Comparison the Biodegradation Efficiency of Cypermethrin by *Pseudomonas aeruginosa* and *Enterobactercloacae*

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Abstract: *Two local bacterial isolates were isolated from agriculture soil contaminated with cypermethrin from AlAbaeje village in Baghdad city,Iraq. Primary screening was done to test bacterial ability to growth and resistance to cypermethrin by using nutrient agar plates containing 500 mg L^{-1}cypermethrin as a sole source of carbon and energy, and incubation at 37 ºC for 72 hours. Secondary screening results showed that these two bacterial isolates have the ability to grow and resistance cypermethrin concentration till 3600 mg L^{-1} in solid MMSM.In lab experiment and under optimal conditions, cypermethrin biodegradation was measured by using FTIR and GC-MS analysis.The results shown that E.cloacae was the best isolate for degrading cypermethrin with percentage 94% while P. aeruginosa with degrading percentage 88%.*

Keywords: Biodegradation**,** Cypermethrin**,** FTIR , GC-MS

1. Introductions

Pyrethroids insecticides are a class of lipophilic esters, with an alcohol and an acid moiety. Although less toxic and persistent than other groups of insecticides, they can still represent a problem. Pyrethroids display high affinity to Na+ channels and its binding to these channels causes a prolonged channel opening that may result in a complete depolarization of the cell membrane thus blocking neuronal activity[1]. The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin. Cypermethrin agricultural products can be applied at various stages of crop development [2].The use of cypermethrin has increased sharply especially in recent years with the restrictions or eliminations of highly toxic organophosphate pesticides, and it has become one of the dominant insecticides among retail sales to consumers [3]. Cypermethrin has an extremely high toxicity to the aquatic environment with concentrations as low as 10 μgL^{-1}, destroying aquatic invertebrate life [4].[5]reported that cypermethrin was found to have carcinogenic activity in both sexes of Swiss albino mice. Pyrethroids are responsible for respiratory effects, immunological or lymphoretical effects, neurological effects, gastrointestinal effects, hematological effects and even cause death to the human being [6].Cypermethrin persistence in environment varies from 14.6 to 76.2 days (half-life) depending on physicochemical properties of soil [7]. Microbial activity in soil also plays vital role in determining the fate and behavior of cypermethrin in soil.

Looking into the facts of toxicity and persistency of this pesticide, it is urgently required to develop some strategiesto eliminate or detoxify cypermethrin and its metabolites from the environment. The biological degradation; involves the use of effective microorganism to degrade the complex pesticide into simple inorganic chemicals [8]. Moreover, this technology is less hazardous, environmentally friendly and economically viable and socially acceptable [9]. The native soil microbial consortia are superior and effective consortia for microbial degradation of pesticide than the non-native strains. Because, the native strains grow very good and have advanced adaptability in particular geographical region [10].native microorganisms are highly adaptable and have the capability to degrade the recalcitrant compounds through evolution of new genes, which encode enzymes that can use these compounds as their primary substrates [11].

Several bacterial strains such as *Pseudomonas aeruginosa*[12], *Streptomyces sp.* [13], *Stenotrophomonas sp.* [14] and *Serratiamarcescens* [15] have been reported to degrade pyrethroid pesticides. The aims of current study, shown the efficiency and different in byproducts produced from biodegradation of cypermethrin by *Pseudomonasaeruginosa* and *Enterobacter cloacae*.

2. Materials and Methods

Chemicals and Media
Commercial grade cypermethrin(10 g L^{-1},Bharat Insecticides Limited, India) was purchased from agricultural chemical dealers anddissolved in acetone to make a stock of 1000 mg L^{-1}. Stock solution was filter sterilized and kept in refrigerator for use. Modified Mineral Salt Media, Nutrient agar, Nutrient broth (pH 7) were used for the isolation and cultivation of pesticide degrading bacterial strains according to [16].

Screening and isolation of cypermethrin degrading bacteria
Soil samples were collected from different sites of agriculture soil contaminated with cypermethrin from AlAbaeje village in Baghdad city, Iraq. The samples were collected randomly from the superficial layer of soil (10-20 cm in depth), these fields were already sprayed with cypermethrin for past few years.These soil samples were used for the isolation of cypermethrin-degrading bacteria. 10 g of soil samples were added to 150 ml MMSM supplemented with 50 mg L^{-1}cypermethrin in 250mL Erlenmeyer flasks, and incubation was carried out at 150 rpm and 30 ºC for five days [17]. After dilution, one ml of soil suspension (10^{-1},10^{-3},10^{-5} and 10^{-7}) was inoculated in

nutrient agar plates supplemented with 50 mg L^{-1}cypermethrin[7], after 3 days of incubation, the discrete bacterial colonies were purified by repeated inoculating them on plates containing nutrients agar.Cypermethrin degrading bacterial cultures were screened from the isolated pure bacterial cultures by:

1) **Primary screening** by growing themon nutrient agar plates containing 500 mg L^{-1}cypermethrin as a sole source of carbon and energy as described by [13].
2) **Secondary screening** by growing them on solid modified mineral salt media containing cypermethrin as sole source of carbon and energy at different concentration ranging from (600 - 3600 mg L^{-1}) and incubated at 37 ºC for 24-48 h The growth of bacterial isolates was examined according to formation of clear zones around the colonies [18].

