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Determination of soil microorganisms carbon transformation of fipronil 5 % w/v sc in loamy sand soil under laboratory condition

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ABSTRACT

The test item Fipronil 5 g/l SC was incubated 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% SC. Control consists of soil treated with equivalent quantity of distilled water was also incubated in the dark along with the treated soil samples.

Carbon transformation was determined by short term respiration of soil microorganisms by amending soil samples with glucose. The oxygen consumption (BOD) during short term respiration of soil microorganisms in soil samples was measured upto 12 consecutive hours following addition of glucose on day 0, 7, 14 and 28 after application of Fipronil 5 g/l SC. The measured values for the carbon transformation in both the treatment levels with Fipronil 5 g/l SC deviated was by less than 25% from the control at 28th day. The dose verification of the Fipronil 5 g/l SC was analyzed by validated high performance liquid chromatographic method (HPLC).

KEYWORDS

Loamy sand soil;
Carbon transformation;
HPLC and Fipronil 5 g/l SC.

INTRODUCTION

Soil microorganisms are very important for the breakdown and transformation of organic matter and its mineralization[1]. Transformation of nitrogen and carbon occurs in all fertile soils. Although the microbial communities responsible for these processes differ from soil to soil, the pathways transformations are basically the same[2]. Long-term interference with these biochemical processes could potentially affect the nutrient cycling thus altering the functionality the soil. The impact of chemicals on the soil microbial community needs to be assessed if products are applied to soil or if an exposure of soil likely.

Living organisms both plants and animals, constitute an important component of soil[3]. The pioneering investigations of a number of early microbiologists showed for the first time that the soil was not an insert static material but a medium pulsating

with life[4]. The soil is now believed to be a dynamic or rather a living system, containing a dynamic population of organisms/microorganisms[5]. Cultivated soil has relatively more population of microorganisms than the fallow land, and the soils rich in organic matter contain much more population than sandy and eroded soils.

Pesticides in soil undergo a variety of degradative, transport, and adsorption/desorption processes depending on the chemical nature of the pesticide and soil properties[6]. Pesticides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes. Microbial biomass is an important indicator of microbial activities and provides direct assessment of the linkage between microbial activities and the nutrient transformations and other ecological processes[7]. Many recent studies reveal the adverse impacts of pesticides on soil microbial biomass or increase in respiration implies the enhanced growth of bacterial population. Some microbial groups are capable of using applied pesticide as source of energy and nutrients to multiply. Whereas the pesticide may be toxic to other Organisms[8]. Likewise sometimes, application of pesticides reduces microbial diversity but increases functional diversity of microbial communities even sometimes demonstrate the tendency of reversible stimulatory/inhibitory effects on soil microorganisms. Pesticides application may also inhibit or kill certain group of microorganisms and outnumber other groups by releasing them from the competition.

An insecticide is a substance used to kill insects. They include ovicides and larvicides used against insect eggs and larvae, respectively. Insecticides are used in agriculture, medicine, industry and by consumers. Insecticides are claimed to be a major factor behind the increase in agricultural, medicine, industry and by consumers. Insecticides are claimed to be a major factor behind the increase in agricultural 20th century's productivity. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; some concentrate along the food chain. Fipronil is phenylprayazole insecticide that was registered for use in 1996. It is a nervous system disruptor effective on contact or ingestion. Fipronil is often used to treat rice seeds, and can be found in several tick and lice control medications for pets.

Fipronil is a Phenylpyrazole insecticide, with toxic to insects by contact or ingestion and is widely used in agriculture. The half-life of Fipronil at different soil water content and temperatures is 122 to 128 days. The microbial biomass in clay loam soil increased with insecticide (Fipronil) treatment during the first 10 days of incubation, but declined from day 14 onward was reported. However, in sandy loam soil, the biomass decreased with an increase of insecticide concentration on day 1, but increased thereafter. In particular, several studies have been carried out on concerns relating to microbial degradation of insecticides.

