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Studying Nitrogen Transformation Test of Picoxystrobin 25% SC (w/v) in loamy Sand Soil

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A B S T R A C T

The effect of Picoxystrobin 25% SC on soil microorganisms nitrogen transformation was assessed in a test that measured nitrogen turnover following the application of Picoxystrobin 25% SC to soil. Picoxystrobin 25% SC was incubated in loamy sand soil over a period of 28 days for the nitrogen turnover at 1-fold and 5-fold concentration, equivalent to 0.340 mg/kg (T1) and 1.700 mg/kg (T2) on product basis, respectively (corresponding to a field application rate of 400 ml/ha and 2000 ml/ha). Control consists of soil treated with equivalent quantity of distilled water, was also incubated in the dark along with the treated soil samples. The measured values for the deviation of nitrogen turnover (transformation) in both the treatment levels with deviated by less than 25 % from the control at 28th day. The dose concentrations (T1 and T2 levels) of Picoxystrobin 25% SCware determined by validated HPLC method.

Introduction

Soil microorganisms are very important for the breakdown and transformation of organic matter and its mineralization (Eppo Decision, 1994). 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 (Gary *et al*., 2009). 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 (Hindumathy *et al*., 2013). 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 (Sahid *et al*.,

1992). The soil is now believed to be a dynamic or rather a living system, containing a dynamic population of organisms/microorganisms (Kiran, 2013). 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 (Lal and Saxena, 1982; Lipika Patnaik *et al*., 2013; Metin and Ferda, 2001). 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 (OECD Guideline, 2004; Rajesh and Chinchmalatpure, 2013). 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 (Wagh *et al*., 2013; Wootton *et al*.,1993; Bartlett *et al*., 2002). 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.

Picoxystrobin is a fungicide belonging to the strobilurin group of chemicals. It is a preventative and curative fungicide with systemic and translaminar movement, acting by inhibition of mitochondrial respiration by blocking electron transfer at the Qocentre of cytochrome Bc1. It is used for control of a range of fungal diseases, including brown rust, tan spot, powdery mildew and net blotch in cereals, pulses and oil seeds. Picoxystrobin is reported to have a high acute toxicity to earthworms (LC50 at 6.7 mg kg-1), and field assays of earthworm toxicity indicte acute acute toxic effects even at recommended doses for use, which might be caused by heavy rain shortly after spraying forcing the earthworm to migrate to the surface. Potentially negative effects on soil microbial nitrogen and carbon mineralization activity are known to be transient (dose: 750 g ha-1, duration: 28 days) (Coleman, 2008; Wu *et al*., 2011). Pycoxystrobin requires continued attention due to its demonstrated potentially negative effects to earthworms (Winding *et al*., 2005).

The importance of soil microbes and their activity in the functioning of soils, justify their thorough investigation in risk assessments. The development of in genomic techniques over the last decades has made detailed studies of the soil microbial community possible, beyond the scope of broad-scale measures like substrate induced respiration (Oyeyola *et al*., 2013; Mariusz and Zofia, 2007). DNA extraction from soil followed by different molecular approaches to determine the genetic diversity and quantify the presence of single organisms or group of related organisms in a soil sample, have been employed successfully in studies of species and functional diversity in agricultural soils. According to OECD guidelines for the testing chemicals carbon and nitrogen

transformation tests (with cut-off criteria of 25% effect) are the recommended methods to assess effects concentrations of chemicals on the soil microbial community (Daniela B *et al*., 2007). In research, soil respiration is commonly used to assess effects of pesticides and other chemicals on soil microbes. Microbial degradation of Picoxystrobin in soil microorganism is an important factor for the complete degradation of Picoxystrobin 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

Standards, Reagents and Samples

The analytical standard of Picoxystrobin(99.9%), 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 tropicacid, Dextrose anhydrous and Phosphoric acid were supplied from Merck Limited and Picoxystrobin 25% SC 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 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 25.16 % 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.