Identification of cypermethrin degrading bacterial strain
The colonies developed on nutrient agar were studied in terms of their shape, colour, odour and their margin, also microscopic examination was achieved for G-stained slides to characterize G-negative bacteria from others. VITEK 2 compact device (Biomerienx, France) was only used to identify the isolates[19,20]. Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions [21]. The results were computerized and recorded by the vitek software after the bacterial suspension was loaded in the vitek cards.

Determination of optimum growth conditions

Optimum pH and Temperature
Overnight bacterial isolate (2%) with (OD=0.5) were seeded into the 250 ml Erlenmeyer flasks containing modified mineral salt medium (100 ml) supplemented with 100 mg l^{-1} of cypermethrin. The pH values of the medium were adjusted to a series of 5, 7 and 9. Three sets of flasks were used each having the unique value of pH. Flasks was inoculated in duplicates in a shaker incubator (160 rpm) at 25,30,35,40 ºC for 8 days, the growth of bacterial isolate was observed by measuring Optical Density (absorbance) at 600 nm in spectrophotometer after culturing for zero time, first, third, fifth and eighth day, respectively [22].

Optimum incubation period
After determine optimum pH and temperature the best incubation period for each isolate determine by inoculated 2% with (OD=0.5) overnight bacterial isolate in Erlenmeyer flask (250 ml) containing 100 ml of modified mineral salts medium, was adjusted to pH 7 and autoclaved at 121ºC for 15 min, then supplemented with100 mg L^{-1} of cypermethrin. The flasks were incubated in a shaker incubator (160 rpm) at 30[0]C for different periods (1, 3, 5, 8, 10, 12, 14,16 days). Experiment carried out in duplicate and the growth of bacterial isolate was observed by measuring Optical Density at 600 nm in spectrophotometer [23].

Biodegradation Experiment
Erlenmeyer flasks (250ml) containing 100ml of the liquid modified mineral salts medium, the pH was adjusted to 7 and autoclaved at 121[0]C for 15 min, supplement with100 mg L^{-1}of cypermethrin. The flasks were inoculated separately with 2% (OD=0.5) from the selected isolates taken from the overnight cultures (one isolate for each flask) [24].

An experiment carried out in duplicate. The flasks were incubated in a shaker incubator with 160rpm at 30[0]C for 14 days. After the incubation period, 5 ml of broth was taken for extraction and analysis of cypermethrin residues according to[25]. Extracted liquid was taken in eppendorff tube and stored at -20[°]C till analysed by FTIR and GC-MS.

Determination of Cypermethrin Residual
Residual analysis of cypermethrin in media was performed according to [25]. Residual pesticide was extracted by adding 5 ml of culture broth to 20 ml of acetone in a flask. The mixture was filtered using Buchner funnel after shaking for 1 h and the obtained residue was filtered again by Millipore filter unit 8µm(Whatman Grade 540 Quantitative Filter Paper, Hardened Ashless) after washing thoroughly with 10 ml acetone. Filtrate was collected in a round-bottom flask [24]. The cypermethrin content was detected using GC-mass and the percentage of cypermethrin biodegradation was measured according to the following equation [26]:

$$\% \, of\ Biodegradation = \frac{Peak\ area\ of\ Zero\ Time(T_0) - peak\ area\ of\ sample(T_1)}{Peak\ area\ of\ Zero\ Time(T_0)} \times 100$$

By FTIR
The use of Fourier transform infrared (FTIR) spectroscopy provided additional information to aid the interpretation of the biodegradation process. Analysis was carried out in labs of chemistry department/college of science/University of Baghdad. Measurement were performed using a Shimadzu FTIR-8400 S using KBr discs containing 2ml of test samples which are prepare performed according [25]. Ten scans were taken for each sample in the range 400 to 4000 cm^{−1}.the measure FTIR for samples done on zero time and fourteenth day of experiment [27].

By GC-MS
Cypermethrin compounds in media were determined using gas chromatography - mass spectrometry (GC-mass). All analysis was carried out in labs of the environment and water directorate/ ministry of Science and Technology.

Statistical analysis
Least significant difference (LSD) was carried out using statistical package for social sciences (SPSS, Version 17.0). Analysis of variance (ANOVA), P – values, tests of significance, was carried out at 95% level of confidence using statistical package for social sciences. P – Values[* (P≤0.05)] were used to determine the significance levels between various treatments and data obtained during the experimental study [28].

