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BIODEGRADATION OF BIFENTHRIN PESTICIDE BY INDIGENOUS BACTERIA FROM PESTICIDE CONTAMINATED SOIL

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ABSTRACT

Pyrethroid group of pesticide is extensively used in agriculture, animal health, home, and garden pest control. Bifenthrin [2-methylbiphenyl-3-ylmethyl-(Z)-(1RS) - 3 -2-chloro-3,3,3 trifluoro- prop -1-enyl)-2,2 dimethylcyclopropane carboxylate] is one of the pesticide among the pyrethroids group of pesticides, which is effective against a broad spectrum of insect pests of economically important crops. It has been considered as greatest toxic component. The present work deals with the biodegradation of bifenthrin by using indigenous bacteria isolated from contaminated soil. It describes the biodegradation of bifenthrin by bacterial isolate IK2a into nontoxic metabolites like benzene 1, 1(methylthio) ethylidine, resorcinol and monochlorotrifluromethane which was confirmed by FTIR and GCMS analysis.

KEYWORDS: **Biodegradation, Pesticide, Bifenthrin, FTIR, GCMS.**

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INTRODUCTION

Application of pesticide has become an important component of modern age agriculture. Their use for the protection of crops from the pests, insects, mites and ticks is increasing constantly.[14,3] Pyrethroid group of pesticide was discovered and commercial developed in the 1970s, since then they have been extensively used in agriculture, animal health, home, and garden pest control.[8] This synthetic pyrethroid was derived from the naturally occurring pyrethrins from chrysanthum flower.[17] The basic characteristic structure of these pesticides is an acid joined to an alcohol by an ester bond. As they have potent neurotoxic activity against insects and low toxic for mammals, they are replaced for more toxic or recalcitrant organochlorines or organophosphates pesticide.[15] Bifenthrin [2-methylbiphenyl-3-ylmethyl-(Z)-(1RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclopropane carboxylate] is one of the pesticide among the pyrethroids group of pesticide, which is effective against a broad spectrum of insect pests of economically important crops.[19,30] It is also extensively used for the control of residential pests such as termites in urban areas.[5] Its half-life in soil is usually between 65 and 125 days, but can range from 2 weeks to over 1 year, depending on the soil type, moisture, pH, temperature, and other conditions.[16,21] Among the pyrethroids group, bifenthrin has been considered as greatest toxic component and is classified as toxicity class II which is moderately hazardous.[12,34] It has contributed most to the observed toxicity in urban sediments.[12,13,32] Bifenthrin is also toxic for the aquatic life and it persists for long days in water and soil.[12,32] Many studies have shown that pyrethroids may have cumulative toxicity, reproductive toxicity, neurotoxicological toxicity.[18,23,1,25,33] Long-term exposure to these kinds of pesticides may lead to some chronic diseases.[30,2] Some of them are considered as a possible human carcinogen.[26,35] However, out of total pesticide applied to agricultural field , 0.1% reaches the target pest and remaining affects the environment.[4]Due to the potential toxic effects to humans, ecosystem and persistence of bifenthrin in the environment, there is an urgent need to develop efficient strategies to remove the pesticide residues. Therefore, the present work was undertaken and it deals with the degradation of bifenthrin pesticide by using indigenous bacteria isolated from contaminated soil.

MATERIALS AND METHODS

Pesticides

Bifenthrin with trade name MARKAR (Dhanuka Agriculture Ltd.) was collected from local market of Sangli. Its composition was bifenthrin 10.00 % w/w.

Enrichment of Soil for isolation of Bifenthrin degrading bacteria:

Soil samples collected from the top 0-15 cm of field plots were air dried to 20% (w/w) moisture content.[7] 50 grams of each sample was placed in six glass plates and covered to maintain moisture conditions. The soil samples were then treated with aqueous solution of Bifenthrin to get final concentration 100 ppm and incubated at room temperature for two weeks by mixing gently. The moisture content was maintained using distilled water. The insecticide treatment was repeated three times at every two week of time interval.

