

In vitro study of mycoremediation of cypermethrin-contaminated soils in different regions of Punjab

Parminder Kaur · Ashish Sharma · Leena Parihar

Received: 22 May 2014 / Accepted: 7 January 2015 / Published online: 27 January 2015
© Springer-Verlag Berlin Heidelberg and the University of Milan 2015

Abstract Cypermethrin is extensively used in cotton, fruit, and vegetable crops as well as in home and garden pest control worldwide. Because of its non-degrading and persisting nature, its extensive use is a great threat to the environment. Therefore, this study was undertaken in order to isolate fungi and evaluate their potential for mycoremediation of cypermethrin. Twenty-eight fungal isolates were purified from agricultural soil and then tested for cypermethrin degradation under lab conditions. Two of the isolates, 2S3 and 4S4, showed the potential to degrade cypermethrin at a concentration of 100 mg L⁻¹ when incubated for 5 days. Strain 2S3 showed a degradation potential of about 66 % with cypermethrin only mixed with water and almost 80 % when media and a carbon source were available. On the other hand, strain 4S4 showed around 70 and 80 %, respectively, under the same conditions. The strains were identified by 18S rRNA sequencing and alignment. The results showed 97 % sequence similarity with *Fusarium oxysporum* strain 8-11P and *Fusarium* sp. zzz1124. Both qualitative and quantitative investigations were conducted on the pesticide residues using FTIR set-up and HPLC analysis to confirm bioremediation of pyrethroid-contaminated environments.

Keywords Mycoremediation · Cypermethrin · Pyrethroids · Pesticides

Electronic supplementary material The online version of this article (doi:10.1007/s13213-015-1033-1) contains supplementary material, which is available to authorized users.

P. Kaur
Department of Biotechnology, School of Biotechnology and Biosciences, Lovely Professional University,
Phagwara 144411, Punjab, India

A. Sharma · L. Parihar (✉)
Botany Department, School of Biotechnology and Biosciences,
Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1),
Phagwara, Punjab 144411, India
e-mail: dr.leena@lpu.co.in

Introduction

Pesticides are chemical compounds which are not only used in agricultural, but also in kitchen gardens and other public areas to control pests (Damalas 2009). Cypermethrin [(+/-)-a-cyano-3-phenoxybenzyl(+/-)-cis, trans-3(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate] is an important synthetic pyrethroid insecticide, which is used worldwide primarily in fruit and vegetable crops, animal health, and kitchen garden pest control (Tallur et al. 2008; Lin et al. 2011). Crop production alone comprises around 26 % of the world's total pesticide use (Dollacker 1991). The usage of pesticides is even higher in developing countries and still continues to increase rapidly. Although pesticides are necessary to protect crops, they are very toxic to living organisms (Tomlin 1997). Use of synthetic pyrethroid insecticides is safe as compared to highly toxic organo-phosphate pesticides (Weston et al. 2009); however, many recent studies showed that continuous and excessive use of synthetic pyrethroid insecticides causes a lot of environmental and health problems (Cuthbertson and Murchie 2010; Cuthbertson et al. 2010). Long-term exposure to pyrethroid pesticides may lead to some chronic diseases (Al-Qurainy and Megeed 2009; Aksakal et al. 2010). For instance, they are regarded as a possible human carcinogen by the US Environmental Protection Agency (EPA), and they also possess genotoxicity, immunotoxicity, neurotoxicity, reproductive toxicity, and endocrine disruption effects (Ansari et al. 2011; Jin et al. 2011; Wang et al. 2011). Most of the applied pesticide gets dispersed through the air, soil and ground water in spite of reaching to its target organism. This results in to the accumulation of their residues and metabolites in soil at toxic levels (Gamon et al. 2003; Shalaby and Abdou 2010). Because of this, cypermethrin has been reported in nearly all sediment samples tested from urban creeks (Riederer et al. 2010; Weston et al. 2009, 2011). Moreover, the waste generated by the pesticide industry has become a serious disposal problem. This disposal problem will continue to be an environmental concern unless proper treatment technology is developed and implemented by the industries

(Fulekar 2005). Among the main consequences derived from soil pollution, we find loss of fertility, which directly or indirectly affects the survival of living beings. Therefore, it is critically necessary to develop an effective and environmentally friendly, rapid treatment method to prevent environmental pollution caused by pyrethroid waste.

Cypermethrin can be removed from soil by a number of methods which include adsorption, oxidation with ozone, heterogeneous oxidation, photolysis, Fenton degradation, and ultrasonic degradation (Rosenheimer and Dubowski 2007; Xie et al. 2011). However, these methods for controlling environmental pollution were less effective, more cumbersome, and more expensive than biological methods of remediation (Yang et al. 2011). In the natural environment, cypermethrin can be degraded through several possible processes, including hydrolysis, volatilization, photolysis, and aerobic degradation by microorganisms (Laskowski 2002). Microbial degradation is considered to be the most significant process determining the fate and behavior of cypermethrin (Fenlon et al. 2011). Much effort has been undertaken to isolate cypermethrin-degrading microbes from soil and polluted water, and a lot of pyrethroid-degrading microorganisms have also been isolated, such as *Pseudomonas* sp. (Jilani and Khan 2006), *Micrococcus* sp. (Tallur et al. 2008), *Serratia* sp. (Zhang et al. 2010), *Streptomyces* sp. (Lin et al. 2011), and *Ochrobactrum* sp. (Chen et al. 2011).

In this study, carried out at the Department of Biotechnology, Lovely Professional University, was undertaken with the objective to isolate potential pyrethroid-degrading fungal species from agricultural soils where the compound was being used, and to test their potential under lab conditions by analyzing the degradation products produced after the action of fungi.

Materials and methods

Chemicals and media Technical grade cypermethrin (94.8 % purity) with pesticide reference standards of pyrethroid pesticides was obtained from Jai Farm Chemicals Pvt. Ltd, India. All other chemicals and reagents used were analytical grade obtained from CDH, Himedia, Loba Chemie, Qualikeins & Sigma, India. Cypermethrin was firstly dissolved in various solvents (including water, normal hexane, and xylene) as stock solutions (100 mg L^{-1}) and rationed into medium to get the desired concentrations.

Microorganisms and cultivation media The pyrethroid-degrading strains 2S3 and 4S4, isolated using an enrichment culture technique from agricultural soil affected by pyrethroids, were maintained in pure cultures. The enrichment medium (Czapek Dox) containing (per litre) 30 g of sucrose, 3 g of NaNO_3 , 0.5 g of KCl, 0.5 g of MgSO_4 , 1 g of K_2HPO_4 , 0.01 g of $\text{Fe}_2(\text{SO}_4)_3$, and 0.5 g peptone, and the Czapek Dox medium without sucrose, were used for isolating fungal

strains. Enrichment and isolation of fungi were performed as described by Lin et al. (2011). Strains KF387626 and KF387627 were designated *Fusarium* sp. 2S3 and *Fusarium* sp. 4S4, respectively, based on the morphology, physio-biochemical characteristics, and 18S rRNA sequence analysis. The partial 18S rRNA gene sequences of strains 2S3 and 4S4 have been deposited in the GenBank database under accession numbers KF387626 and KF387627, respectively. Czapek Dox medium (CDM) with sucrose and media without sucrose using cypermethrin as the sole carbon source were used for the characterization of degrading strains. Cypermethrin used was at concentrations 25, 50, 70, and 100 mg L^{-1} and was added just before inoculation.