Treatment levels of test item

Sandy soil with the following study groups in three replicates each was tested:

Soil treated with distilled water (control)

Soil treated with and Picoxystrobin 25% SC@ 0.340 mg/kg soil dry weight equivalent to

400 ml/ha (1-fold) (T1)

Soil treated with and Picoxystrobin 25% SC@ 1.700 mg/kg soil dry weight equivalent to

2000 ml/ha (5-fold) (T2)

The calculations are presented below

Application Rates Tested	400 ml/ha - MUR 2000 ml/ha -5 x MUR
Soil volume/ha (V)	Depth area = 0.05 m 10 000 m^2 = 500 m^3/ha
Soil density (ρ)	1.5 g dry wt/cm^3 = 1.5 kg/m^3
Soil mass/ha	Vρ = 750000 kg soil dry weight/ha
Application rate	400 ml/750000 kg soil dry weight = 0.340 mg/kg soil dry weight
	2000 ml/750000 kg soil dry weight = 1.700 mg/kg soil dry weight

Abbreviation: ha - Hectare, V-Volume, ρ – Density, MUR – Maximum used Rate

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 NO_3^--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 < 10^oC), added 3 ml of 0.1% chromotropic acid prepared in conc.H_2SO_4 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 upto 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 curve was taken including correlation co-efficient. 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 89.50 and 96.09 at low and high levels, respectively. Details were presented in Table 1 and Table 2.

Application of test item

Both treatment solutions of Picoxystrobin 25% SCwere prepared by dissolving 22.98 mg of test item into a 100 ml volumetric flask. 0.8 ml of acetonitrile was added to the volumetric flask and sonicated to dissolve the content and 50 ml of distilled water was added to the flask and sonicated to dissolve the test item and the volume of the flask was made upto the mark with distilled water to homogenize the contents and coded as T2. 10 ml of T2 solution was pipette out in a 50 ml volumetric flask made upto the mark with distilled water which was coded as T1. 22.120 ml of T1 solution was used to treat soil (T1) meant for 0.340 mg/kg of soil dry weight 22.120 ml of T2 solution was used to treat soil (T2) meant for 1.700 mg/kg of soil dry weight.

Control soil consisted of soil treated with 22.120 ml of distilled water. After treatment, soil in test containers was thoroughly mixed. Each treatment group contained approximately 3755 g of soil on dry weight basis for the carbon transformation test. Test systems were incubated as bulk samples for each treatment and control.

Chromatographic separation parameters

The HPLC-PDA system used, consisted Waters Alliance Series with e2695separations module and 2998 photodiode array detector with Empower2 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 pH 2.5 phosphoric acid adjusted HPLC water (70:30 (v/v)). The flow- rate used was kept at 1.5 mL/min. A detector wavelength was 240 nm. The retention time of Picoxystrobin about 4.8 min. The slope intercept method was used for this analysis.

Validation of analytical method for Picoxystrobin analysis

Specificity

The prepared solutions of standard solution, sample solution, mobile phase solvents i.e., HPLC Water and acetonitrile solvent, distilled water were injected into HPLC and checked for specificity and it was observed that there was no interference found with the main peak.

Linearity

Accurately weighed 5.35 mg of Picoxystrobin reference standard into a 10 mL volumetric flask and 5 mL of acetonitrile was added to dissolve the content and sonicated and made up to the mark with the same solvent. This was 535.46 mg/L solution. A series of calibration solutions were then prepared by diluting the appropriate volume of stock solution into different 10 mL volumetric flasks and bringing to volume with acetonitrile. The prepared calibration solution 0.01 mg/L , 0.1 mg/L, 1.0 mg/L, 2.0 mg/L, 5.0 mg/L and 10.0 mg/L were analyzed by HPLC at 245 nm. The details were given in the Table 3and Table 4. A linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined. A calibration curve showed in Fig 1.

