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Biodegradation of Fipronil by *Paracoccus* sp. in Different Types of Soil

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Abstract In the present studies potential of microorganisms isolated from the cotton fields was explored for the bioremediation of fipronil contaminated soils. The cultures of microbes were grown in Luria broth as shake culture maintained at 28°C. After that Dorn's broth enrichment culture supplemented with fipronil was used and isolated two bacterial cultures viz. *Paracoccus* sp. and Gamma Proteobacteria. The performance of both bacteria was evaluated for degradation of fipronil in soil. *Paracoccus* sp. was found better than the Gamma Proteobacteria as far as degradation of fipronil in soil is concerned. The samples of soil were extracted and cleaned up by following a standardized methodology. Fipronil was quantified by gas liquid chromatography and confirmed by gas chromatography mass spectrometer. The limit of quantification of fipronil was worked out to be 10 μg kg^{-1}. By using this methodology, the mean recoveries of fipronil in different types of soil were found to be more than 85%. The residues of fipronil were found to persist only up to 10 days in soils fortified with fipronil @ 20 μg kg^{-1} and amended with *Paracoccus* sp. while in the soils fortified @ 80 μg kg^{-1} fipronil, residues persisted up to 20, 30 and 30 days in loamy sand, sandy loam and clay loam, respectively. Therefore, the use of *Paracoccus* sp. can further be explored for the bioremediation of fipronil contaminated soils.

Keywords Degradation · Fipronil · GLC · *Paracoccus* sp

Fipronil [5-Amino-3-cyano-1-(2, 6-dichloro 4 trifluoromethylphenyl)-4-trifluoromethyl sulfinyl pyrazole] is a phenyl pyrazole insecticide first synthesized by Rhône Poulenc Ag Company (now Bayer Crop Science) in 1987, introduced for use in 1993 and registered in the U.S. in 1996 (Tomlin 2000; Ware 2000; Tingle et al. 2003). Fipronil is labeled for use in large number of crops and is effective against a wide range of insect pests. It has been evaluated against over 250 insect pests and on more than 60 crops worldwide (Anonymous 2004). Fipronil, as marketed under the name Regent, used against lepidopteran and orthopteran pests on a wide range of field and horticultural crops and coleopteran larvae in soils (Chanton et al. 2001; Aajoud et al. 2003). Fipronil is registered for non-agricultural as well as agricultural use in many countries. Its application rate 0.6–200 g a.i. ha^{-1} is lower than the conventional insecticides (Connelly 2001). It acts on gamma amino butyric acid (GABA) receptor, the principal nerve transmitter in insects, preventing the inhibition of GABA. Biological studies have shown that fipronil interferes with the passage of chloride ions through the gamma amino butyric acid disrupting central nervous system (CNS) activity. Biochemical assay also indicated that fipronil bind to the insect GABA receptor with a higher affinity than the vertebrate site (Cole et al. 1993). Fipronil is very highly toxic for crustaceans, insect and zooplankton, as well as bees, termites, rabbits, the African tilapis, the fringe-toed lizard and certain groups of gallinaceous birds (Anonymous 2000). Technical grade fipronil is considered moderately toxic by ingestion with an oral LD_{50} of 97 mg kg^{-1} in rats and an oral LD_{50} of 95 mg kg^{-1} in mice (Dange 1994).

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The half-life of fipronil in soil varies greatly, ranging from 3 days to 7 months. Many factors influence the rates of degradation of fipronil, including temperature, moisture, formulation and most notably, soil composition. However, the knowledge on the fate of these degradates in the environment is obscure. (Ying and Kookana 2002). Due to its higher persistence of residue in soil bioremediation is a promising approach to degrade the pesticide (Singh 2008).