Enrichment and isolation of pyrethroid-degrading strains Agricultural soil samples were collected as the inocula from various regions of Punjab, India. One gram of soil was transferred into 250-mL Erlenmeyer flasks containing 100 mL of sterilized Czapek Dox broth (CDB) with sucrose and enrichment medium without added sucrose. One percent cypermethrin (v/v, aqueous solution) at various concentrations (25, 50, 75, 100 ppm) was added to the medium as a carbon source and incubated at 28°C for 8 days on a rotary shaker (National Scientific Works Pvt. Ltd, India) at 100 rpm (Lin et al. 2011).

The final cultures from each of the flasks were streaked on CDM agar plates with and without sucrose, enriched with cypermethrin. The plates were incubated at $28\pm 2^\circ\text{C}$ for 5 to 7 days, colonies were picked and purified by restreaking three times and were assigned code numbers. The pure isolate with highest cypermethrin degradation efficiency was selected for further study. The isolated strains were maintained at 4°C . The ability of isolates to degrade cypermethrin was determined by high performance liquid chromatography (HPLC) of extracts as described by Chen et al. (2011).

Morphological and physiological characteristics Identification of the isolates was performed on the basis of the nature of mycelium and growth patterns as described in a fungi manual (Gilman 1971). Fungal isolates were examined for cultural characteristics, such as cell shape, margin, elevation, surface appearance of the colonies, and colour change in the medium

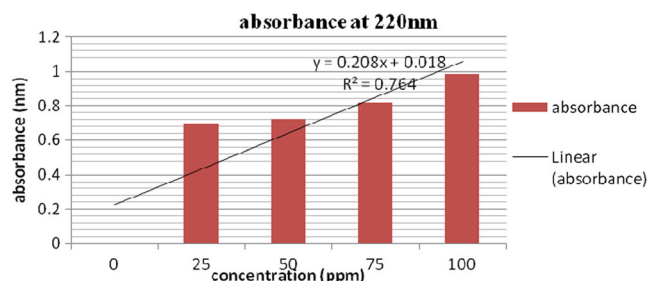
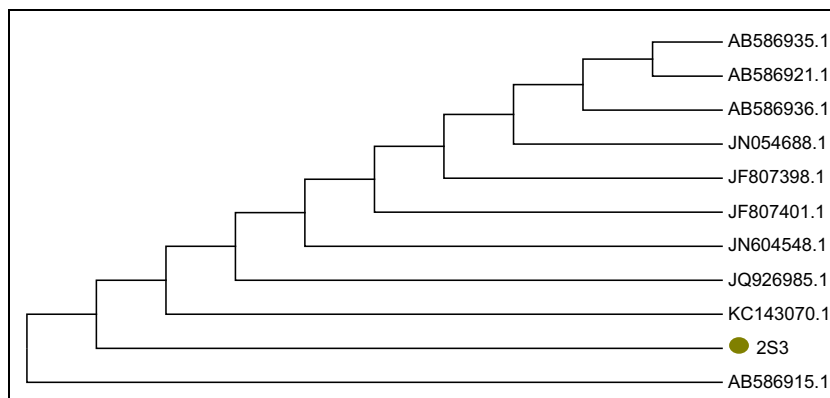


Fig. 1 Pesticide Calibration Curve at 220 nm

Fig. 2 Phylogenetic tree constructed by the neighbour-joining method based on 18S rRNA gene sequences of strain 2S3 and the type strains of related species



because of pigment formation (Molla et al. 2002). Morphological features of the cells and spores were also observed with microscope.

Taxonomic identification of the pyrethroid-degrading strain Five-day-old cultures were studied under the microscope for their morphology. Molecular identification of fungal isolates was done by Samved Biotech Pvt. Ltd, Ahmedabad, India. Genomic DNA was extracted from both cultures (2S3 and 4S4) using a microbial DNA isolation kit (Sambrook and Russell 2001). Evaluation of the quality was done on 1.2 % agarose gel. The 18S rRNA gene was amplified from the genomic DNA by polymerase chain reaction (PCR). Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with DF and DR primers using BDT v3.1 Cycle Sequencing Kit on the ABI 3730xl Genetic Analyzer. Consensus sequence of 609 bp rRNA gene was generated from forward and reverse sequence data using aligner software. The 18S rRNA gene sequence was used to carry out BLAST with the nr database of NCBI genbank database. A distance matrix was generated using the RDP database and phylogenetic analyses of the sequence data was performed using software Mega 4. An unrooted tree was built using the neighbor-joining method (Tamura et al. 2007).

Biodegradation of cypermethrin Biodegradation studies were performed using FTIR spectroscopy. Cypermethrin was extracted in xylene and filtered. FTIR spectrum was obtained using

FTIR-8400S, SHIMADZU, Luzchem Solsim v1.2, intensity approximately AM1.5 solar spectrum, provided wavelengths of 280–800 nm for the study. IR solution software (Shimadzu's FTIR instrument control) was used for data analysis.

Spectral analysis of pyrethroid degradation Several microorganisms were isolated and identified, but only the isolates with high growth rates were used for the pyrethroid degradation study. Spores harvested from the stock culture were added to three different media: the first medium comprised CDB, cypermethrin, and fungus (2S3 and 4S4); the second comprised CDB (without sucrose), cypermethrin, and fungus; the and third contained distilled water along with cypermethrin and fungus, incubated at 28 ± 2 °C on shaking incubator for 5 days. For efficient spectrophotometric detection of the tested pyrethroid, the optimum wavelength (λ max) of cypermethrin was determined by recording its UV absorption using a spectrophotometer (Saeed 2012). Optical density (OD) was observed at 220 nm using a UV spectrophotometer (SL-159, ELCO UV/Vis spectrophotometer) (Fig. 1; ESM Table 1) to plot a pesticide calibration curve for further comparison of cypermethrin degradation in all the media. At regular intervals of 0, 1, 2, 3, 4, and 5 days, about 3 mL aliquots were aseptically removed from all the experimental tubes and the OD was measured at 280 nm to study the degradation of cypermethrin in all the samples.