Assay accuracy and precision

The analytical method was validated for the recovery of the standard at two fortification levels i.e., LOQ and 10 times LOQ level (SANCO Guidelines, 2009).

Preparation of standard solution

The standard prepared for the linearity was used.

Preparation of 0.01 mg/L Fortification Level

0.1 ml aliquots of 1.0 mg/L Standard solution was transferred into a 10 ml volumetric flask and fortified with distilled water. The sample was extracted with 100 ml of n-hexane, and then the extract was evaporated upto dryness at 70 ºC using rotary evaporator and then, the sample was then transferred into 10 ml volumetric flask and diluted upto the mark with acetonitrile and the samples were coded as T1R1, T1R2, T1R3, T1R4 and T1R5. Control samples were also maintained and coded as T0R1 and T0R2.

Preparation of 0.1 mg/L Fortification Level

0.1 ml aliquots of 10 mg/L Standard solution was transferred into a 10 ml volumetric flask and fortified with distilled water. The sample was extracted with 100 ml of n-

hexane, and then the extract was evaporated upto dryness at 70 ºC using rotary evaporator and then, the sample was then transferred into 10 ml volumetric flask and diluted upto the mark with acetonitrile and the samples were coded as T2R1, T2R2, T2R3, T2R4 and T2R5. Control samples were also maintained and coded as T0R1 and T0R2.

The above preparations were injected into HPLC and checked for accuracy (% Recovery) and precision (%RSD) was calculated by injecting five times of one replication of each fortified concentration into HPLC. The results were given in the Table 5.

Dose verification

2.574 ml of T1 solution was taken into a 10 ml volumetric flasks and made upto the mark with acetonitrile and 0.514 ml of T2 solution was taken into a 10 ml volumetric flasks and made upto the mark with acetonitrile to bring the concentrations into linear range of the analytical method and analyzed the solutions with a validated HPLC method. The concentration of the solution was about 7.77 mg/L for T1 and 38.85 mg/L for T2.Dose verification details were presented in Table 6.

The typical T1 and T2 dose chromatograms are showed in Fig 2.and Fig 3.

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 7and Table 8respectively.

Nitrogen turnover

Soil nitrification was determined measuring the NO_3--N content of aqueous soil extracts. The concentrations of NO_3--N in the soil were then calculated from the measured values. 10 g soil in triplicate from each treatment was added 100 ml of deionized 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 $CaCO_3$ 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 Ag_2SO_4 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 70°C. Extract was

transferred into 10 ml standard flask carefully and kept the flask in cold water bath (<10°C) for five minutes followed by addition of 3 ml of 0.1% chromotropic acid prepared in conc.H_2SO_4.

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 NO_3--N in the soil was expressed as mg of NO_3--N per kg soil dry weight. For all test samples, NO_3--N was determined using following formula.

$$NO_3^-\text{-}N = \frac{C \times F_1 \times 100\% \times 1000g}{\text{Soil D.W} \times 10\ g}$$

NO_3^-N = Content of NO_3^--N in soil [mg/kg dry weight]

C = Content of NO_3^- in solution [mg/10g/100ml]

$$F_1 = \frac{\text{Molecular weight of N}}{\text{Molecular weight of } NO_3^-} = \frac{14.006}{62.003} = 0.226$$

Soil D.W = Soil dry weight (%)

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

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.

Results and Discussion

The effect of the test item on nitrogen turnover was investigated in a sandy soil. The application rates of test item were 0.340 mg/kg of soil dry soil (1-fold concentration) and 1.700 mg/kg of soil dry weight (5-fold concentration) on product basis, corresponding to a field application rates of 400 ml/ha and 2000 ml/ha.