The microorganisms can be helpful when it comes to the elimination of pesticide pollutants in the soil environment. The knowledge of microbial degradation of fipronil in soil, which is an important part of our environment, is necessary both for agricultural practices and environmental safety (Fenet et al. 2001). The increasing number of pesticides used in agriculture has recently acquired great importance due to the contamination of the environment. Degradation studies in soils are essential for evaluation of persistence of pesticides and their breakdown products. Data on the rate of pesticide degradation are extremely important as they permit prediction of the potential risk associated with exposure. Information on the nature and amounts of pharmacological active of fipronil and its degradation in soil is necessary to know the fate of pesticide. Since, no information on microbial degradation of fipronil in different types of soil is available, therefore the present studies were conducted.

Materials and Methods

Fipronil MB-46030 (97.5%) and Regent 0.3 G were supplied by M/S Bayer Crop Science, Mumbai. Acetone, Anhydrous sodium sulphate, Sodium chloride, Charcoal, Dichloromethane, Hexane and Silica gel were procured from Merck Pvt. Ltd, Mumbai. Dorn's broth media were used for culturing the microbes. The composition Dorn's broth were $Na_2HPO_412{\cdot}H_2O$ 3.0 g, KH_2PO_4 1.0 g, $(NH_4)SO_4$ 1.0 g, $MgSO_4.7H_2O$ 10.0 g, $CaCl_2.2H_2O$ 2.0 g, $MnSo_4$. H_2O 3.0 g, $FeSO_4.7$ H_2O 0.2 g, Ammonium ferric citrate 0.01 g, Yeast extract 0.1 g, Distilled water, pH 7.0. All the above used solvents were of laboratory grade and were redistilled using all glass distillation apparatus. Running reagent blanks before actual analysis ensured the suitability of solvents and other chemicals.

To study the persistence of fipronil, three types soil (clay loam, sandy loam and loamy sand) collected randomly from the field of Punjab Agricultural University. Stone and plant debris were discarded from soil samples. Then the soil samples were air-dried, crushed and passed through a 2 mm diameter mesh. The soil samples were autoclaved to destroy the microbes responsible for degradation of pesticides. The soils had the following physiochemical properties: in clay loam pH 6.5, organic matter 0.69, electrical conductivity 0.61, in sandy loam pH 6.9, organic matter 0.62, electrical conductivity 0.54 and in loamy sand pH 7.4, organic matter 0.39, electrical conductivity 0.51.

The degradation studies of fipronil by using soil microbes were conducted at the Pesticide Residue Analysis Laboratory and Insect Molecular Biological Laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana. Different soil types, viz. clay loam, loamy sand and sandy loam were used for experimentation. Soil samples collected from different cotton growing areas with extensive use of pesticide history served as source of pesticide degrading microbes. Different soil samples were pooled by thorough mixing. Around 200 gm of pooled sample was suspended in 1 L of sterilized distilled water and suspension filtered by sieving through two layers of muslin cloth. The microbial biomass in the filtrate was recovered in pellet by centrifugation at 10,000 rpm for 10 min and resuspended in 0.5 mL of sterile distilled water. Bacterial cultures were isolated by inoculating the soil samples in synthetic media i.e. Dorn's broth and Dorn's medium supplemented with fipronil. The pH of the medium was adjusted before autoclaving at 15 psi for 20 min. Stock solutions of carbon sources were prepared and their pH values were adjusted at 7.0 and sterilized separately. Sterilized carbon sources and mineral salt solutions were added to the medium at the time of pour plating to get appropriate final concentration. For studies on growth of cultures and degradation of fipronil, actively growing cultures were inoculated in 250 mL flasks containing 100 mL of sterilized medium having fipronil as the sources of carbon and nitrogen. Flasks were incubated on shaker at 150 rpm at 28 ± 1°C. Growth was measured as increase in optical density. Samples (5 mL) were drawn at regular intervals, and spun to prepare a cell palette, which was suspended in 5 mL of distilled water for measuring optical density at 540 nm. Microorganism that showed growth in specified carbon sources were streaked on Dorn's agar medium containing same pesticide as the carbon source. Cultures were purified by repeated streaking on synthetic media and nutrient agar. Pure cultures were further sub cultured on Dorn's agar and maintained at 4°C. Taxonomic identification of bacterial isolates showing potential for fipronil metabolization was based upon 16s ribosomal RNA (16s rRNA) nucleotide sequence homology.