Fig. 3 Phylogenetic tree constructed by the neighbour-joining method based on 18S rRNA gene sequences of strain 4S4 and the type strains of related species

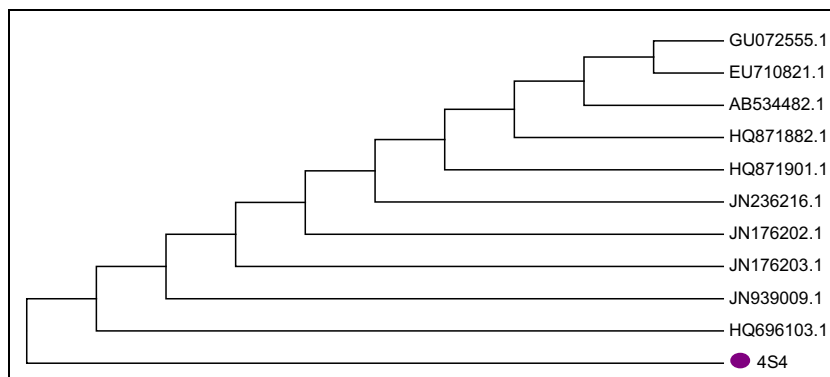
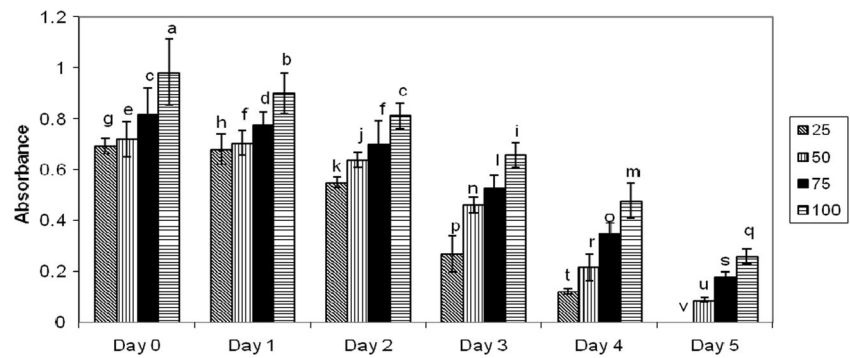


Fig. 4 Degradation of cypermethrin treated with fungal isolate (2S3) in water (without any carbon source) in five successive days. Each point represents the mean of three replicates. Means followed by common letter in the columns are not significantly different

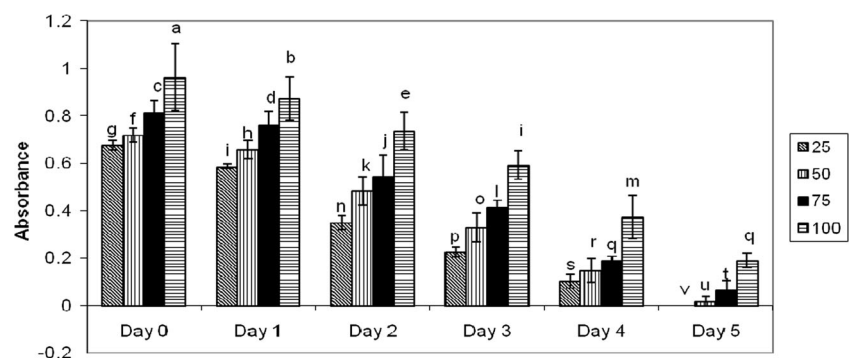


Residual quantification analysis of cypermethrin degradation Soil sub-samples were taken from three soil microcosms, for subsequent pesticide extraction and HPLC analysis to estimate the concentration of pesticide remaining in the soil. Pure cypermethrin (as a control) was used for comparing the results obtained after degradation of cypermethrin by fungal isolates.

The HPLC analysis of cypermethrin was done at the National Institute of Pharmaceutical Education & Research, Mohali, Punjab. The residual analysis of cypermethrin for the standard solutions and the sample (aliquots) was performed using an HPLC chromatographic system (Shimadzu, Japan) equipped with a UV/Vis detector, C18 column (4.6 × 250 mm), and MeOH as the mobile phase at a flow rate of 1 mL min⁻¹ with a total run time of 30 min. The temperature was maintained at 32 ± 2 °C. Quantification of the pesticides was performed by comparing the area of the peaks of treated pesticide samples to a calibration curve of the standard (Frenich et al. 2005).

Statistical analysis The data on various parameters were analysed by analysis of variance in accordance with the complete randomized design (three replications) using SPSS-16 statistical software to quantify and evaluate the sources of variation. The treatment means were compared at a significance level of 0.05 using Duncan's multiple range test (Cypreïn and Kumar 2012).

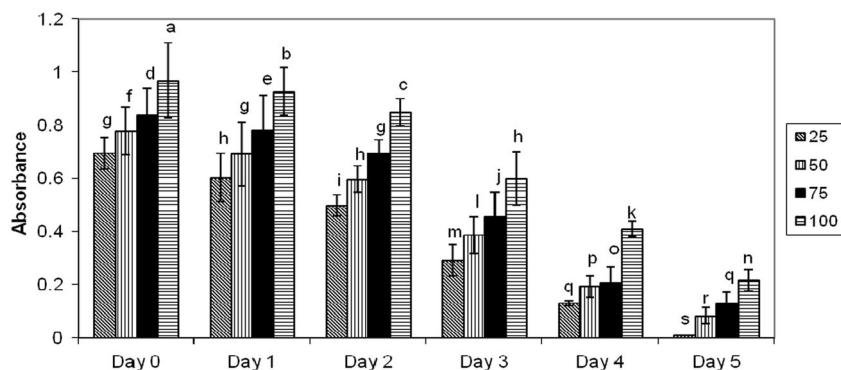
Fig. 5 Degradation kinetics of cypermethrin treated with fungal isolate (2S3) in CDB medium. Means followed by common letter in the columns are not significantly different



Results

Isolation and characterization of the pyrethroid-degrading strains By screening isolates that were able to grow with cypermethrin in the medium, we identified two fungal strains, designated 2S3 and 4S4, which were able to completely degrade 100 mg L⁻¹ of cypermethrin within 5 days. These strains showed an aerobic growth habit when grown on CDM plates, with pale yellow colonies of 2S3 and olive green colonies of 4S4. Colonies showed septate hyphae/hyaline septate hyphae bearing conidiophores, phialides, and single celled microconidia, macroconidia (single/multicelled), boat-shaped with septation. Sequences of the 18S rRNA genes from 2S3 and 4S4 showed 97 % similarity to the *Fusarium oxysporum* strain 8-11P (GenBank accession number KC143070.1) and *Fusarium* sp. zzz1124 18S ribosomal RNA gene (GenBank accession number HQ696103.1) based on nucleotide homology and phylogenetic analysis respectively. The sequence of the 18S rRNA gene (GenBank accession numbers KF387626 and KF387627) places strains 2S3 and 4S4 into the *Fusarium* group. Based on the results of morphological, physiological, and molecular analyses, the isolates were designated *Fusarium* sp. 2S3 and *Fusarium* sp. 4S4. Phylogenetic trees constructed by the neighbour-joining method based on 18S rRNA gene sequences of both the fungal strains (2S3 and 4S4) are shown in Figs. 2 and 3.

Fig. 6 Degradation kinetics of cypermethrin treated with fungal isolate (2S3) in sucrose-free medium. Means followed by common letter in the columns are not significantly different



Optimization of culture conditions for cypermethrin degradation by strains 2S3 and 4S4 Spectrophotometric analysis was performed to study the degradation of cypermethrin, for both sets of Czapek Dox medium (with and without sucrose), and for water also, inoculated with spore suspensions of the two isolated fungal strains (2S3 and 4S4) containing cypermethrin at different concentrations (Figs. 4, 5, 6, 7, 8, and 9). Uninoculated Czapek Dox medium served as control. Cypermethrin exhibited higher UV absorption at 220 nm (Saeed 2012), while the degradation of cypermethrin through fungal isolates showed maximum absorption at 280 nm. The absorbance of standard cypermethrin indicated that absorption increases linearly with increase in concentration (Fig. 1). The overall effect of the different media on the degradation response of cypermethrin was further analyzed where fungal strain 2S3 showed higher degradation than 4S4. The profile of the degradation response was plotted with the fixed value of inocula amount (the non-significant variable) at different concentrations of cypermethrin (25, 50, 75, 100 ppm).