3. Results and Discussion

Isolation and characterization of cypermethrin degrading bacteria
In the present study two isolates*Pseudomonas aeruginosa* and *Enterobacter cloacae*were only selected due to their ability to utilize cypermethrin as a carbon source. Characteristic of Bacterial colonies growing on nutrient agar plates give in table 1.

Table 1: Morphological characteristic, Gram stain, ID and probability value of bacterial isolates.

Characteristics / Isolates No.	Colony shape	Colony color	Cell shape	Gram stain	ID of isolate By Vitek 2 compact	probability value
1	Smooth flat	yellow	Rod	Negative	*Enterobacter cloacae*	99%
2	Smooth convex	green	Rod	Negative	*Pseudomonas aeruginosa*	99%

According to VITEK 2 compact result the dominate isolate in soil contaminated with cypermethrin was *Pseudomonas aeruginosa* about 87.5% while *Enterobacter cloacae* present in 12.5%.

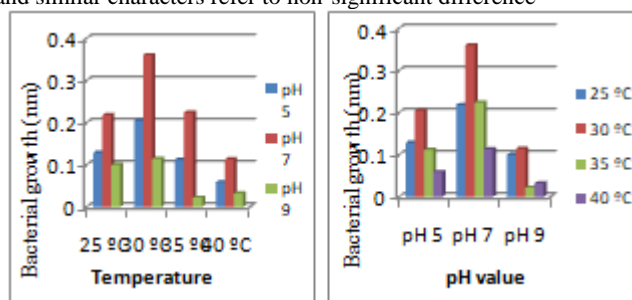## Optimization of growth conditions

Optimum pH and Temperature

Effect of pH and Temperature on growth of *Pseudomonas aeruginosa*
The results in Table 2 explains that there are significant difference ($p<0.05$) of bacterial growth at different pH and temperature degrees. Under 25 ℃, 30 ℃, 35 ℃ and 40 ℃ the best and significant means bacterial growth recorded at pH 7 were 0.218, 0.360, 0.224 and 0.133 nm respectively. Also under pH 5, pH 7 and pH 9 the best and significant means of bacterial growth recorded at 30 ℃ as 0.205, 0.360 and 0.114 nm respectively. The highest mean value for *P.aerogenosa* growth recorded at 30 ℃ and pH 7 was 0.360 nm, while lowest mean value recorded at 35 ℃ and pH 9 was 0.022 nm. As shown in fig 2.

Table 2: Mean value of *Pseudomonas aeruginosa* growth at different pH and temperature value after 8 days of incubation

pH / Temp.	pH 5	pH 7	pH 9	LSD ≤ 0.05
25 ℃	0.129 ± 0.046 Bb	0.218 ±0.062 Ab	0.100 ± 0.047 Bab	0.064
30 ℃	0.205 ± 0.051 Ba	0.360 ± 0.098 Aa	0.114 ± 0.114 Ba	0.126
35 ℃	0.112 ± 0.058 Bb	0.224 ± 0.100 Ab	0.022 ± 0.016 Bc	0.073
40 ℃	0.059 ± 0.021 Bb	0.113 ± 0.039 Ab	0.032 ± 0.032 Bb	0.035
LSD ≤ 0.05	0.074	0.122	0.070	

*Uppercase refer to comparison between means with same raw, lowercase refer to comparison between means with same column and similar characters refer to non-significant difference

Figure 2: Mean value of *Pseudomonas aeruginosa* growth at different pH and temperature value after 8 days of incubation.

Effect of pH and Temperature on growth of *Enterobacter cloacae*
The results in Table 3 present that there are significant difference ($p<0.05$) in bacterial growth at different pH and temperature degrees. Under 25 ℃, 30 ℃ and 35 ℃ the best and significant means bacterial growth recorded at pH 7 were 0.186, 0.345, and 0.155 nm, respectively. While no significant difference was observe at 40 ℃ at pH 5, 7 and 9. Also at pH 5, pH 7 and pH 9 the best and significant means of bacterial growth recorded at 30 ℃ were 0.223, 0.345 and 0.225 nm respectively. The highest mean value 0.345 nm for *Enterobacter cloacae* growth was recorded at 30 ℃ and pH 7, while lowest mean value 0.036 nm was recorded at35 ℃ and pH 9. As shown in fig 3.