Screening and selection of Bifenthrin degrading bacteria

The enriched soil samples (5 to 10 gm) were inoculated in mineral salt medium supplemented with bifenthrin pesticides in 10 ppm concentration for the enrichment of bifenthrin degrading bacteria. It was kept on rotary shaker operating at 250 rpm for seven days at room temperature (ranged from 25 - 28°C). A loop full of enriched culture was streaked on minimal agar plates supplemented with varying concentrations of bifenthrin (upto 100ppm) and incubated at 37°C for 24 - 48 hr. Individual colonies were sub cultured on minimal agar plates containing same concentration of bifenthrin until pure culture was isolated. The isolates showing the highest degree of tolerance were maintained on agar slant at 4°C and sub cultured after every three months.

Medium for biodegradation

The Mineral Salt Medium (MSM) containing (g L^{-1}) $(NH_4)2SO_4$, 2.0; MgSO4.7H2O, 0.2; CaCl2.H2O, 0.01; FeSO4.7H2O, 0.001; Na2HPO4.12H2O, 1.5; KH2PO4, 1.5, pH 7.2 and supplemented with 10 ppm of bifenthrin as a sole source of carbon and nitrogen was used to study the degradation.

Biodegradation of Bifenthrin

The isolates were incubated at an ambient temperature of 30^0C at shaking (150 rpm in an orbital shaker) conditions for 8 days. The degradation bifenthrin was determined after every two days by measuring decrease in λmax of the compound at 270 nm. For this, the samples were collected after every two days of incubation and centrifuged at 10000 rpm for 12 minutes in cooling centrifuge adjusted to 4^0C. The supernatant was taken, filtered through 0.2 µm membrane filter and then the filtrate was scanned in the UV- Vis Spectrophotometer (Cyberlab UV 100). The band width was set to 1 nm during scanning program. Control flask containing synthetic medium but without inoculum was run parallel along with the test flask. The degradation activity was expressed as percent degradation which was calculated by using formula,[24]

Percent degradation = Ab – Aa /Ab X 100,

Where,

Ab is absorbance of compound at 270 nm before degradation and

Aa is absorbance at same wavelength after degradation.

EXTRACTION

FTIR analysis

The biodegradation was also confirmed by Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum 65) analysis.[22] For this, after 8 days of incubation, the culture broth was centrifuged at 6000 rpm for 10 min. and supernatant was separated. Equal

volume of ethyl acetate was added to this supernatant and the organic phase containing extracted metabolites collected. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum flash evaporator. It was then mixed with spectroscopically pure KBr in the ratio of 5:95 and pressed to obtain IR- transparent pellet. The pellet was placed in sample holder and the analysis was carried out in the mid IR region of 500 - 3500 cm^{-1} with 16 scan speed.

GCMS analysis

For this, the dried metabolites obtained were dissolved in HPLC grade methanol and filtered through 0.2 µm membrane filters. The filtrate was then analyzed by Gas chromatography (Helwett Packard 984-BMS) engine with a Resteck column (0.25 mm × 30 mm; XTI-5) attached to mass spectrometry. The temperature programming mode was adjusted and samples were injected in splitless mode. During analysis the initial temperature of column was maintained at 80^0C for 2 minutes, increasing rate was by 10^0C and the final temperature was 290^0C holding for 5 minutes. Helium was used as carrier gas. The compounds were identified on the basis of mass spectra and were compared using National Institute of Standards and Technology (NIST) library.

Statistical analysis

All the experiments were carried out in triplicate. Analysis of the variants was carried out on all data at $P< 0.05$ using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

