Acta Ecologica Sinica 36 (2016) 50–55

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

Soil microorganisms' carbon transformation test for Picoxystrobin 25% SC (*w*/*v*) in loamy sand soil

Nageswara Rao Tentu [a,*], Manohara Naidu Tentu [b]

[a] Department of Chemistry, Krishna University, Machilipatnam, AP, India
[b] Department of Nuclear Physics, Andhra University, Visakhapatnam, AP, India

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 16 December 2015
Accepted 31 December 2015

Keywords:
Loamy sand soil
Carbon transformation
HPLC
BOD
Picoxystrobin 25% SC

ABSTRACT

The effect of Picoxystrobin 25% SC on soil microorganisms (carbon transformation) was assessed in a test that measured induced respiration rate 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 carbon turnover at 1-fold and 5-fold concentrations, equivalent to 0.340 mg/kg dry weight (T1) and 1.700 mg/kg dry weight (T2) on product basis, respectively (corresponding to a field application rates of 400 mL/ha and 2000 mL/ha). Control consists of soil treated with equivalent quantity of distilled water and it 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 up to 12 consecutive hours following addition of glucose on days 0, 7, 14 and 28 after application of Picoxystrobin 25% SC. In loamy sand soil respiration study at the end of the 28th day, deviations in respiration rates compared to controls after applying the test item to soil were −18.34% and −19.55% for the test concentrations of 0.340 mg and 1.700 mg of Picoxystrobin/kg soil dry weight, respectively. The dose concentrations (T1 and T2 levels) of Picoxystrobin 25% SC ware determined by validated HPLC method.

1. 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 [2,3]. 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 [4–7]. 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 [8–10]. The soil is now believed to be a dynamic or rather a living system, containing a dynamic population of organisms/microorganisms [11]. 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 [12]. 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 [13]. 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. 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 Qo centre of cytochrome Bc1 [14]. 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 [15]. 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.

The purpose of this study was to describe the effects of strobilurin fungicides application on the population of microorganisms in soil. The strobilurin fungicide chosen was Picoxystrobin commonly used for the control of fungi in cereal crops field. The effect of Picoxystrobin on soil microflora was conducted under laboratory condition that measured nitrogen and respiration following an application of the sample to loamy sand soil. The compound was incubated in loamy sand soil over a

* Corresponding author at: Mugada, Badangi, Vizianagaram, Andhrapradesh 535578, India.
E-mail addresses: tentu6581@rediffmail.com (N.R. Tentu), t.manoharanaidu@rediffmail.com (M.N. Tentu).

http://dx.doi.org/10.1016/j.chnaes.2015.12.008
1872-2032/

period of 28 days for carbon at concentrations of 0.340 mg and 1.700 mg of formulation/kg soil dry weight. The concentrations tested were based on the maximum field application rates of 400 mL/ha and 2000 mL/ha of Picoxystrobin/ha.

2. Experimental

2.1. Materials and methods

BOD meter is supplied by Lovibond, Germany.

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

Hot air oven is supplied by Universal Engineering Co.

pH meter is supplied by Eutech Instruments Private Limited, Singapore.

Test sieve (2 mm) is supplied by Jayant Scientific Ind.

Sonicator (Ultra) is supplied by Fast Clean.

Rotary evaporator is supplied by Heidolph LR.

Distilled water unit is supplied by Stone-fin.

Digital Hygro Thermometer is supplied by TFA Germany.

Centrifuge is supplied by Eltek.

UV/Vis spectrophotometer, Model UV-1700, Shimadzu.

HPLC, Model UV-1700, Prominence, Shimadzu.

2.1.1. Standards, reagents and samples

The analytical standard of Picoxystrobin (99.9%), was obtained from Sigma Aldrich. Acetonitrile (HPLC Grade), ammonium acetate, ammonia, and sodium hydroxide were purchased from Rankem, New Delhi. Analytical grade reagents, copper sulfate pentahydrate, 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 Picoxystrobin 25% SC was purchased from local market.

2.2. 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.

2.3. 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 25.16% 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.

Table 1
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 2
Detector linearity with Picoxystrobin standard.

Concentration (mg/L)	Peak area AU-sec
0.01	238
0.1	1608
1	15,881
2	31,979
5	77,354
10	157,152
Slope	15,666.56
Intercept	81.75
Correlation coefficient	1.0000

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 0.2, 0.3 and 0.4 g of glucose per 100 g of soil dry weight. Mean respiratory response found in terms of O_2 consumed was 43.67, 54.67 and 60.92 mg/L at respective doses. The maximum respiratory response was found at 4.0 g of glucose per kg of soil dry weight and the same dose of glucose was used for glucose induced respiration.

2.4. Application of test item

Both treatment solutions of Picoxystrobin 25% SC were 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 up to 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 up to 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.

2.5. Chromatographic separation parameters

The HPLC–PDA system used, consisted of Waters Alliance Series with e2695 separations module and 2998 photodiode array detector with Empower2 software, equipped with a reversed phase C18 analytical column of 250 mm × 4.6 mm and particle size 5 μm (PhenomenexLuna-C18) Column temperature was maintained at 30 °C. The injected sample

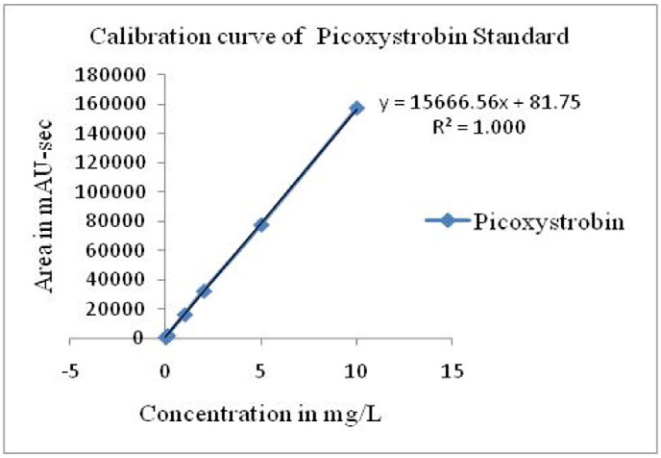

Fig. 1. Representative calibration curve of Picoxystrobin standard.