A loopful of bacterial mass taken from an isolated clone on LB-agar plate was inoculated into 3 mL of Luria Broth (LB) and allowed to grow for 48 h at 28 ± 1°C on an orbital shaker (150 rpm). The bacterial cell mass was harvested in pellet by centrifugation at 10,000 rpm, for 1 min. The cells were suspended in 10 mM Tris EDTA buffer (TE) containing 20 μg mL^{-1} lysozyme, incubated at RT for 10 min. The celluar proteins in the lyzate were

removed by three times extractions with phenol:chloroform (1:1 v/v) followed by chloroform: isoamyl alcohol (25:1 v/v). The total DNA in the aqueous phase was precipitated with equal volume of isopropanol. The bacterial DNA was collected by centrifugation at 10,000 rpm, for 5 min. The DNA in the pellet was washed with 70% ethanol and allowed to air dry at room temperature. The dried DNA pellet was dissolved in 100 μL of TE buffer and stored at −20°C until used. The quality of DNA isolated from bacteria was determined by horizontal agarose 0.7% containing ethidium bromide @1 μg per mL gel electrophoresis in 1× TAE buffer at 75 V for 1 h. The DNA bands were visualized and photographed under a UV transilluminator in 'Ultra cam Gel documentation system.

Specific amplification of 16s rRNA was performed using universal 16s rRNA primers (Forward:CGGCAGGCTTAA CACATGCA and Reverse:TCTACGAATTTCACCTC TACACT) as per Weisburg et al. (1991). The primers were custom synthesized through facility of Integrated DNA Technologies, Inc, Coralville, IA, USA. Each primer was dissolved in 1/10 TE buffer (stock solution) and diluted to provide 10 μM of working primer solution before use. PCR amplifications was performed in 0.5 mL PCR tube the reaction mixture in which was consisted of bacterial DNA-10 ng), Forward and Reverse primers- 1.5 μL each, 10× Taq reaction buffer (5.0 μL), Taq polymerase (5 units), 5 mM dNTPs mix (2.0 μL) and distilled water (to make 50 μL). PCR-amplification was accomplished in a programmable DNA thermalcycler (Mastercycler Gradient-eppendorf^{TM}) using the following PCR program: 95°C for 5 min, (95°C for 1 min, 53°C for 2 min, 72°C for 2 min) ×28 cycles, 72°C for 5 min and Store at 4°C. 10 μL aliquote from the PCR amplification product was resolved by horizontal agarose gel electrophoresis using 0.7% (w/v) agarose gel (supplemented with ethidium bromide @ 1.0 mg/L) TAE buffer. 10 μL of PCR product was mixed with 2 μL of 6× 'gel loading buffer' (0.25% bromophenol blue-0.25% xylene cyanol-30% glycerol in water) and mixture loaded into the wells of agarose gel. The gel was subjected to electrophoresis at constant voltage (75 V) for 1 h. The molecular size of the amplified products in the gel were visualized under UV-transilluminator (using low intensity of UV light) and recorded with a Gel documentation system (Ultra cam). Size of amplified bands was ascertained by co-running a molecular weight standard (1,000 bp DNA ladder plus, Fermentas Life Sciences) along with the samples in the gel. The 40 μL of amplified reaction mixture was submitted to M/S Xcelris Labs Limited, Ahmedabad for custom sequencing (both strands) of amplified DNA using the universal 16s rRNA forward and reverse primers. The final sequence of 16s rRNA genes were edited using DNA software chromaslite 201. Establishing Taxonomic identity through Blast search of homologous sequence: In order to determine the taxonomic identity, the individual 16s rRNA sequences were processed for sequence homology through Blast function (Taxonomy Report) of NCBI Gene Bank database of NCBI (National Center for Biotechnology Information). *The specie was identified as the one showing closest homology with the unknown target bacterial species*.