RESULT AND DISCUSSION

Screening of bifenthrin degrading bacteria

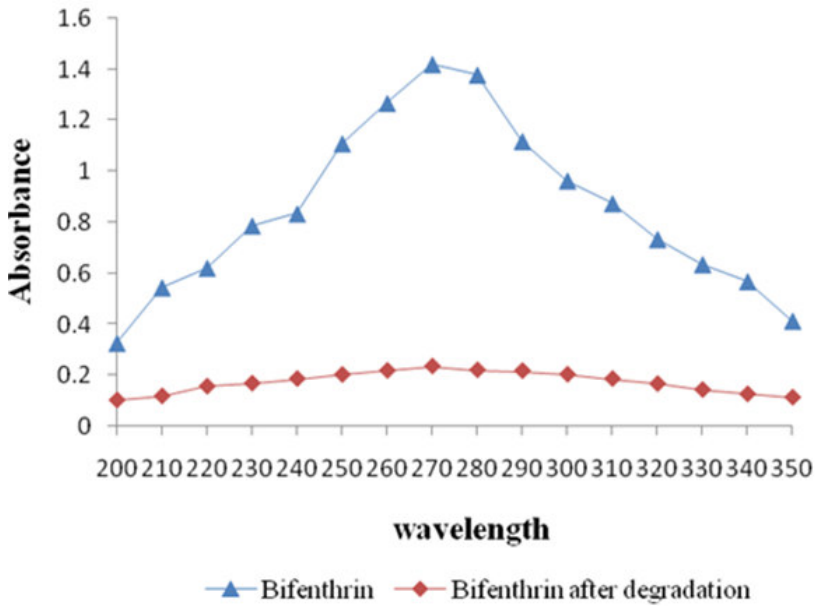
Bifenthrin was used as a sole carbon source in mineral salt medium for the isolation of pyrethroid degrading strains by enrichment technique. In the isolation procedure, two strains were able to grow well on MSM agar plates containing 10 ppm of bifenthrin. Pesticide tolerance abilities of the stains were checked by providing higher concentration of pesticide respectively 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm concentration and the highest concentration tolerating bacteria was selected and coded as IK2a. This isolate was used for further degradation study.

UV-Vis analysis of bifenthrin

UV-Vis spectral analysis of cell free broth at 200 to 400 nm wavelength was carried out to confirm the degradation of bifenthrin. Fig.1 shows the change in the absorbance spectra of bifenthrin before and after degradation by isolate IK2a. Degradation of bifenthrin was found to be 83.38%.

Graph 1

UV-Vis spectra of bifenthrin degraded metabolites after 8 days incubation.



At every 2,4,6 and 8 days of incubation the percentage degradation was calculated and it was found to be increasing with decrease in concentration of bifenthrin (Table 1).

Table 1

Percentage degradation of bifenthrin after 2,4,6 and 8 days incubation with isolate IK2a

	Before incubation	After 2 days of incubation	After 4 days of incubation	After 6 days of incubation	After 8 days of incubation
Wavelength maxima	270	270	270	270	270
Percentage degradation	0%	20.91±0.0023%	38.16±0.0023%	68.02±0.0023%	83.38±0.0023%

Values are mean of ±SEM of three experiments

FTIR analysis

The difference in FTIR spectrum of Bifenthrin (Fig.2A) and metabolites obtained after its degradation (Fig.2B) confirms biodegradation.