Table.1 Linearity with potassium nitrate solutions

S. No.	Concentration in mg/L	Absorbance
1	0.10	0.0225
2	0.50	0.1142
3	1.00	0.2374
4	2.00	0.4948
5	3.00	0.7485
6	5.00	1.3165
7	10.00	2.5765
8	Slope	0.2600
9	Intercept	-0.0150
10	CC	0.9998

Table.2 Recovery of nitrate in nitrate free soil

Replication No.	Fortified concentration of Nitrate in soil (mg/kg)	Recovered concentration of Nitrate in soil (mg/kg)	% of Recovery	Mean Recovery %
T1R1	0.30	0.27	90.83	89.50
T1R2	0.30	0.27	88.94	
T1R3	0.30	0.27	88.71	
T2R1	5.00	4.79	95.77	96.09
T2R2	5.00	4.75	94.94	
T2R3	5.00	4.88	97.57	

Table.3 Serial dilutions for linearity standard solutions

Stock solution concentration (mg/l)	Volume taken (ml)	Final volume (ml)	Obtained concentration (mg/l)
535.46	0.187	10	10.0
10	5.0	10	5.0
10	2.0	10	2.0
10	1.0	10	1.0
10	0.1	10	0.1
1.0	0.1	10	0.01

Table.4 Detector linearity with Picoxystrobin standard

Concentration (mg/l)	Peak area AU-sec
0.01	238
0.1	1608
1	15881
2	31979
5	77354
10	157152
Slope	**15666.56**
Intercept	**81.75**
Correlation Coefficient	**1.0000**

Table.5 Recoveries of the picoxystrobin from aqueous distilled water samples (n=6)

Sample ID	Sample area	Slope	Intercept	Dilution Factor	Fortified Concentration (in mg/L)	Recovered Concentration (in mg/L)	% of Recovery
Standard 0.1 mg/L	1597	15666.56	81.75	1	-	-	-
T0R1	-	15666.56	81.75	1	-	-	-
T0R2	-	15666.56	81.75	1	-	-	-
T1R1	234	15666.56	81.75	1	0.01	0.0097	97.18
T1R2	232	15666.56	81.75	1	0.01	0.0096	95.90
T1R3	233	15666.56	81.75	1	0.01	0.0097	96.54
T1R4	236	15666.56	81.75	1	0.01	0.0098	98.46
T1R5	234	15666.56	81.75	1	0.01	0.0097	97.18
T2R1	1602	15666.56	81.75	1	0.1	0.0970	97.04
T2R2	1598	15666.56	81.75	1	0.1	0.0968	96.78
T2R3	1605	15666.56	81.75	1	0.1	0.0972	97.23
T2R4	1597	15666.56	81.75	1	0.1	0.0967	96.72
T2R5	1601	15666.56	81.75	1	0.1	0.0970	96.97

	0.01 mg/L	**0.1 mg/L**
Mean Recovery (%)	97.05	96.95
Standard Deviation (%)	0.95	0.20
Relative Standard Deviation (%)	0.98	0.21

Table.6 Dose verification Results

Sample ID	Sample area	Slope	Intercept	Dilution Factor	Final volume (ml)	Aliquote volume (ml)	Concent-ration of aliquote (mg/l)	Recovered concentra-tion (mg/l)	% of Recovery	Mean Recovery %
Distilled Water control	-	15666.56	81.75	-	-	-	-	-	-	-
Standard 2 mg/L	31771	15666.56	81.75	-	-	-	-	-	-	-
D1R1	30728	15666.56	81.75	1	10	2.564	7.80	7.63	97.82	
D1R2	30689	15666.56	81.75	1	10	2.564	7.80	7.62	97.70	97.78
D1R3	30723	15666.56	81.75	1	10	2.564	7.80	7.63	97.81	
D2R1	30734	15666.56	81.75	1	10	0.513	38.99	38.14	97.82	
D2R2	30742	15666.56	81.75	1	10	0.513	38.99	38.15	98.84	97.84
D2R3	30749	15666.56	81.75	1	10	0.513	38.99	38.16	98.87	