Degradation of cypermethrin in water Degradation of cypermethrin in water without any carbon source differed from other media. On the very 1st day, degradation was almost negligible for both the strains (2S3 and 4S4). From the 2nd day onwards, rapid changes occurred in the degradation rate. Complete degradation was observed at a concentration of 25 ppm. At higher concentrations degradation was comparatively less than the media supplemented with and without

carbon source. At higher concentrations, inhibition by cypermethrin became prominent (Fig. 4 and 7).

Effect of sucrose on the degradation of cypermethrin in CD medium Strain 2S3 efficiently used cypermethrin at almost all the tested concentrations. Degradation started rapidly within 1 day. Cypermethrin was completely degraded by both the strains after 5 days up to the level of 75 ppm with the presence of sucrose in CD medium (Figs. 5 and 8). On the other hand, degradation of cypermethrin in CDB media without sucrose differed from the medium containing sucrose (Figs. 6 and 9). In the absence of sucrose, degradation was less than the sucrose-containing medium during the first 2 days. Degradation was almost equal after 5 days of incubation. The results showed that the method exhibited linearity in the tested range of concentrations. The experiment clearly indicated that the degradation proceeded comparatively faster in the presence of sucrose than in sucrose-free medium. Similarly, enhanced degradation in the presence of added carbon sources was observed by Anwar et al. (2009) and Cycon et al. (2009).

Spectral analysis of the cypermethrin through FTIR Cypermethrin IR spectra recorded before and after degradation of cypermethrin through fungal isolates are shown in Figs. 10, 11, and 12. Untreated cypermethrin solution was analyzed to check for the presence of organic bonds. It was observed that in sample 2S3, the absorbance at the bands associated with ether-cyanate, the ester group (1,125 and 1,

Fig. 7 Cypermethrin treated with fungal isolate (4S4) in water. Means followed by common letter in the columns are not significantly different

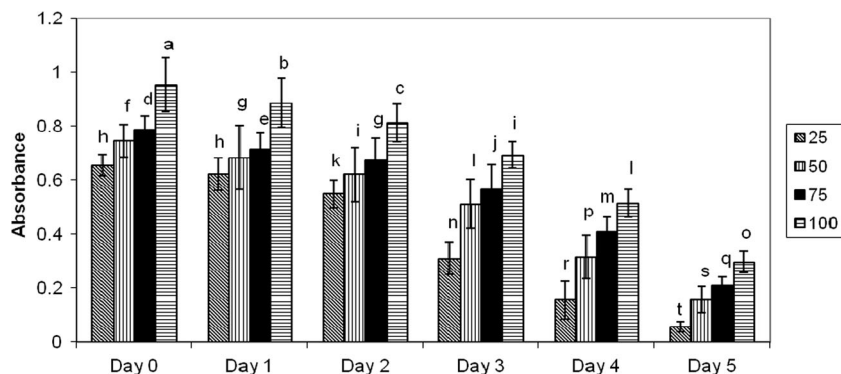
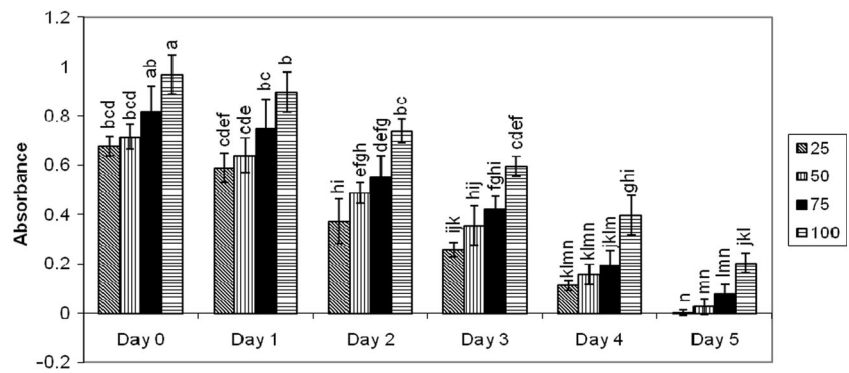


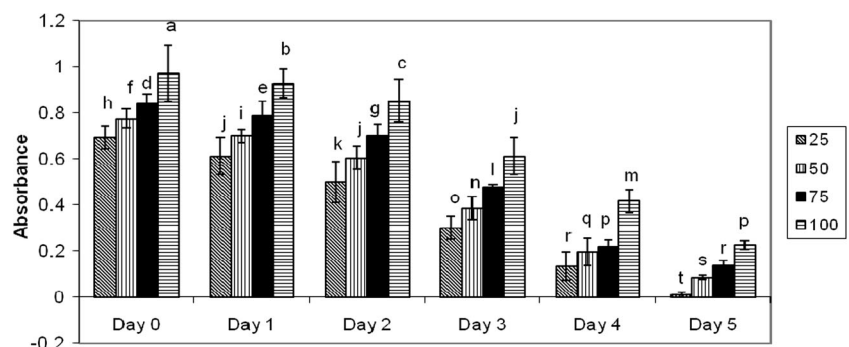
Fig. 8 Degradation kinetics cypermethrin treated with fungal isolate (4S4) in CDB medium. Means followed by common letter in the columns are not significantly different



076 cm^{-1} , respectively), decreases. A decrease of the cypermethrin carbonyl band ($1,740 \text{ cm}^{-1}$) was also observed, in parallel to a slight increase of carbonyl signals adjacent to it (around $1,650\text{--}1,700 \text{ cm}^{-1}$ and $1,760\text{--}1,780 \text{ cm}^{-1}$) due to carbonyl stretching, C=C stretching in chloroalkenes, ring vibration of benzene, CH_2 deformation in $\text{R-CH}_2\text{-CN}$ structure, and (C=O)-O-stretching. As seen in Figs. 10, 11, and 12, a shift is observed in the 750 cm^{-1} absorbance band (associated with the cyclopropane ring) (Lin-Vien et al. 1991). Figure 12 is the observed spectra of superposition of all the samples (parent cypermethrin and cypermethrin treated with both fungal isolates). In the present study, the spectra showed major changes in the range $1,210\text{--}1,160 \text{ cm}^{-1}$; and also in the region of $3,018\text{--}2,731 \text{ cm}^{-1}$, indicating degradation of pesticide (Rosenheimer et al. 2011). Similarly, Rosenheimer and Dubowski (2008) performed photolysis of thin films of cypermethrin using in situ FTIR monitoring; the identified photoproducts include 3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, acetonitrile, and cypermethrin isomers on the surface and formic acid, CO_2 , and CO in the gas phase.