Each soil type (clay loam, loamy sand and sandy loam) sample was fortified using two doses of fipronil @ 20 and 80 μg kg^{-1} along with isolated 45 × 10^7 microbe cells. Each treatment was replicated thrice. From each fortified (insecticide + microbes) sample, 50 g soil sample was taken and filled in plastic cup. The cups were moistened daily with water. The whole experiment was conducted at 25 ± 2°C under laboratory conditions. Fortified soil samples along with control samples were taken at each sampling at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 days after inoculation of isolated microbes.

Representative soil samples (50 g each) were dipped in acetone (100 mL) and kept over night. The extracted samples were re-extracted with 50 mL of acetone and filtered. Finally, a washing was given to the beaker with acetone to remove traces of residues of fipronil. The combined extract, thus obtained, was transferred to a separatory funnel of 1 Litre capacity and diluted with 600 mL of 2% aqueous solution of sodium chloride. To this 100 mL of dichloromethane was added and shaken gently for 1 min. The contents were allowed to stand till a clear separation of two phases was obtained. The lower layer of dichloromethane was drained into a 500 mL beaker through one and half-inch layer of anhydrous sodium sulphate supported on a pre-washed glass wool in a funnel. The aqueous layer was re-extracted with 100 mL of dichloromethane and twice with 50 mL of hexane each time and the upper organic phase was passed through the same anhydrous sodium sulphate and combined with the contents already obtained. The sodium sulphate was washed with the additional 25 mL of dichloromethane. The combined extracts thus obtained were concentrated to 2–3 mL in vacuum in a rotary evaporator at a temperature below 35°C. The extracts were cleaned up by column chromatography using silica gel as an adsorbent. Before use, the silica gel was activated at 110°C for 2 h. A glass column (60 × 1.5 cm i.d.) was packed with activated silica gel (10–12 g), mixed with 0.5 g of charcoal in between the two small layers of anhydrous sodium sulphate supported on glass wool. The column was pre-washed with hexane, following which the concentrated extract was poured over it. The glass beaker was rinsed with acetone and the extract was transferred to the column. The extract was eluted with a freshly prepared solvent mixture of dichloromethane and acetone (1:1, v/v). The eluate was concentrated to near dryness in a rotary evaporator under vacuum and transferred to 5 mL of acetone for further analysis.

Standard solution of fipronil ranging from 1 ng to 10 ng were injected into the gas liquid chromatograph (GLC) system and checked for linearity of the instrument which was found to be satisfactory even at low concentrations. The cleaned up extract of 50 g soil sample could be concentrated to 12.5 mL, out of which 1 μL (4 mg equivalent soil), when injected did not give interfering peaks in the region of the compounds analyzed. Under the operating conditions described earlier, 0.2 ng of fipronil gave half scale deflection. Based on twice the noise level, the limit of determination of fipronil in soil was worked out to be 10 μg kg^{-1}.

Recovery experiments were (more than 85%) carried out to evaluate the efficiency of the analytical method used. Samples of soils from control plots were spiked with the insecticide at level of 10, 50, 100, 500 and 1,000 and μg kg^{-1}. These were extracted, cleaned up and analyzed following the method already described. The control samples from untreated plots and reagent blanks were also processed in the same way so as to find out the interferences, if any, due to the substrate and reagents, respectively.

The sample extracts were analyzed using gas liquid chromatograph (GLC), model Shimadzu 2010, equipped with electron capture detector (ECD) and capillary column DB-5 (30 m × 0.25 mm i.d. × 0.25 μm film thickness of 5% diphenyl 95% dimethyl polysiloxane). The working conditions of GC were: injector temperature 280°C, column initial hold temperature 200°C for 5 min, followed by 220°C for 10 min and detector temperature 300°C, Column flow @ 1 mL min^{-1} with split ratio 1:10. Before use, the column was primed with several injections of standard solution of fipronil till a consistent response was obtained. Suitable aliquots of the cleaned samples were then injected into the ECD mode of the detector. The compound in the sample was identified and quantified by comparison of the retention time and peak heights of the sample chromatograms with that of standard run under identical operating conditions. The results were confirmed by gas chromatograph mass spectrometer (GC MS) in single ion monitoring mode. The gas chromatograph (GC) used was Shimadzu-QP 2010 equipped with mass spectrometer (MS) and a capillary column Rtx-5 Sil MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The system software used was GCMS solution version 2.5. The GCMS operating conditions were: oven (program) initial temperature was 150°C and held for 2 min, ramped 10°C min^{-1} to 220°C and held for 5 min, then again ramped 1°C min^{-1} to 230°C, held for 10 min; injector temperature was 280°C, column temperature was 150°C and detector temperature was 290°C. Helium was used as a carrier gas with a flow rate of 0.7 mL min^{-1}.

