 | 84.12 | 83.96 | 84.76 |
| **Deviation from control (%)** | | | | |
| NO3- - N | -2.61 | -4.03 | -4.43 | -2.58 |
| **T2 (8.9 mg/kg soil dry weight on active basis)** | | | | |
| $NO3^-$ - N | 84.07 | 78.85 | 82.09 | 84.39 |
| **Deviation from control (%)** | | | | |
| $NO3^-$ - N | -3.01 | -10.05 | -6.55 | -3.01 |

**Table 7** Effect of test item on the nitrate formation rate

| Assessment interval Day | Control (Distilled water) NO3- -N [mg/kg dry weight / day]# | | | |
|---|---|---|---|---|
| 0-7 | 0.139 | | | |
| 0-14 | 0.083 | | | |
| 0-28 | 0.011 | | | |
| **Assessment Interval Day** | **T1(1.78 mgkg dry weight on active basis)** | | **T2 (8.9 mg/kg soil dry weight on active basis)** | |
| | **NO3- -N [mg/kg dry weight / day]** | **Deviation from control [%]** | **NO3- -N [mg/kg dry weight / day]** | **Deviation from control [%]** |
| 0-7 | -0.043 | -131.06 | -0.747 | -638.97 |
| 0-14 | -0.033 | -139.46 | -0.141 | -270.08 |
| 0-28 | 0.012 | 6.49 | 0.011 | -1.31 |

**Table 8** Limit of quantification (LOQ) for nitrate (mg/kg soil dry weight).

Lowest quantity of nitrate found at each occasion was reported as Limit of quantification of nitrate at that particular occasion.

| OCCASION | LOQ (mg/kg soil dry weight) |
|---|---|
| 0th Day | 84.07 |
| 7th Day | 78.85 |
| 14th Day | 82.09 |
| 28th Day | 84.39 |

**Table 9** Nitrogen transformation test: effect of fipronil 5% SC on nitrification of soil microorganisms

| Day | Control (Distilled water) | | | T1(1.78 mg/kg dry weight on active basis) | | | T2 (8.9 mg/kg dry weight on active basis) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean Nitrification in terms NO3- N produced (mg/kg) | SD | RSD | Mean Nitrification in terms NO3- N produced (mg/kg) | SD | RSD | Mean Nitrification in terms NO3- N produced (mg/kg) | SD | RSD |
| 0 | 86.68 | 0.38 | 0.44 | 84.42 | 0.44 | 0.52 | 84.07 | 0.39 | 0.46 |
| 7 | 87.65 | 0.29 | 0.33 | 84.12 | 0.45 | 0.53 | 78.85 | 0.49 | 0.63 |
| 14 | 87.85 | 0.39 | 0.44 | 83.96 | 0.67 | 0.80 | 82.09 | 0.45 | 0.54 |
| 28 | 87.00 | 0.28 | 0.33 | 84.76 | 0.42 | 0.50 | 84.39 | 0.53 | 0.62 |

## Results and Discussion

The effect of the check object on nitrogen turnover was investigated in a Loamy sand soil. The software rates of take a look at object had been 1.seventy eight mg/kg of soil dry soil (1-fold attention) and 8.nine mg/kg of soil dry weight (five-fold awareness) on lively foundation, similar to a field software quotes of 350 g a.i/ha and 1750 g a.i/ha. 28 days after incubation, the bottom treatment organization deviated by -2.58 % and maximum treatment institution deviated with the aid of -three.01 % from manage. The deviation costs had been 6.forty nine% and -1.31% respectively. Deviation costs of the each the check companies in nitrogen transformation from manage check systems become beneath the brink value of <±25% for this reason, the examine was terminated.

large inhibitory effect in nitrogen transformation turned into determined upto 14 days after application of check item at each the remedy groups (1-fold and 5-fold concentrations of 28 days after utility of test object, the values for both utility costs have been below the edge price given within the OECD guideline 216. The percent deviation among soil dealt with test item and manipulate becomes 6.49% for 1-fold utility charge and -1.31% for 5-fold utility rate 28 days after application.

## Conclusion

The effect of fipronil 5% w/v SC on soil microorganisms was assessed in a test that measured nitrogen turnover following the application of fipronil 5% w/v SC to soil. The test was conducted in accordance with OECD Guideline 216. The measured values for the deviation of nitrogen turnover in both the treatment levels with fipronil 5% w/v SC deviated by less than 25 % from the control at 28$^{th}$ day. Based on this result, it is concluded fipronil 5% SC has no long-term effect on nitrogen transformation of soil microorganisms.

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