Residual quantification analysis of cypermethrin by HPLC with UV/VIS detector After a 15-day interval, the residual concentration of cypermethrin was determined by HPLC. The pesticide solution mixture was extracted in xylene. Percentages of recovery were given in the respective data with technical standards. Figure 13a, b, c shows the HPLC chromatograms of untreated cypermethrin (2S0) and cypermethrin treated with both fungal isolates (2S3 and 4S4). Similarly,

Fig. 9 Degradation kinetics of cypermethrin treated with fungal isolate (4S4) in sucrose-free medium. Means followed by common letter in the columns are not significantly different



efficient diastereomeric separation and quantification of cypermethrin, resmethrin, and permethrin through RP-HPLC were performed by Saeed (2012).

Discussion

In the present investigation, where two fungal isolates were evaluated for their efficiency in degrading the pesticide cypermethrin, the observed results showed that the fungal isolates were able to use cypermethrin as a carbon source and were able to grow on it. 18S rRNA analysis showed that the fungal isolates belonged to the genera *Fusarium*. This degradation of pesticide is mainly due to the production of extracellular enzymes which break the complex structure of pesticide and convert it into small, usable components. Also, at present, more than 30 isolates of pyrethroid-degrading strains have been reported. Most of them are bacteria including *Pseudomonas* sp., *Serratia plymuthica*, *Acidomonas* sp., *Micrococcus* sp., *Bacillus* sp., and *Ochrobactrum lupini* (Grant et al. 2002; Mandar et al. 2005; Tallur et al. 2008; Chen et al. 2011), while few are fungi, including *Aspergillus niger* (Liang et al. 2005), and only one belongs to actinomycetes (*Streptomyces* sp.) (Lin et al. 2011). To our knowledge, 2S3 and 4S4 are the first reported strains in the genus *Fusarium* capable of degrading cypermethrin. In a similar type of study, both bacteria and fungi were compared for their efficiency of survival and degrading pesticides in contaminated soil. In the non-contaminated soil bacterial population was higher than the fungal population. In contrast, in pesticide-contaminated soil the

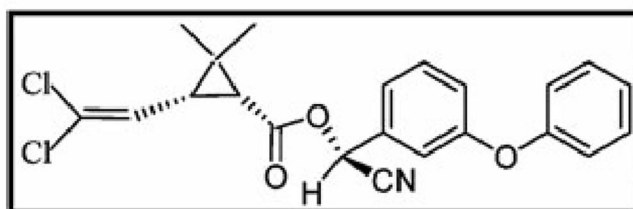
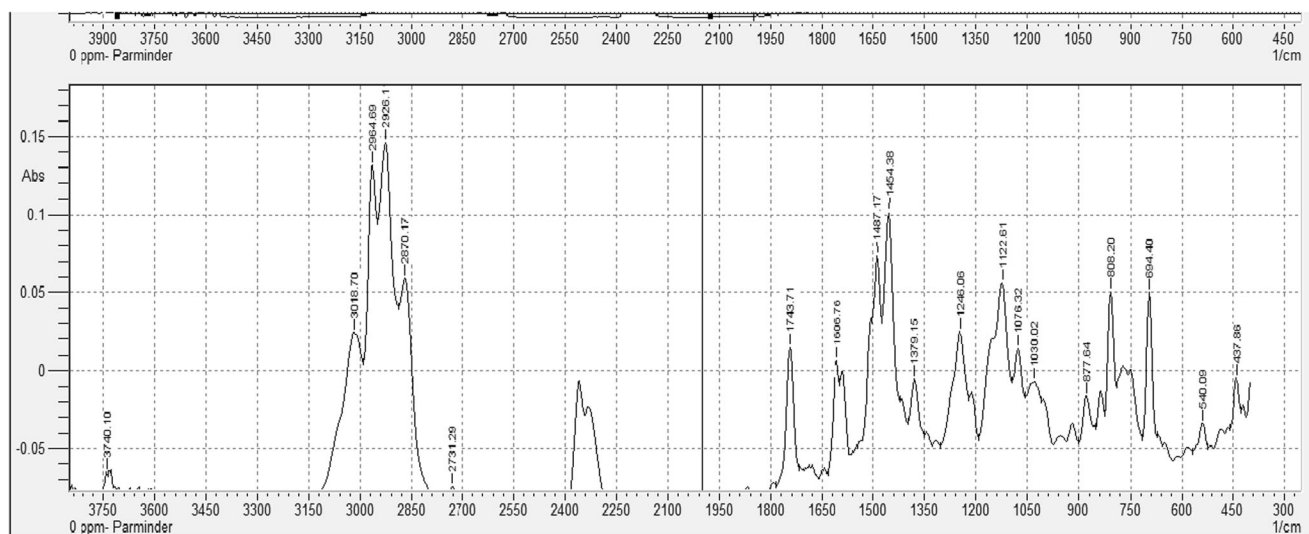


Fig. 10 IR spectra of untreated cypermethrin (as control) between 430 and 3,800 cm⁻¹ cypermethrin molecular structure is shown below the plot

bacterial populations were greatly suppressed, and the fungal population increased and became dominant. These results show that fungal strains were more resistant and possibly had the capacity to degrade into harmless metabolites (Moon et al. 2011; Chen et al. 2011).

The degradation abilities of both the fungal isolates were compared in three different conditions viz. CD medium (with and without sucrose) and water (with cypermethrin as the sole carbon source). In the case of water with cypermethrin used as medium, the rate of

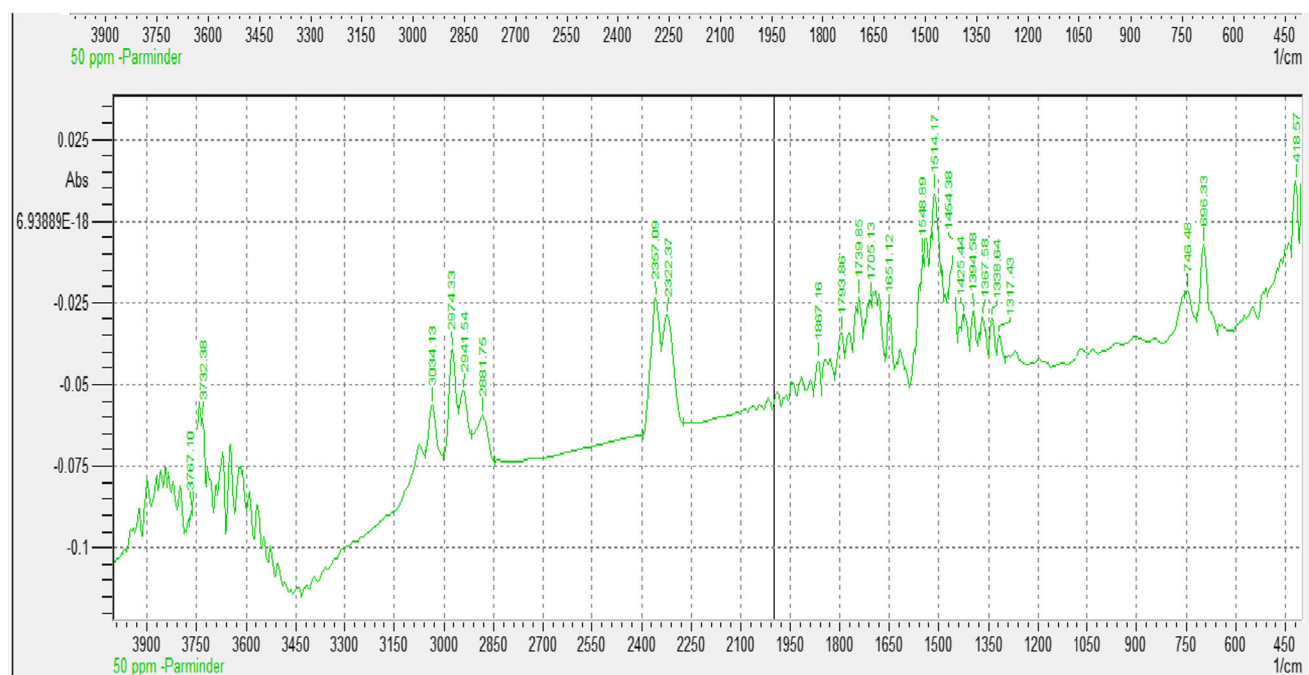


Fig. 11 IR spectra of cypermethrin treated with fungal isolate 2S3 in the range of 450 and 4,000 cm⁻¹