The residue of fipronil was confirmed by using gas chromatograph mass spectrometer (GC MS). The mass/ions ratio (m/z) and retention time (Rt) were used for the confirmation of fipronil in different types of soil viz. loamy sand, sandy loam and clay loam. The samples were injected and confirmed on electron ionization (EI) mode. The compound were identified based on m/z ratio of total ions chromatograph (TIC) and fragmentations of selective ions monitoring (SIM) compared with fragmentations of different mass number obtained with fipronil standard. In loamy sand soil 7 day after application of fipronil @ 20 μg kg^{-1} only parent compound was observed which has molecular mass 437.1, confirmed by m/z: 351, 367 at Rt 16.40 min.

Results and Discussion

Enrichment of microbial biomass obtained from soil under cotton fields in Dorn's medium containing fipronil (10 mg mL^{-1}) as sole source of carbon and nitrogen resulted in active proliferation of only two types of bacterial colonies. These bacterial species were purified by streak plating on Dorn's agar medium containing fipronil as above and stored in Stabs of LB-agar medium and stored at 4°C. Both of the bacterial species were gram negative and formed tiny colonies with shining surface and entire colony margin. However, whereas colonies of bacterial isolate RA-1 were white in colour, those of bacterial isolate RB-1 were yellowish- white. Pure cultures were further sub-cultured on Dorn's agar and maintained. 16s rRNA gene sequences represent the default sequences for taxonomic identification of different bacterial species by CCDB (Canadian centre on DNA barcoding). Amplification of 16s rRNA Gene Region and Sequence Determination: Amplification of total DNA from bacterial species RA-1 and RB-1 with sequence specific primers resulted in amplification of single DNA fragments of expected size of around 650 bp (Fig. 1).

Establishing taxonomic identity through blast search of homologous sequence: In order to determine the taxonomic identity, the individual 16s rRNA sequences were processed for sequence homology through Blast function. *The species was identified as the one showing closest homology with the unknown target bacterial species*. *B*oth the bacterial species were found to have high sequence homology scores with 16s rRNA from uncultured bacterial species. Bacterial isolate RA-1 was found to belong to *Paracoccus* species an α proteobacteria. However, though the nucleotide sequence homology could not assign a genus status to bacterial isolate RB-1, on the basis of close homology with 196 reported sequences was found to be a Gamma proteobacterium. The homology reports suggest that though 16s r RNA sequences are available for an enormously high number of bacterial species, these species have not been

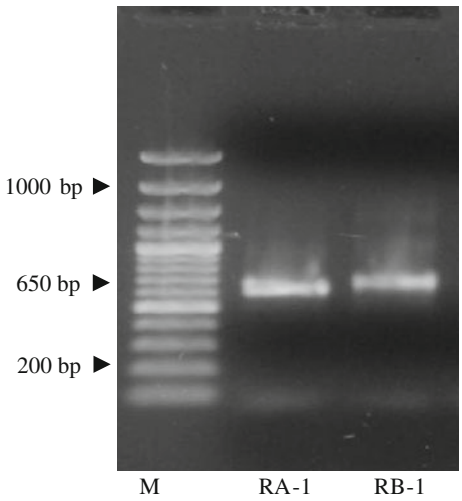

Fig. 1 PCR amplification of 16s rRNA gene region (~650 bp) from total DNA of RA-1 and RB-1 bacterial species. M- is 1,000 bp DNA ladder plus

cultured and hence are not available. In this respect, a successful isolation of bacterial species with potential for bioremediation of fipronil contaminated soils. The both bacterial culture were evaluated for degradation capacities of fipronil, in which *Paracoccus* sp was found better than the Gamma Proteobacteria.