Table.7 pH Values

Sample ID	pH Measurement during Nitrogen Transformation at			
	Day 0 (25.3° C)	Day 7 (25.2° C)	Day 14 (25.0° C)	Day 28 (25.4° C)
Control (Distilled water)	5.47	5.67	5.84	5.89
T1 (1.78mg/kg soil dry weight on active basis)	5.51	5.81	5.61	5.73
T2 (8.9 mg/kg soil dry weight on active basis)	5.65	5.74	5.82	5.77

Table.8 Moisture content Values

Sample ID	Moisture content (%) at			
	Day 0	Day 7	Day 14	Day 28
Control (Distilled water)	18.12	19.06	19.15	18.79
T1- (1.78mg /kg soil dry weight on active basis)	18.54	18.96	19.25	18.92
T2 -(8.9mg/kg soil dry weight on active basis)	18.26	19.19	18.99	19.08

Table.9 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	89.06	88.05	88.00	88.96
T1 (0.565 mg/kg of soil dry weight basis)				
$NO3^-$ - N	88.50	86.91	86.20	88.39
Deviation from control (%)				
$NO3^-$ - N	-0.63	-1.30	-2.05	-0.64
T2 (2.825 mg/kg soil dry weight basis)				
$NO3^-$ - N	88.06	85.48	85.53	87.96
Deviation from control (%)				
$NO3^-$ - N	-1.13	-2.91	-2.81	-1.12

Table.10 Effect of test item on the nitrate formation rate

Assessment interval Day	Control (Distilled water)			
	NO_3^- -N [mg/kg dry weight / day]#			
0-7	-0.145			
0-14	-0.076			
0-28	-0.004			
Assessment Interval Day	**T1(0.565 mg/kg dry weight basis)**		**T2 (2.825 mg/kg soil dry weight basis)**	
	NO3- -N [mg/kg dry weight / day]	**Deviation from control [%]**	**NO3- -N [mg/kg dry weight / day]**	**Deviation from control [%]**
0-7	-0.227	56.70	-0.368	14.06
0-14	-0.164	16.54	-0.181	13.81
0-28	-0.004	1.14	-0.004	-7.31

Table.11 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)
0^{th} Day	88.06
7^{th} Day	85.48
14^{th} Day	85.53
28^{th} Day	87.96

Table.12 Nitrogen transformation test: effect of picoxystrobin 25% w/v sc on nitrification of soil microorganisms

Day	Control (Distilled water)			T1(0.565 mg/kg dry weight basis)			T2 (2.825 mg/kg dry weight basis)		
	Mean Nitrification in terms NO_3^- N produced (mg/kg)	SD	RSD	Mean Nitrification in terms NO_3^- N produced (mg/kg)	SD	RSD	Mean Nitrification in terms NO_3^- N produced (mg/kg)	SD	RSD
0	89.06	0.30	0.34	88.50	0.67	0.75	88.06	0.47	0.53
7	88.05	0.65	0.74	86.91	0.36	0.41	85.48	0.77	0.90
14	88.00	0.78	0.89	86.20	0.59	0.68	85.53	0.67	0.78
28	88.96	0.12	0.13	88.39	0.24	0.28	87.96	1.22	1.39