The maintenance of soil fertility depends on the size and activity of soil microbial biomass, which is of fundamental importance in the biological cycles of almost all major plant nutrients. Microbial breakdown is the breakdown of chemicals by microorganism such as fungi and bacteria. The degradation of soil microorganism on the benzene ring of the insecticide hydrolysis product was reported. Factors such as soil temperature, humidity, pH, and organic content affecting the degradation of insecticide in soil have also been reported[9, 10]. Microbial degradation of Fipronil in soil microorganism is an important factor for the complete degradation of Fipronil in 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

BOD meter supplied by Lovibond, Germany

Laboratory balance, Sartorious Mechatronics India Private Limited, Bangalore, India.

Hot Air Oven, supplied by Universal engineering Co

pH mete r, 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

Cetrifuge, supplied by Eltek

V/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, AllylThiourea was purchased from Lovibond 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.

Preparation of soil

Prior to the experiment initiation, the stored soil which was collected from the field was sieved through a mesh of particle size 2 mm. After determining moisture content and maximum water holding capacity (MWHC) of the soil, moisture content of soil was adjusted to 22.5 % which was 50% of MWHC with distilled water. For carbon transformation test, 3000 g of soil on dry weight basis was taken into each test system. Pre-incubation was carried out as bulk samples for all the three test systems at 20±2°C in aerobic and dark conditions.

Amount of glucose needed to elicit a maximum respiratory response in the test soil was determined in the pre-test in which respiratory response was checked at 0.2g, 0.3g and 0.4g of glucose per 100 g of soil dry weight. Mean respiratory response found in terms of O_2 consumed was 39.33, 52.88 and 61.75 mg/l at respective doses. The maximum respiratory response was found at 4g of glucose per kg of soil dry weight and the same dose of glucose was used for glucose induced respiration.

Application of test item

Both treatment solutions of Fipronil 5% w/v SC were prepared by dissolving 0.3042 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. 25 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 3674 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 tempera-

ture 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. 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[11]. The details were given in the TABLE 1 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.

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-2. The typical T1 and T2 dose chromatograms are showed Figure. 2 and 3.

TABLE 1 : Detector linearity with fipronil standard

Concentration (mg/L)	Peak Area (mAU-sec)
0.01	197
0.1	1885
0.5	9523
1	18368
5	88995
10	181205
Slope	18054.32
Intercept	48.46
Correlation coefficient	0.9999

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±2°C. 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. 3 and TABLE. 4 respectively.

Short term respiration

Triplicate samples of 100 g each from treated and untreated soil were sampled for analysis after mixing the soil thoroughly at each sampling point. Based on dry weight of soil, glucose mixed fine sand was added at the rate of 4 g per kg of soil dry weight.