Three types of soil (clay loam, loamy sand and sandy loam) were fortified with fipronil @ 20 and 80 μg kg^{-1} along with 45 × 10^7 microbe cells. The samples of each type of soil were analyzed at different time interval. The residues of fipronil at level of 10.9, 11.5 and 12.2 μg kg^{-1} were present in loamy sand, sandy loam, and clay loam soil samples respectively, after 10 days of application of fipronil @ 20 μg kg^{-1} along with *Paracoccus* sp. while the same fortified sample along with the Gamma proteobacteria persisted up to 20 days (Table 1). Thereafter, these residues were not detected at the detection limit of 10 μg kg^{-1}. Similarly, fipronil residues were found to be present up to 20 days in loamy sand and in sandy loam and clay loam soils it were persisted up to 30 days with fortification of different soils @ 80 μg kg^{-1} along with *Paracoccus* sp. while the same fortified sample along with the Gamma proteobacteria persisted up to 60 days (Table 2). Microorganisms are of great importance in environmental cleaning and insecticide degradation. The half-life of fipronil in soil varies greatly, ranging from 3 days to 7 months. Many factors influence the rates of degradation of fipronil, including temperature, moisture, formulation and most notably, soil composition (Ying and Kookana 2002). In case of fipronil one bacterial *Paracoccus* sp. was identified for the degradation of fipronil contaminated soils. The *Paracoccus* sp. have been described as bacteria capable of utilizing carbon and nitrogen as source of energy from the fipronil.

In different types of soils viz. loamy sand, sandy loam and clay loam fortified with fipronil @ 20 μg kg^{-1} only parent compound, fipronil was detected up to 30 days. When the same fortified soils were amended with *Paracoccus* sp. then the fipronil was found to persisted up to 10 days. The same fortified soils were amended with Gamma proteobacteria then the fipronil was persisted up to 20 days (Table 1). Similarly, when these soils were fortified with only fipronil @ 80 μg kg^{-1}, it was persisted up to 80, 90 and 90 days in loamy sand, sandy loam and clay loam, respectively and when amended with *Paracoccus* sp. then fipronil persisted up to 20 days in loamy sand, and 30 days in sandy loam and clay loam. The same fortified soil when amended with Gamma proteobacteria then it was persisted up to 60 days in loamy sand, sandy loam and clay loam (Table 2). These results show that the bacterium *Paracoccus* sp. has a great role in the degradation of

Table 1 Residues of fipronil in different types of soil fortified @ 20 μg kg^{-1} with unamended, amended with *Paracoccus* sp. and Gamma proteobacteria

Days after treatment	Type of soil	Unamended	*Paracoccus* sp.	Gamma proteobacteria
10	Loamy sand	18.0 ± 1.1*	10.9 ± 1.3*	17.1 ± 1.2*
	Sandy loam	19.1 ± 2.3	11.5 ± 1.1	18.4 ± 2.1
	Clay loam	20.3 ± 1.4	12.2 ± 1.2	19.3 ± 1.3
20	Loamy sand	12.5 ± 1.4	BDL	10.1 ± 1.1
	Sandy loam	12.9 ± 1.3	BDL	12.6 ± 1.2
	Clay loam	13.4 ± 1.2	BDL	13.2 ± 1.0
30	Loamy sand	10.3 ± 1.5	BDL	BDL
	Sandy loam	10.7 ± 1.2	BDL	BDL
	Clay loam	11.8 ± 1.4	BDL	BDL
40	Loamy sand	BDL	BDL	BDL
	Sandy loam	BDL	BDL	BDL
	Clay loam	BDL	BDL	BDL

Table 2 Residues of fipronil in different types of soil fortified @ 80 μg $kg^{-1}$with unamended, amended with *Paracoccus* sp. and Gamma proteobacteria