Table 3: Mean value of *E. cloacae* growth at different pH and temperature value after 8 days of incubation

pH / Temp.	pH 5	pH 7	pH 9	LSD ≤ 0.05
25 ℃	0.071 ± 0.008 Bb	0.186 ± 0.114 Ab	0.082 ± 0.044 Bb	0.053
30 ℃	0.223 ± 0.053 Ba	0.345 ± 0.093 Aa	0.225 ± 0.043 Ba	0.113
35 ℃	0.130 ± 0.073 ABb	0.155 ± 0.089 Ab	0.036 ± 0.044 Bb	0.107
40 ℃	0.115 ± 0.011 Ab	0.086 ± 0.071 Ab	0.055 ± 0.021 Ab	0.114
LSD ≤ 0.05	0.070	0.143	0.055	

*Uppercase refer to comparison between means with same raw, lowercase refer to comparison between means with same column and similar characters refer to non-significant difference
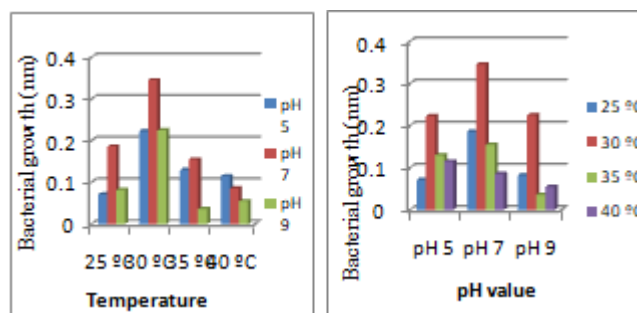
Figure 2: Mean value of *E. cloacae* growth at different pH and temperature value after 8 days of incubation.

So the pH 7 is optimum pH for growth of two selected bacterial isolates in MMSM contain cypermethrin, this finding is supported by [32], who reported that isolated *Pseudomonas* strain can grow and retain their degradation ability in a wide range of pH with optimum growth at pH around 7. Regarding temperature 30℃ is optimum for growths of two selected bacteria isolate. During Cypermethrin degradation, the direct correlation was found between temperature and microbial activity, significant removal occurred when biosimulator was operated at 28 - 30 ℃ [29].

Effect of Incubation period on growth and biodegradation
The results in table 4 showed significant difference ($p<0.05$) among means of bacterial growths for different incubation periods. The two selected bacterial isolates had highest and significant growth after 10 days of incubation.From day 1 to

day 10 there are gradually increase in growth rate of two selected bacterial isolates after 10 days of incubation the growth start to decrease. The highest mean growth value 0.457 and 0.517 recoded for *E.cloacae* and *P. aeruginosa* respectively. Also there are significant difference (p<0.05) between mean growth of two selected isolates within same day and this difference start significantly after 3 days of incubation.. As shown in fig 4.

Table 4: Mean values ± SD of Bacterial growth at 600nm at different incubation periods, and LSD value incubation

Incubation period Days	Mean± SD of bacterial growth		LSD ≤ 0.05
	P. aerogenosa	E. cloacae	
First Day	0.207 ± 0.015 Ag	0.227 ± 0.023 Ae	0.036
Third Day	0.340 ± 0.011 Af	0.317 ± 0.015 Bd	0.025
Fifth Day	0.367 ± 0.015 Ae	0.370 ± 0.026 Ac	0.038
Eighth Day	0.487 ± 0.006 Ab	0.443 ± 0.006 Ba	0.015
Tenth Day	0.517 ± 0.006 Aa	0.457 ± 0.015 Ba	0.026
Twelfth Day	0.470 ± 0.010 Ab	0.407 ± 0.015 Bb	0.024
Fourteenth Day	0.437 ± 0.006 Ac	0.367 ± 0.015 Bc	0.031
Sixteenth Day	0.400 ± 0.010 Ad	0.327 ± 0.015 Bd	0.041
LSD ≤ 0.05	0.018	0.030	

*Uppercase refer to comparison between means with same raw, lowercase refer to comparison between means with same column and similar characters refer to non-significant difference
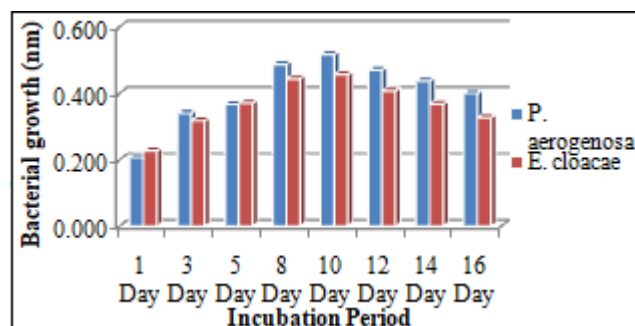
Figure 4: Mean value of Bacterial growth at different incubationperiods.

It is well known the increasing of incubation period which results in to increase viable counts [30] particularly on media with low nutrient concentrations, and due to depletion of contaminate concentration and produce of intermediates compounds and metabolic products that result in decrease the pH of media and then decreases the growth of bacteria [31].