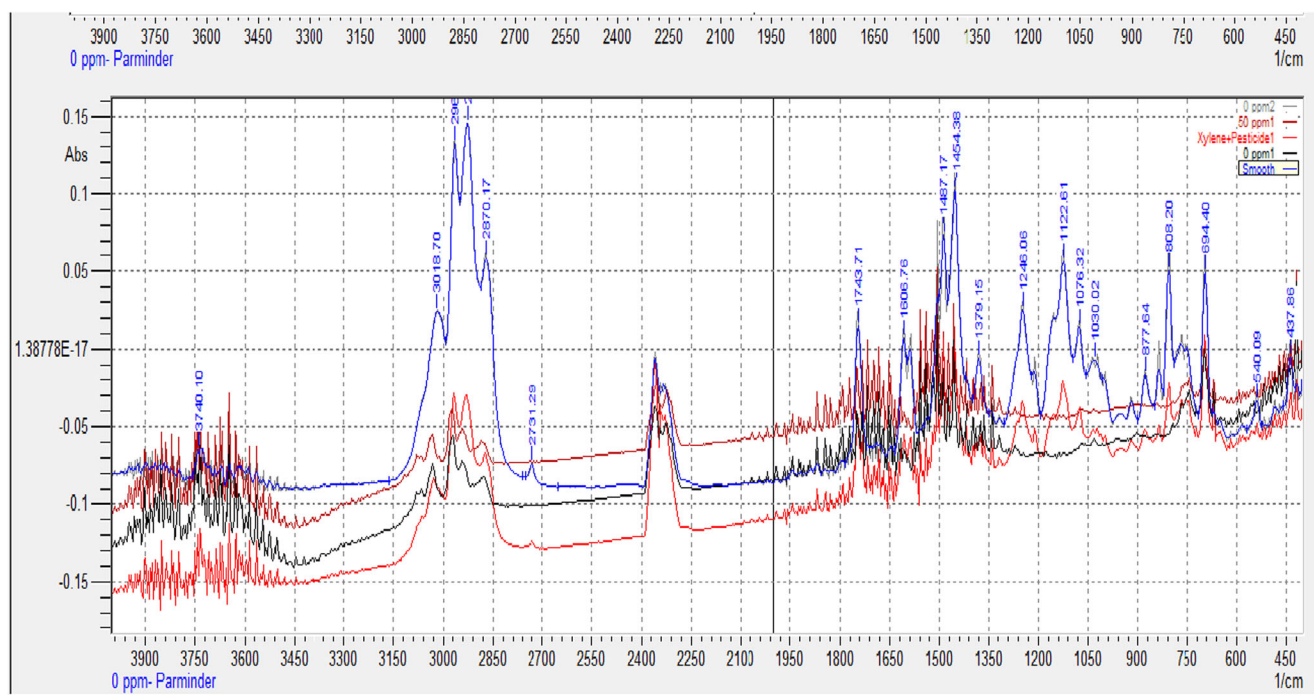


Fig. 12 Spectra of superimposition of all the samples (parent cypermethrin and cypermethrin treated with fungal isolate 2S3 and 4S4, respectively). Blue lines represent parent cypermethrin before treatment,

dark pink showing cypermethrin + xylene, while black and red represents cypermethrin treated with fungal isolates (4S4 and 2S3), respectively