Days after treatment	Type of soil	Unamended	*Paracoccus* sp.	Gamma proteobacteria
10	Loamy sand	72.4 ± 4.0*	23.5 ± 3.5*	63.6 ± 3.2*
	Sandy loam	76.7 ± 3.2	28.4 ± 2.2	72.2 ± 4.7
	Clay loam	79.3 ± 2.1	31.1 ± 3.3	74.0 ± 3.4
20	Loamy sand	58.5 ± 3.2	12.5 ± 2.4	49.4 ± 3.5
	Sandy loam	67.0 ± 4.3	18.0 ± 3.3	57.2 ± 2.2
	Clay loam	68.3 ± 3.5	21.3 ± 2.4	61.7 ± 3.8
30	Loamy sand	38.2 ± 4.2	BDL	40.3 ± 3.0
	Sandy loam	46.3 ± 3.2	10.4 ± 1.4	47.1 ± 2.1
	Clay loam	47.1 ± 2.3	11.9 ± 1.2	51.3 ± 1.2
40	Loamy sand	30.6 ± 3.2	BDL	31.7 ± 2.4
	Sandy loam	37.3 ± 2.2	BDL	41.3 ± 2.2
	Clay loam	40.6 ± 3.2	BDL	42.5 ± 1.2
50	Loamy sand	19.1 ± 1.2	BDL	20.6 ± 1.0
	Sandy loam	28.3 ± 1.3	BDL	22.2 ± 1.3
	Clay loam	32.2 ± 2.0	BDL	28.0 ± 2.5
60	Loamy sand	12.1 ± 1.3	BDL	11.3 ± 1.2
	Sandy loam	17.5 ± 1.2	BDL	12.1 ± 1.1
	Clay loam	19.6 ± 1.1	BDL	13.4 ± 1.3
70	Loamy sand	13.0 ± 1.2	BDL	BDL
	Sandy loam	13.7 ± 1.6	BDL	BDL
	Clay loam	14.4 ± 1.4	BDL	BDL
80	Loamy sand	12.2 ± 1.2	BDL	BDL
	Sandy loam	12.9 ± 1.0	BDL	BDL
	Clay loam	13.0 ± 1.6	BDL	BDL
90	Loamy sand	BDL	BDL	BDL
	Sandy loam	10.4 ± 1.0	BDL	BDL
	Clay loam	10.8 ± 1.2	BDL	BDL
100	Loamy sand	BDL	BDL	BDL
	Sandy loam	BDL	BDL	BDL
	Clay loam	BDL	BDL	BDL

BDL Below detectable limit (<10 μg kg^{-1})

* Means ± SD of three replications

fipronil contaminated soils. The studies are in agreement with Kumar et al. (2008) who demonstrated the role of microorganisms in the biodegradation and bioremediation of endosulfan contaminated soils. The cultures of *Orchrobacterium* sp., *Arthrobacter* sp. and *Burkholderia* sp. isolated and identified on the basis of 16s rDNA gene sequences, individually showed in situ biodegradation of α-endosulfan in contaminated soil by 61, 73 and 74%, respectively, whereas degradation of β-endosulfan was 63, 75 and 62% respectively, after 6 weeks of incubation over the control which showed 26 and 23% degradation of α-endosulfan and β-endosulfan, respectively. Goswami et al. (2009) studied the biodegradation of endosulfan and its metabolites using fungal strains. Maximum degradation capability was shown by *Aspergillus sydoni* which degraded 95% of α-endosulfan and 97% β-endosulfan in 18 days of incubation.

Based on the present studies, it can be concluded that biodegradation is a natural process, whereas the degradation of a pesticide by an organism is primarily a strategy for their own survival. Most of these microbes work in natural environment but some modifications can be brought about to encourage the organisms to degrade the pesticide at a faster rate in a limited time. The bacterial culture of *Paracoccus* sp. seems to have a great potential to be used for bioremediation of fipronil contaminated soils. Knowledge of physiology, biochemistry and genetics of the desired microbes may further enhance the microbial

process to achieve bioremediation with precision and with limited or no scope for uncertainty and variability in microbial functioning.

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