4. Biodegradation of Cypermethrin

FTIR analysis of biodegraded cypermethrin
Between 600 and 1800 cm^{-1} In this region, the main absorption bands of cypermethrin are assigned to the carbonyl asymmetric stretching (1720- 1740 cm-1), C=C stretching of the aromatic rings (1450 and 1600 cm^{-1}), CH2 deformation in R–CH2–CN structure (1400-1450 cm^{-1}) and the (C=O) –O– stretching (1050-1095 cm^{-1}). The band at 1200-1275 cm^{-1}, caused by aryl–O of diphenyl ether, involves aryl–O stretch, out-of-phase C–O–C stretching and ring vibrations. An additional important representing band of the molecule is observed at 1125-1205 cm^{-1} and is related to the C–O stretching of the cyanate group (–O–C=N). The band at 910-990 cm^{-1} was assigned to the asymmetric

wagging vibrations of the terminal dihalovinyl group, and the band at 810-910 cm^{-1} was assigned to the deformation vibrations of the cyclopropane ring [32].

In current study, we focused on change in transmission of cypermethrin bonds because according to Beer-Lambert Law "IR transmitted through a solution changes in an inverse logarithmic relationship to the sample concentration" [33].

The results in table5appears clear differences in transmission of cypermethrin bands between zero time and after 14 days of biodegradation. For *P. aeruginosa*,it was clearly note formation new three peaks, which belong to Carbonyl asymmetric, CH2 deformation in R–CH2–CN structure and Aryl–O of diphenyl ether. Peak at 1335 belong to N-O symmetric stretch (nitro compound), Peak 1649 belong to N-H amines group and 1689 belong to C=O (carboxylic acid) cannot be detected after 14 days of biodegradation.For *E. cloacae,* We note formationnew four peak which belong to CH2 deformation in R–CH2–CN structure, Aryl–O of diphenyl ether, (C=O) –O– stretching and Cyclopropane ring, while carbonyl asymmetric band (C=O), C-H rocks AlKanes group and N-O symmetriv stretch (nitro compounds) can't detected by FTIR spectra after 14 days of treatment with *E. cloacae*,(Fig 5& 6).

Changes in peak pattern of cypermethrin under bacterial treatment as compared to zero time were observed in table 5, which indicate to ability of *P.aerogenosa* and *E. cloacae* to increase transmission of cypermethrin bands, which indicate that decrease in concentration of compound which composed from these bands according to Beer-Lambert Law. Difference in the FTIR spectrum of zero time and after 14 days of pesticide aqueous solution indicates degradation of the organic bonds of the pesticide, also Stretching in C=C chloroalkenes, ring vibration of benzene, CH2 deformation in R–CH2–CN structure and (C=O)–O–stretching in cypermethrin were reported by [34] using *Fusariumsp* which approve occurrence of biodegradation.

Table 5: Change in transmission of Cypermethrin 100 mg L^{-1} bands after treatment with *Pseudomonas aerogenosaand E. cloacae* for 14 days

Band	Wave Number	Change in Bands transmission %			
		P. aeruginosa		E. cloacae	
		Zero time	Day 14	Zero time	Day 14
Carbonyl asymmetric stretching	1720-1740 cm^{-1}	Zero	30	Zero	Not detected
C=C stretching of the aromatic rings	1450-1600cm^{-1}	24	60	3	94
CH2 deformation in R–CH2–CN structure	1400-1450 cm^{-1}	Zero	30	Zero	86
Aryl–O of diphenyl ether	1200-1275 cm^{-1}	zero	28	Zero	80
C–O stretching of the cyanate group (–O–C=N)	1125-1205 cm^{-1}	38	66	6	94
(C=O) –O–Stretching	1050-1095 cm^{-1}	4	39	Zero	92
Terminal dihalovinyl group	910-990 cm^{-1}	34	68	6	92
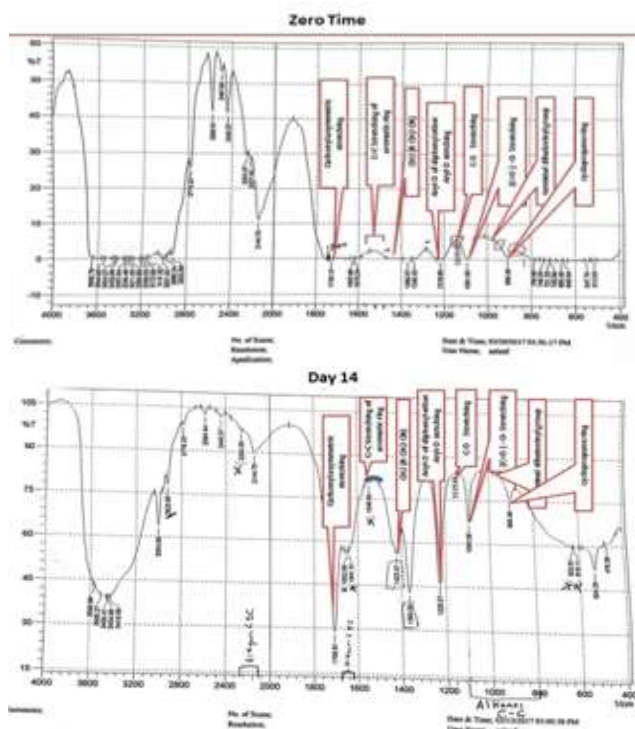
Figure 5: FTIR Results of Cypermethrin 100 mg L^{-1} after treatment with *Pseudomonas aerogenosa* for 14 days
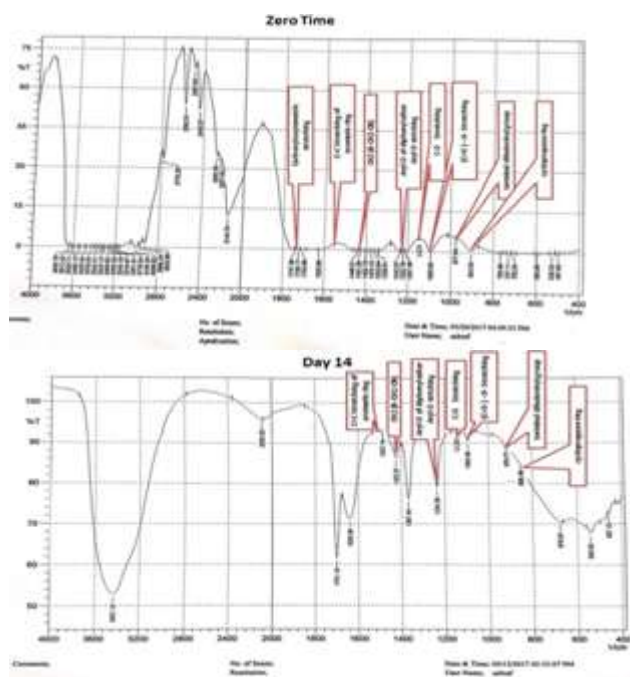
Figure 6: FTIR Results of Cypermethrin 100 mg L^{-1} after treatment with *Enterobacter cloacae* for 14 days