Fig.1 Representative Calibration curve of Picoxystrobin standard

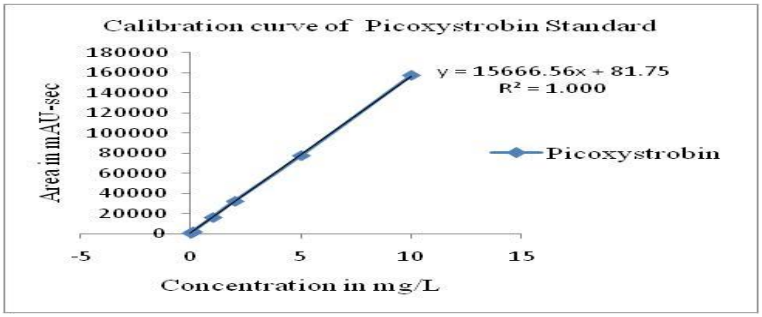


Fig.2 Representative Chromatogram T1 Dose verification sample

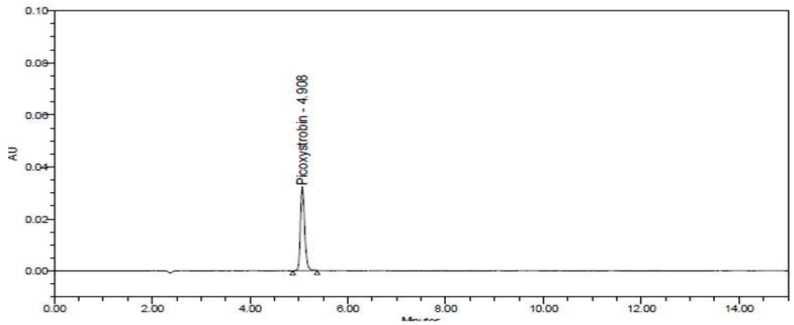


Fig.3 Representative Chromatogram T2 Dose verification sample

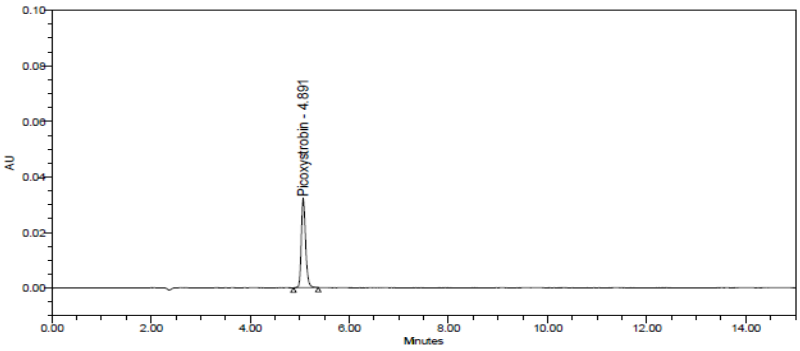
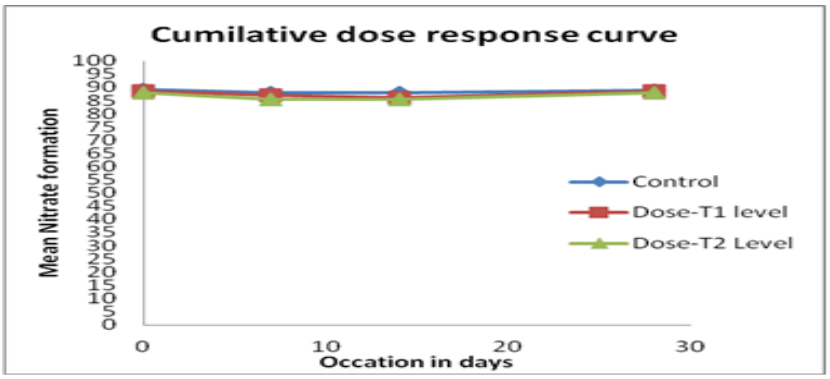


Fig.4 Cumulative respiratory curve



28 days after incubation, the lowest treatment group deviated by -0.64% and highest treatment group deviated by -1.12 % from control. The deviation rates were 1.14 % and -7.31% respectively. Deviation rates of the both the test groups in nitrogen transformation from control test systems was below the threshold value of <±25%. Hence, the study was terminated. Significant inhibitory effect in nitrogen transformation was observed upto 14 days after application of test item at both the treatment groups (1-fold and 5-fold concentrations of 28 days after application of test item, the values for both application rates were below the threshold value given in the OECD guideline 216. The percent deviation between soil treated with test item and the control was 1.14 % for 1-fold application rate and -7.31% for 5-fold application rate 28 days after application. The results of the nitrogen turnover were presented in Tables 9 to12. The cumulative dose response curve showed in Fig 4.