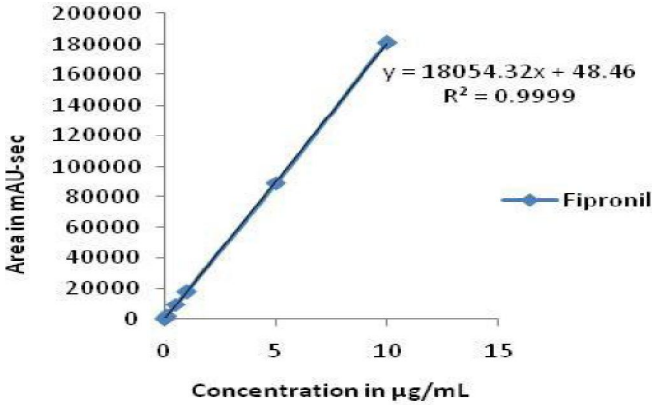

Figure 1 : Representative calibration curve of fipronil standard

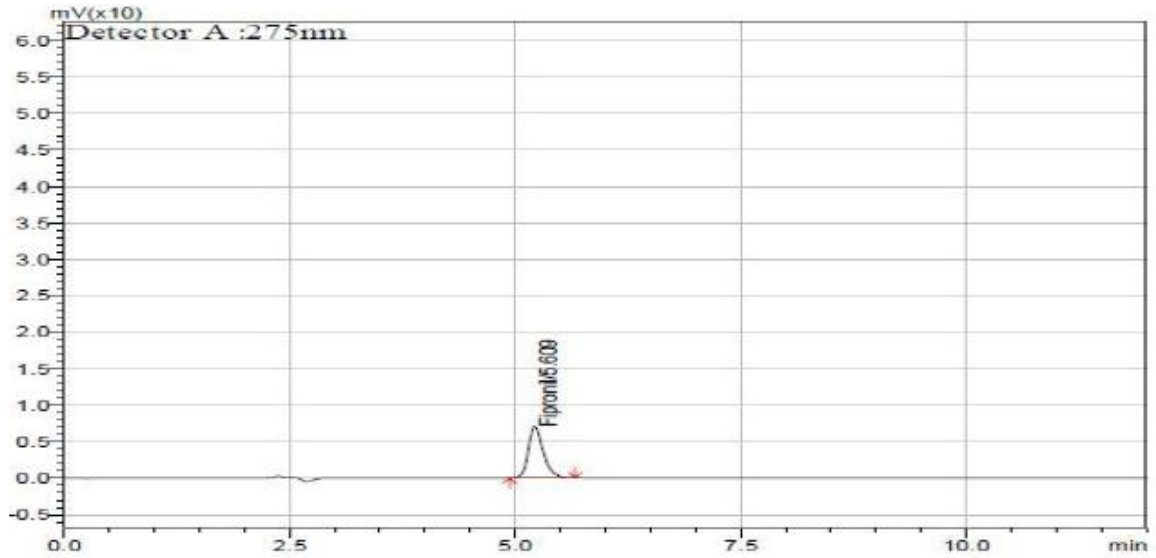

Figure 2 : Representative chromatogram T_1 dose verification sample

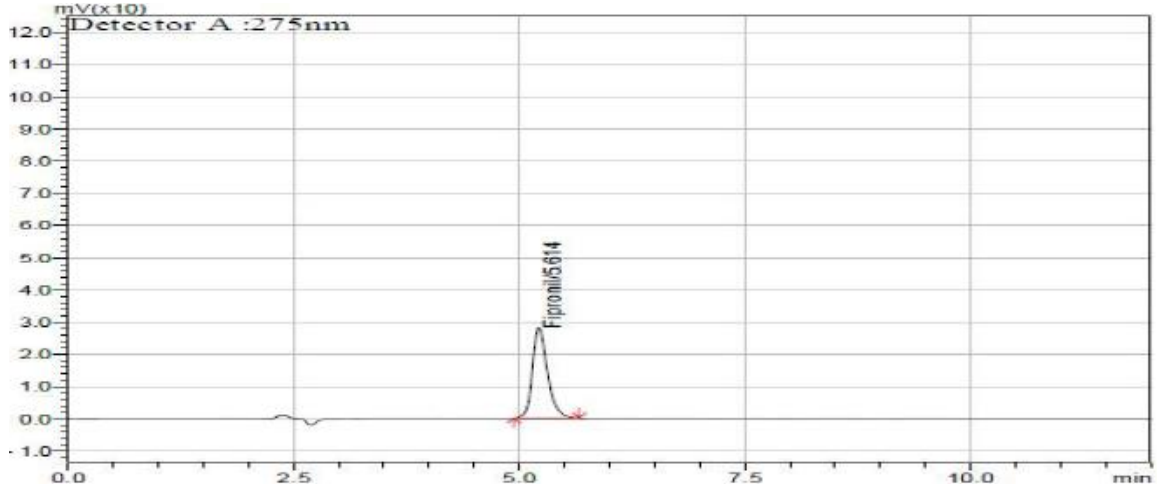

Figure 3 : Representative chromatogram T_2 Dose verification sample

After addition of Allyl thiourea, soil samples were mixed well and transferred into BOD bottles within 2 hours after amending with glucose. 2 ml of 2M KOH was taken into gaskets carefully, sensors were set and loaded onto the instruments. The instrument was incubated in a thermostatic condition of 20±2°C and the oxygen consumption (BOD) was measured for 12 consecutive hours using BOD meter. The carbon dioxide produced during short term respiration was calculated by multiplying the BOD value with a factor of 1.375. (1 mg of consumed O_2 corresponds to 1.375 mg of CO_2). Respiration curve was drawn between consecutive hours and consumed O_2 in mg/kg of soil dry weight. The values were calculated as the mean of 3 replicate determinations. The inhibition or stimulation of short term respiration was calculated by comparing the values of the treated with those of untreated soil samples. The results are presented in TABLE 5.