5. GC-MS analysis of biodegraded cypermethrin

The results of analysis of MMSM containing cypermethrin at concentrations 100 mg L^{-1} by GC-MS treated separately with each one of two selected isolates showed the presence of cypermethrin and its Intermediate metabolites. These compounds were identified based on their retention time and molecular weight with those of corresponding authentic compounds in the database, also the comparison with standard library of Wiley Registry of Mass Spectral Data

version-7 confirmed the matching of mass/charge ratio v/s relative intensity.

The results showed variation in bacterial capacity for degradation of cypermethrin and also variation in intermediate metabolites after 14 day of incubation, the percentage of its degradation were 88 % for *P. aerogenosa* and 94 % for *E. cloacae* according to biodegradation equation [26].

% of Biodegradation by *P. aerogenosa* $= \frac{698539 - 83825}{698539} \times 100 = 88\ \%$

% of Biodegradation by *E. cloacae* $= \frac{698539 - 41912}{698539} \times 100 = 94\ \%$

P. aeruginosa in current study provides the evidence of efficient degradation pathway of cypermethrin. The bacteria converted cypermetrin into smaller molecular weight compounds which can further be mineralized under natural environmental conditions. Intermediates compounds formed from cypermethrin biodegradation by *P. aerogenosa* are present in table 6.

Table 6: Retention time, Peak area and M. wt. of intermediates metabolites formed from cypermethrin at 100 mg L^{-1} biodegraded by *P. aerogenosa*

NO	Retention time	Peak Area	M. wt.	Intermediate metabolites
1	4.973	120459	106	1,3-Dimethylbenzene
2	9.07	448468	142	2-Methylnonane
3	10.966	905981	94	Phenol
4	10.968	816099	138	4-Hydroxybenzoate
5	11.809	477692	314	1,2-Benzenediol,
6	12.251	3409699	188	1,8-Cyclotetradecadiyne
7	12.522	426879	138	4-Isopropenylcyclohexanone
8	15.191	1160967	148	4-Propylbenzaldehyde
9	15.43	1138664	150	Phenol, M-tert-butyl-
10	15.963	927213	226	3-Methylpentadecane
11	16.331	8487199	198	n-Tetradecane
12	16.357	1175678	164	2-Tert-pentylphenol
13	16.881	600633	220	Butyric acid
14	17.751	991174	186	1-Dodecanol
15	20.568	8882501	225	a-Hydroxy-3- phenoxy-benzeneacetonitrile
16	20.571	703191	198	3-Phenoxy-benzaldehyde,
17	21.749	1125347	270	Isopropyl myristate
18	22.787	2281725	298	Hexadecanoic acid, methyl ester
19	24.895	524846	282	Oleic Acid
20	25.76	2099301	270	Isoamyllaurate
21	30.543	882673	256	Phenyl ester of o-phenoxy benzoic acid
22	30.801	83825	415	Cypermethrin
23	30.943	3049045	236	3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate