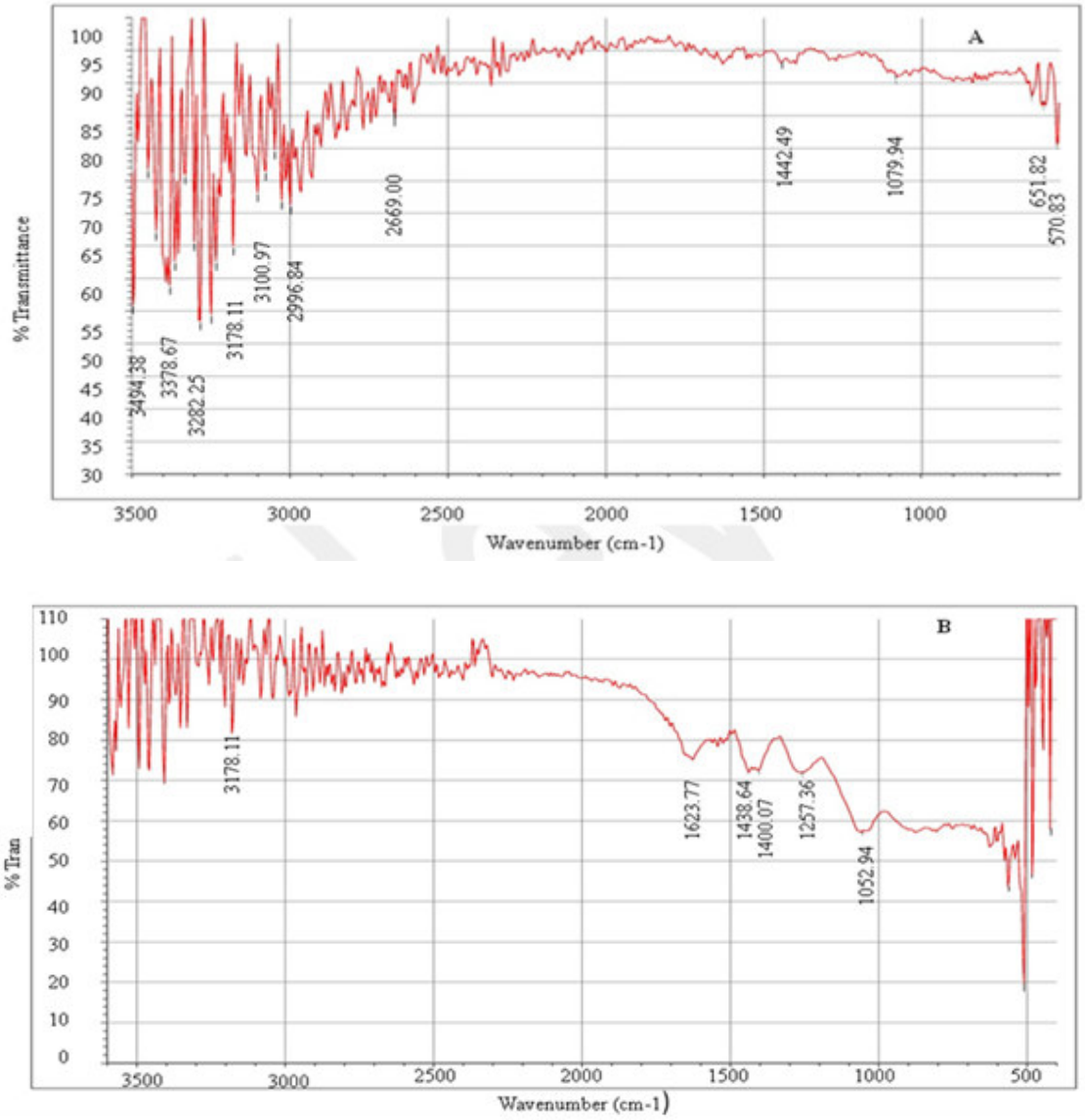


Fig 2

2 A. ***FTIR spectrum of control Bifenthrin,***

2 B. ***FTIR spectrum of metabolites obtained after degradation Bifenthrin***

The FTIR analysis is shown in Fig. 2.A. (control peak of Bifenthrin) and Fig 2.B (degraded peak of Bifenthrin). Comparison of these figures indicates C=O at 1623.77cm^{-1} disappears. This indicates complete breakdown of carboxylic group. C=C stretch was observed from 1442.49 to 1438.64 cm^{-1} and C-F stretch was observed from 1079.94 to 1052.94 cm^{-1}. The metabolites formed after degradation of Bifenthrin were further identified by GCMS analysis and degradation pathway was proposed.

PROPOSED DEGRADATION PATHWAY

GCMS anlysis of Bifenthrin shows retention time of 37.717 minutes. The result obtained were matched with NIST library database where it showes retention time of Bifenthrin. The result were matched with respect to mass/charge ratio v/s relative intensity. The result clearly showes the formation of benzene 1, 1(methylthio)ethylidine, resorcinol and monochlorotrifluromethane from Bifenthrin degradation by isolate IK2a (Fig 3).