RESULTS AND DISCUSSION

The effect of the test item on short term respiration of soil microorganisms was investigated in a Sandy soil. The application rates of test item were 1.78 mg/kg of soil dry soil (1-fold concentration) and 8.9 mg/kg of soil dry weight (5-fold concentration) on active basis, corresponding to a field application rates of 350 g a.i/ha and 1750 g a.i/ha. 28 days after incubation, both the treatment groups deviated by less than 25% from control which was the threshold value established by the guideline. So the

TABLE 2 : Dose verification results

Sample ID	Area	Slope	Intercept	Dilution factor	Nominal Concentration (µg/mL)	Recovered concentration (µg/mL)	% of Recovery	Mean Recovery %
Standard –0.5 mg/l	9625			-	-	-		
Control	-			-	-	-		88.29
T1R1	1474	18054.32	48.46	1	0.09	0.079	87.73	
T1R2	1492			1	0.09	0.080	88.84	
T2R1	7598			1	0.45	0.418	92.92	93.03
T2R2	7616			1	0.45	0.419	93.15	

TABLE 3 : pH values

Sample ID	pH Measurement during Nitrogen Transformation at			
	Day 0 (25.0° C)	Day 7 (25.1° C)	Day 14 (25.1° C)	Day 28 (25.3° C)
Control (Distilled water)	5.81	5.88	5.87	5.77
T1 (1.78mg/kg soil dry weight on active basis)	5.83	5.76	5.75	5.76
T2 (8.9 mg/kg soil dry weight on active basis)	5.74	5.79	5.71	5.78

TABLE 4 : Moisture content values

Sample ID	Moisture content (%) at			
	Day 0	Day 7	Day 14	Day 28
Control (Distilled water)	20.45	19.46	19.52	19.91
T1- (1.78mg /kg soil dry weight on active basis)	18.78	20.39	20.38	19.67
T2 -(8.9mg/kg soil dry weight on active basis)	19.59	18.74	18.59	19.79

TABLE 5 : Carbon transformation test: effects of fipronil 5% SC on induced respiration rates of soil microorganisms

Day	Control (Distilled water)				1.78 mg /kg Fipronil 5% SC- T1					8.9 mg. /kg Fipronil 5% SC- T2				
	Respiration rate in terms CO_2 produced (mg/kg/hr)	Mean Respiration rate	SD	RSD	Respiration rate in terms CO_2 produced (mg/kg/hr)	Mean Respiration rate	SD	RSD	% D	Respiration rate in terms $CO`_2$ produced (mg/kg/hr)	Mean Respiration rate	SD	RSD	% D
	88.17				87.15					84.79				
0	89.89	88.17	6.71	7.61	87.75	87.10	0.68	0.78	-6.3	85.96	83.38	3.50	4.20	-10.45
	100.56				86.39					79.39				
	87.74				90.12					81.49				
7	93.09	87.74	5.72	6.52	89.71	89.40	0.92	1.03	-4.71	78.71	79.50	1.74	2.19	-15.36
	99.17				88.36					78.29				
	87.26				82.41					83.49				
14	91.79	87.26	3.74	4.29	82.16	83.12	1.45	1.75	-9.04	83.45	81.24	3.86	4.75	-10.89
	94.69				84.79					76.79				
	97.29				86.47					73.47				
28	96.16	97.29	0.58	0.60	86.13	85.19	1.94	2.27	-11.79	76.96	77.94	5.03	6.46	-19.31
	96.48				82.96					83.39				

study was terminated. Significant inhibitory effect in short term respiration was observed up to 14 day after application of test item at both the treatment groups (1-fold and 5-fold application rates of

Fipronil 20 SC). 28 days after application of test item, the values for both application rates were below threshold value given in guideline OECD-217[12]. The percent deviation between soil treated with test item and control was -11.79% for 1-fold application rate and -19.31% for 5-fold application rate. The soil microorganisms respiration rates that found at 1 fold and 5 fold application rates were 85.19 and 77.94 mg/kg/hr respectively.

CONCLUSION

Based on the test results, the test item Fipronil 5% SC has no long-term effect on (carbon transformation) induced respiration rates of soil microorganisms.

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