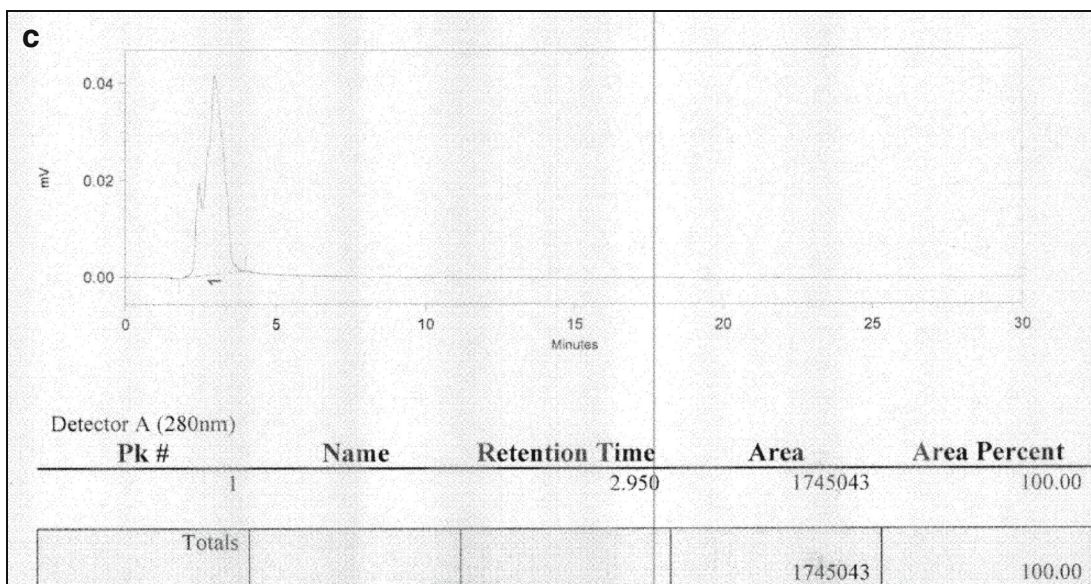
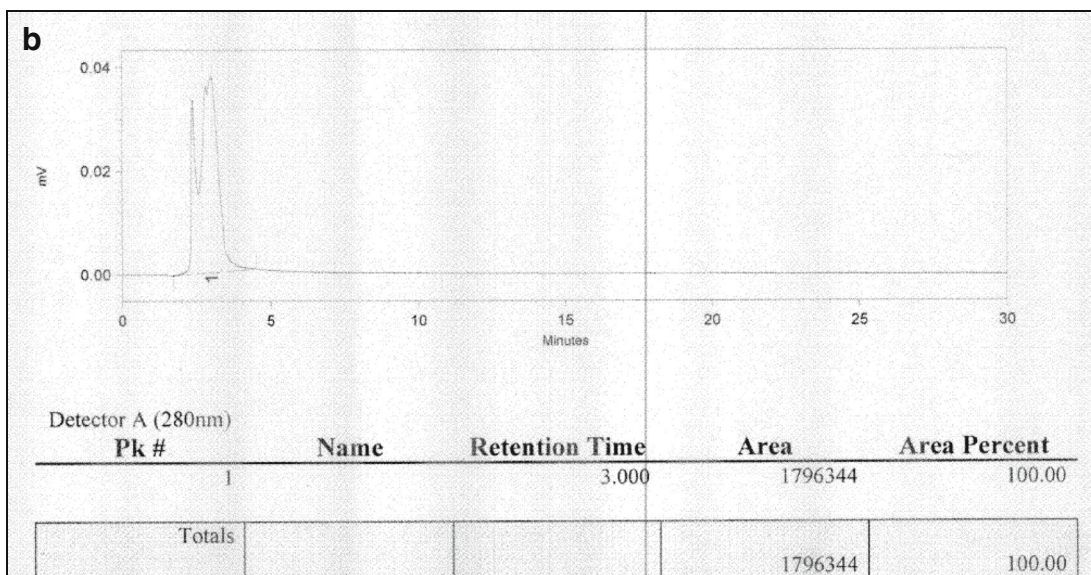
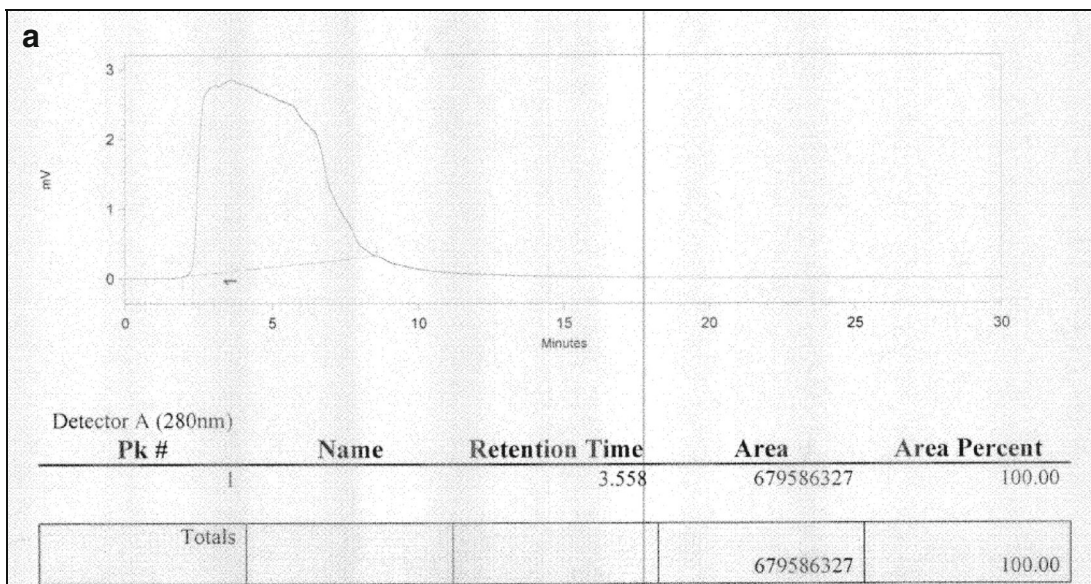
degradation was slower for both strains on initial days, but later on showed complete degradation at a concentration of 25 ppm. At higher concentrations, inhibition by cypermethrin became prominent. At higher concentrations, degradation was comparatively less than the media supplemented with and without carbon source. On the other hand, cypermethrin (at almost all the tested ranges of concentrations) in CD medium was efficiently used by both the strains. Degradation proceeds at a comparatively faster rate from the very beginning. Cypermethrin was almost completely metabolized by both strains after 5 days up to the level of 75 ppm with the presence of sucrose in CD medium. In the absence of sucrose, degradation was slightly less than the sucrose-containing medium. The results showed that the method exhibited linearity in the tested range of concentrations. Similar experiments were performed by Anwar et al. (2009) and Cycon et al. (2009), in which they showed an enhanced rate of degradation in the presence of added carbon sources.

The results of the spectral analysis of the cypermethrin through FTIR were recorded for both untreated and treated samples of cypermethrin. The study of the spectra showed major changes in the range 1,210–1,160 cm^{-1} , and also in the region of 3,018–2,731 cm^{-1} , indicating degradation of pesticide. A decrease in the absorbance at the bands associated with the ether-cyanate, the ester group (1,125 and 1,076 cm^{-1} , respectively), was also

observed in the sample of cypermethrin treated with fungal isolate 2S3; similar results were obtained by Lin-Vien et al. (1991) and Rosenheimer et al. (2011). In another experiment, Rosenheimer and Dubowski (2008) performed photolysis of thin films of cypermethrin using in situ FTIR monitoring; the identified photoproducts include 3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, acetonitrile, and cypermethrin isomers on the surface, and formic acid, CO_2 , and CO in the gas phase.

To verify the results of cypermethrin degradation obtained through both the spectral analysis and FTIR spectroscopy, HPLC analysis was done for the residual quantification of cypermethrin. After comparing the peak areas occupied and retention indices for both the treated and untreated samples of cypermethrin, it confirmed that the results were in compliance. Similarly Frenich et al. (2005) did the same experiment for the residual quantification analysis. In the same way, efficient diastereomeric separation and quantification of cypermethrin, resmethrin, and permethrin through RP-HPLC were performed by Saeed (2012).

Fig. 13 HPLC chromatograms of cypermethrin extracts obtained after 15 days of incubation with both fungal strains (2S3 & 4S4). **a** Parent cypermethrin (control), **(b)** incubation with strain *Fusarium* 2S3, **(c)** incubation with strain *Fusarium* 4S4



Conclusion

Cypermethrin is a potent pyrethroid pesticide which shows persistence in the environment owing to its complex structure. Therefore, the aim of the present study was to identify the fungal isolates that can degrade this pyrethroid compound from the agricultural micro-environments. The isolated fungi were able to degrade cypermethrin in water and media efficiently and convert it into less harmful compounds. The degradation was confirmed by fungal culture bioassays, with further confirmation from FTIR and HPLC studies. Therefore, we can conclude that fungal isolates can be used efficiently for the degradation of pyrethroid-containing compounds.

Acknowledgment We are thankful to Lovely Professional University, Phagwara, Punjab, India, for financial assistance for phylogenetic analysis of microorganisms.