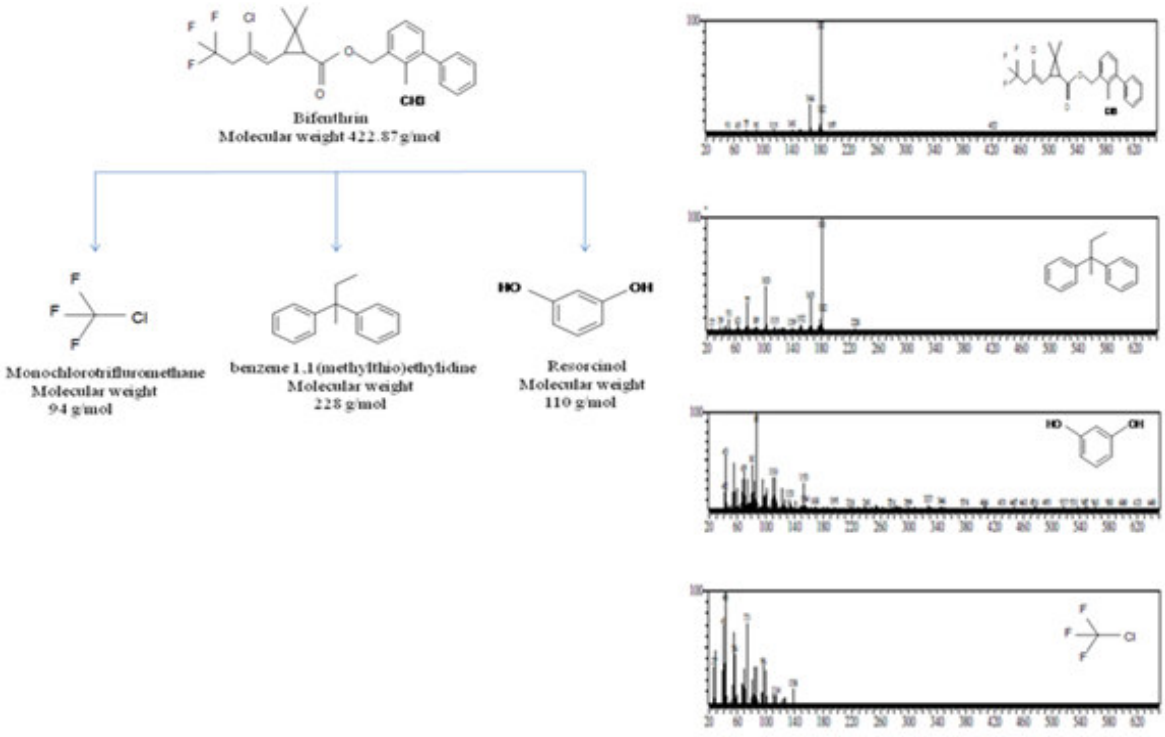


Figure 3. Proposed degradation pathway of Bifenthrin by Isolate IK2a.

DISCUSSION

The biodegradation of bifenthrin by bacterial isolate IK2a which degrades bifenthrin into non toxic metabolites like benzene 1, 1(methylthio) ethylidine, resorcinol and monochlorotrifluromethane which was confirmed by FTIR and GCMS analysis. Biodegradation is the potential of microbes to metabolize organic pollutants into nontoxic and environment friendly products that can enter into tropic levels of food chain without posing any threat to life. The release of bifenthrin to surface waters or sediments is subjected to hydrolysis, photodecomposition, volatilization, and aerobic degradation by microorganisms.[16] The microbial degradation is considered to be the most significant process for determining the fate of bifenthrin and other pyrethroids in nature.[9] An effective, cheap, and safe approach to clean up contaminated environments by using bacteria.[27] The presence of some pyrethroid degrading microorganisms such as *Bacillus cereus*.[20] The *Pseudomonas fluorescens* have ability to degrade pyrethroid pesticide.[10] Some pyrethroid degrading microorganisms such as *Micrococcus spp*. CPN 1.[28] The *Sphingobium spp.* JZ-2 have ability to degrade pyrethroid pesticide.[11] Some pyrethroid-degrading microorganisms such as *Serratia spp*. JCN13 and *Ochrobactrum tritici* pyd-1 and *Cladosporium sp.* HU have ability to degrade pyrethroid.[35,31,6] However, there was very little information available on bifenthrin degrading microorganism. The present work is in relation with the above mentioned work.

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CONLFICT OF INTEREST

Conflict of interest declare none.

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