In conclusion, based on the test results, the test item Picoxystrobin 25% w/vhas no long-term effect on (Nitrogen transformation) induced respiration rates of soil microorganisms.

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References

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., and Parr-Dobrzanski, B. 2002.The strobilurin fungicides. *Pest Manag. Sci*., 58: 649-662.

Coleman, D.C. 2008. Linkages between soil biota and their influences on ecological processes. *Soil Biol. Biochem*., 40: 271-289.

Daniela, B., Cristina, A., Fabrice, M., Najoiel, A., Mara, G. 2007.Studies on the response of microflora to the application of the fungicide fenhexami. *Int. J. Environ. Anal. Chem.*, 87: 949-956.

Eppo. 1994. Decision-making scheme for the environmental risk assessment of plant protection chemicals- Soil Microflora. *EPPO Bulletin*, 24**:** 1-16.

Gary, R., Alison, P., Fabrice, P., Alan, S. 2010. The effect of soil moisture content on nitrogen transformation using test guideline 216.*Applied soil Ecol*., 46: 478-482.

Hindumathy, C.K., Gayathri, V. 2013. Effect of Pesticide (Chlorpyrifos) on soil microbial flora and pesticide degradation by strains isolated from contaminated soil. *Bioremed. Biodeg*., 4: 178.

Kiran, G.C. 2013. Studies of the physicochemical parameters of soil samples. *Adv. Appl. Sci. Res., 4*: 246-248.

Lal, R., Saxena, D.M. 1982. Accumulation, metabolism and effects of organochlorine insecticides on microorganisms. *Microbio. Rev*., 46: 95.

Lipika Patnaik, *et al.* 2013. Physico chemical and heavy metal characterization of soil from industrial belt Cuttack, Orissa. *Asianj. Exp. Biol. Sci*., 4: 219-225.

Metin, D., Ferda, U. 2001. Effect of some organ phosphorus insecticides on soil microorganisms. *Turk. J. Biol*., 25: 51-58.

OECD. Guideline for testing of chemicals 2004. No.217.

Oyeyola, G.P., Agbaje, A.B.,Adetunji, C.O. 2013. Determination of the soil microflora of a soil near Microbilogy

Laboratory at the University of Ilorin Main campus. *Egypt Acad. J. Biolog. Sci.*, 5: 35-41.

Rajesh, P.G., Chinchmalatpure, P.G. 2013. Physicochemical assessment of soil in RajurBazar in Amravati District of Maharastra (India). *IJCEPR*, 4: 46-49.

Sahid, I., Ainon, H., Aris, P.M. 1992. Effects of Paraquat and alchlor on soil microorganisms in peat soil. *Pertanika*, 15:121-125.

SANCO Guidelines. Method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/10684/2009.

Wagh, G.S., Chavhan, D.M., Sayyed, M.R.G. 2013. Physicochemical analysis of solisfrom Eastern part of Pune city. *Universal J. Environ. Res. Technol.*, 3: 93-99.

Winding, A., Hund-Rinke, K., Rutgers, M. 2005.The use of microorganisms in ecological soil classification and assessment concepts. *Ecotox Environ. Safe*, 62: 230-248.

Wootton, M.A., Kremer, R.J.,Keaster A. 1993. J Effects of carbofuran and cornrhizosphere on growth of soil microorganisms. *Bull. Environ. Contam.Toxicol.*, 50: 49-56.

Wu, L., Wang, H., Zhang, Z., Lin, R. 2011.Comparative metaproteomic analysis on consequtively Rehmann iaglutinosa-monocultured rhizosphere soil. *PloS ONE*, 6: e20611.

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