References

- Aksakal E, Ceyhun SB, Erdogan O, Ekinci D (2010) Acute and long-term genotoxicity of deltamethrin to insulin-like growth factors and growth hormone in rainbow trout. *Comp Biochem Phys C* 152: 451–455
- Al-Qurainy F, Megeed AA (2009) Phytoremediation and detoxification of two organophosphorous pesticides residues in Riyadh area. *World Appl Sci J* 6:987–998
- Ansari AR, Rahman S, Kaur M, Anjum S, Raisuddin S (2011) In vivo cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch. *Ecotoxicol Environ Saf* 74: 150–156
- Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S (2009) Biodegradation of chlorpyrifos and its hydrolysis product 3, 5, 6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. *J Hazard Mater* 168:400–405
- Chen SH, Hu MY, Liu JJ, Zhong GH, Yang L, Rizwan-ul-Haq M, Han HT (2011) Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupini* DG-S-01. *J Hazard Matter* 187:433–440
- Cuthbertson AGS, Murchie AK (2010) Ecological benefits of *Anystis baccarum* in an orchard ecosystem and the need for its conservation. *Int J Environ Sci Tech* 7:807–813
- Cuthbertson AGS, Blackburn LF, Northing P, Luo W, Cannon RJC, Walters KFA (2010) Chemical compatibility testing of the entomopathogenic fungus *Lecanicillium muscarium* to control *Bemisia tabaci* in glasshouse environment. *Int J Environ Sci Tech* 7:405–409
- Cycon M, Wojcik M, Piotrowska-Seget Z (2009) Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. *Chemosphere* 76:494–501
- Cypreïn M, Kumar V (2012) A comparative statistical analysis of rice cultivars data. *J Reliab Stat Stud* 5:143–161
- Damalas CA (2009) Understanding benefits and risks of pesticide use. *Sci Res Essay* 4:945–949
- Dollacker A (1991) Pesticides in third world. *Pflanzl Nachr Bayer* 44:89–99
- Fenlon AK, Andreou K, Jones CK, Semple TK (2011) The extractability and mineralisation of cypermethrin aged in four UK soils. *Chemosphere* 82:187–192
- Frenich AG, Manuel J, Rodriguez GA, Jose L, Martinez V, Francisco J, Arrebola FJ, Torres M (2005) A study of the disappearance of pesticides during composting using a gas chromatography-tandem mass spectrometry technique. *Pest Manag Sci* 61:458–466
- Fulekar MH (2005) Bioremediation technologies for environment. *Indian J Environ Prot* 25:358–364
- Gamon M, Saez E, Gil J, Boluda R (2003) Direct and indirect exogenous contamination by pesticides of rice-farming soils in a Mediterranean wetland. *Arch Environ Contam Toxicol* 44:141–151
- Gilman JC (1971) *A Manual of Soil Fungi*. Biotech books publisher.
- Grant RJ, Daniell TJ, Betts WB (2002) Isolation and identification of synthetic pyrethroid-degrading bacteria. *J Appl Microbiol* 92:534–540
- Jilani S, Khan MA (2006) Biodegradation of Cypermethrin by *Pseudomonas* in a batch activated sludge process. *Int J Environ Sci Tech* 3:371–380
- Jin YX, Zheng SS, Fu ZW (2011) Embryonic exposure to cypermethrin induces apoptosis and immunotoxicity in zebrafish (*Danio rerio*). *Fish Shellfish Immunol* 30:1049–1054
- Laskowski DA (2002) Physical and chemical properties of pyrethroids. *Rev Environ Contam T* 174:149–170
- Liang WQ, Wang ZY, Li H, Wu PC, Hu JM, Li NL, Cao X, Liu YH (2005) Purification and characterization of a novel pyrethroid hydrolase from *Aspergillus niger* ZD11. *J Agric Food Chem* 53:7415–7420
- Lin QS, Chen SH, Hu MY, Haq MR, Yang L, Li H (2011) Biodegradation of cypermethrin by a newly isolated actinomycetes HU-S-01 from waste water sludge. *Int J Environ Sci Tech* 8(1):45–56
- Lin-Vien D, Colthup NB, Fateley WG, Grasselli JG (1991) *The handbook of infrared and Raman frequency of organic molecules*. Academic, San Diego, pp 277–306
- Mandar P, Manish J, Dileep D (2005) Biodegradation of allethrin, a pyrethroid insecticide, by an *Acidomonas* sp. *Biotechnol Lett* 27: 1909–1913
- Molla AH, Fakhru'l-Razi A, Abd-Aziz S, Hanafi MM, Roychoudhury PK, Alam MZ (2002) A potential resource for bioconversion of domestic wastewater sludge. *Bioresour Technol* 85:263–272
- Moon C, Lee CH, Sang BI, Um Y (2011) Optimization of medium compositions favouring butanol and 1, 3-propanediol production from glycerol by *Clostridium pasteurianum*. *Bioresour Technol* 102: 10561–10568
- Riederer AM, Hunter RE, Hayden SW, Ryan PB (2010) Pyrethroid and organophosphorus pesticides in composite diet samples from Atlanta, USA adults. *Environ Sci Technol* 44:483–490
- Rosenheimer MS, Dubowski Y (2007) Heterogeneous ozonolysis of cypermethrin using real-time monitoring FTIR techniques. *J Phys Chem* 111:11682–11691
- Rosenheimer MS, Dubowski Y (2008) Photolysis of thin films of cypermethrin using in situ FTIR monitoring: products, rates and quantum yields. *J Photochem Photobiol* 200:262–269
- Rosenheimer MS, Linker R, Dubowski K (2011) Heterogeneous oxidation of the insecticide cypermethrin as thin film and airborne particles by hydroxyl radicals and ozone. *Phys Chem Chem Phys* 13: 506–517
- Saeed AS (2012) Development of a reversed-phase high performance liquid chromatographic method for efficient diastereomeric separation and quantification of cypermethrin, resmethrin and permethrin. *Res J Chem Sci* 2:26–31
- Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*, 3rd edn. Cold Spring Harbor Laboratory, NY
- Shalaby SEM, Abdou GY (2010) The influence of soil microorganisms and bio-organic fertilizers on dissipation of some pesticides in soil and potato tube. *J Plant Protect Res* 50:86–92

- Tallur PN, Megadi BV, Ninebark ZH (2008) Biodegradation of cypermethrin by *Micrococcus* sp. strain CPN 1. *Biodegradation* 19:77–82
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Tomlin CDS (1997) *The pesticide manual*, 11th edn. British Crop Protection Council, London, pp 128–141
- Wang H, Wang SF, Ning H, Ji YL, Zhang C, Zhang Y, Yu T, Ma XH, Zhao XF, Wang Q, Liu P, Meng XH, Xu DX (2011) Maternal cypermethrin exposure during lactation impairs testicular development and spermatogenesis in male mouse offspring. *Inc Environ Toxicol* 26:382–394
- Weston DP, Holmes RW, Lydy MJ (2009) Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environ Pollut* 157: 287–294
- Weston DP, Asbell AM, Hecht SA, Scholz NL, Lydy MJ (2011) Pyrethroid insecticides in urban salmon streams of the Pacific Northwest. *Environ Pollut* 159:3051–3056
- Xie JM, Wang PL, Liu J, Lv XM, Jiang DL, Sun C (2011) Photodegradation of lambda-cyhalothrin and cypermethrin in aqueous solution as affected by humic acid and/or copper: intermediates and degradation pathways. *Environ Toxicol Chem* 30:2440–2448
- Yang L, Chen SH, Hu MY, Hao WN, Geng P, Zhang YB (2011) Biodegradation of carbofuran by *Pichia anomala* strain HQ-C-01 and its application for bioremediation of contaminated soils. *Biol Fertil Soils* 47:917–923
- Zhang C, Jia L, Wang SH, Qu J, Xu LL, Shi HH, Yan YC (2010) Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. *Bioresour Technol* 101:3423–3429