Cypermethrin could be metabolized by *P. aerogenosa* into two major compounds (ά -hydroxy-3-phenoxy- benzene acetonitrile and 3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate). ά-hydroxy-3- phenoxy- benzene acetonitrile is unstable and spontaneously transformed to yield 3-phenoxy benzaldehyde [32,35]. 3-phenoxybenzaldehyde has antimicrobial activity, but does not affect producing culture and enhances biodegradation in

soil or media [36]. 3-phenoxybenzaldehyde is transformed into 4-propylbenzaldehyde which again converts to 4-hydroxybenzoate. Afterward 4-hydroxybenzoate was metabolized by *P. aerogenosa* to form phenyl ester of o-phenoxy benzoic acid. Two intermediate metabolites (3-phenoxybenzoic acid and 3-phenoxybenzaldehyde) are the key metabolites of pyrethroids [37].

The GC-MS chromatogram revealed that primary metabolites of degraded cyermethrin by *E. cloacae* are presented in table 7.

Table 7: Retention time, Peak area and M. wt. of intermediates metabolites formed from cypermethrin at 100 mg L-1 biodegraded by E. cloacae

NO	Retention time	Peak Area	M. wt.	Intermediate metabolites
1	4.973	100459	106	1,3-Dimethylbenzene
2	8.353	24600	184	11-Methyldodecane
3	9.07	398345	142	2-Methylnonane
4	10.966	8755857	94	Phenol
5	10.968	816099	138	4-Hydroxybenzoate
6	15.191	1160967	148	4-Propylbenzaldehyde
7	15.43	988415	150	Phenol, M-tert-butyl-
8	15.963	907213	226	3-Methylpentadecane
9	16.331	8477199	156	Hendecane
10	16.357	1175678	164	2-Tert-pentylphenol
11	17.853	991174	197	5-methoxy-2-nitrobenzoic
12	20.486	8882501	237	3,5-dimethoxybenzamide
13	20.537	703191	181	5-aminoisophthalic acid
14	21.749	1125347	270	Isopropyl myristate
15	22.787	2273702	530	Phthalic acid
16	24.895	386691	376	Sulfurous acid
17	30.801	41912	415	Cypermethrin
18	30.892	3049045	72	cyclopropanemethanol

From the results in table 7, In the molecular structure of cypermethrin there is an ester bond which is not as firm as other chemical bonds. Previous studies indicated that the first step in the microbial degradation and detoxification of cypermethrin is the hydrolysis of its carboxyl ester linkage [36]. It is evident from GC/MS results that E. cloacae degraded cypermethrin by reductive dechlorination, oxidation or/and hydrolysis to transform to other metabolites. On other hand 3-phenoxybenzoic acid (3-PBA) wasn't detected in the metabolites by GC-MS after 14 days of treatment, while 3-PBA was generally regarded as the major metabolite after hydrolysis of SPs in soil and water [11]. Chen reported that fenvalerate was degraded by hydrolysis of the carboxylester linkage to yield 3-PBA, and then the intermediate was further utilized for bacterial growth by strain ZS-S-01, finally resulted in complete mineralization [17]. So, we speculated that carboxylesterases and oxidoreductases involved in degradation of cypermethrin by *E. cloacae*, which needed to be testified by further experiments.

Pollution by pyrethroids has become a very important problem in pesticide-treated areas. Currently, one of the major environmental problems is pesticide contamination caused by activities related to agricultural applications. Different mechanical and chemical methods are rarely used to remove pesticides from contaminated fields due to limited efficiency and the expense. Biodegradation is an encouraging process for the treatment of pesticide-contaminated areas due to the rapid and complete mineralization of pesticides. An isolated bacterial strain might be useful for bioremediation of cypermethrin-polluted soil and water environments.

6. Conclusion

*P. aerogenosa 1 and E. cloacae*have been proved to possess good degradation performance on cypermethrin. The Intermediate metabolites result from two selected isolates were not only proficiently to degrade cypermethrin but also converted the metabolites compound into nontoxic forms.

References

[1] Ortiz-Hernández, M. L., Sánchez-Salinas, E., Olvera-Velona, A., and Folch-Mallol, J. L. (2011). *Pesticides in the environment: impacts and their biodegradation as a strategy for residues treatment. Pesticides-formulations, effects, fate*. ISBN, 978-953.

[2] EPA. (2006). *Cypermethrin: Reregistration Eligibility Decision for Cypermethrin (RED); -HQ-OPP-2005-0293-0036;U.S.* Environmental Protection Agency, Office of Prevention, Pesticides and toxic substance, office of pesticide Programs, U.S. Government Printing Office: Washington, DC.

[3] Weston, D.P., Holmes, R.W., and Lydy, M.J. (2009). Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environ. Pollut*. 157, 287–294.

[4] Vinodhini, R. and Narayanan, M. (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinuscarpio (Commoncarp). *Int. J. Environ. Sci. Tech*., 5 (2), 179-182 (4 pages).

[5] Shukla,Y., Yadav, A., and Arora, A. (2002). Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin. *Cancer Lett*., 182 (1), 33-41 (9 pages).

[6] Javed, M., Majeed, M. Z., Khaliq, A., Arshad, M., and Bakar, M. A. (2015). Review on exposure, absorption and elimination of pyrethroids in humans.

[7] Akbar, S., Sultan, S., and Kertesz, M. (2015). Determination of cypermethrin degradation potential of soil bacteria along with plant growth-promoting characteristics. *Current microbiology*, 70(1), 75-84.

[8] Wood, T. K., (2008). Molecular approaches in bioremediation. *CurrOpinBiotechnol* 19:572–578.

[9] You, M., and Liu, X. (2004). Biodegradation and bioremediation of pesticide pollution. *Chin J Ecol* 23:73–77.

[10] Porto, A. M., Melgar, G. Z., Kasemodel, M. C., and Nitschke, M. (2011). Biodegradation of pesticides, pesticides in modern world-pesticides use and management. [online] http://www.intechopen. com/books/pesticides-in-the-modern-world-pesticidesuse- and-management/biodegradation-of-pesticides.

[11] Suenaga, H., Mitsuoka, M., Ura, Y., Watanable, T., and Furukawa, K. (2001). Directed evolution of biphenyl dioxygenase: emergence of enhanced degradation capacity for benzene, toluene and alkylbenzenes. *J Bacteriol*.183:5441–5444.

[12] Zhang, C., Wang, S. H., Yan, Y. C. (2011). Isomerization and biodegradation of beta-cypermethrin

by Pseudomonas aeruginosa CH7 with biosurfactant production. *BioresourceTechnol* 102:7139–7146.

[13] Lin, Q. S., Chen, S. H., Hu, M. Y., Rizwan-ul-Haq, M., and Yang, L., and Li, H. (2011). Biodegradation of cypermethrin by a newly isolated Actinomycetes HU-S-01 from wastewater sludge. *Int J Environ SciTechnol* 8:45–56.

[14] Chen, S. H., Lai, K. P., Li, Y. N., Hu, M. Y., Zhang, Y.B., and Zeng, Y. (2011). Biodegradation of deltamethrin and its hydrolysis product 3-phenoxybenzaldehyde by a newly isolated Streptomyces aureus strain HP-S-01. *ApplMicrobiolBiotechnol* 90:1471–1483.

[15] Cycon´, M., Zmijowska, A., and Piotrowska-Seget, Z. (2014). Enhancement of deltamethrin degradation by soil bioaugmentation with twodifferent strains of Serratiamarcescens. *Int J Environ SciTechnol* 11:1305–1316.

[16] Negi, G., PankajSrivastava, A., and Sharma, A. (2014). In situ biodegradation of endosulfan, imidacloprid, and carbendazim using indigenous bacterial cultures of agriculture fields of Uttarakhand, India. *Int J Biol Food Vat AgricEng* 8(9):935–943.

[17] Chen, S., Lai, K., Li, Y., Hu, M., Zhang, Y., and Zeng, Y. (2011). Biodegradation of deltamethrin and its hydrolysis product 3-phenoxybenzaldehyde by a newly isolated *Streptomyces aureus* strain HPS- 01. *ApplMicrobiolBiotechnol* 90:1471–1483. doi:10.1007/s00253-011-3136-3.

[18] Murugesan, A. G., Jeyasanthi, T., and Maheswari, S. (2010). Isolation and characterization of cypermethrin utilizing bacteria from Brinjal cultivated soil. *African Journal of Microbiology Research*, 4(1), 010-013.

[19] Thomson, K. S. and Sanders, C. C. (1992). Detection of extended- spectrum ß-lactamases in members of the family Enterobacteriaceae – comparison of the double-disk and 3- dimensional tests. *Antimicrob Agents Chemother* 36, 1877– 1882.

[20] Shah, K., Shrimali, G., and Mulla, S. (2016). Comparison of Double Disc Diffusion Method and Vitek 2 Compact System to Screen the ESBL Producers in Intensive Care Unit in Hospital. *National Journal of Community Medicine*, 7(9), 789-791.

[21] Gibb, A. P. and Crichton, M. (2000). Cefpodoxime screening of Escherichia coli and Klebsiella spp. by Vitek for detection of organisms producing extended-spectrum ß-lactamases. *DiagnMicrobiol Infect Dis* 38, 255–257.

[22] Zhang, H., Zhang, Y., Hou, Z., Wang, X., Wang, J., Lu, Z. and Pan, H. (2016). Biodegradation potential of deltamethrin by the *Bacillus cereus* strain Y1 in both culture and contaminated soil. *International Biodeterioration and Biodegradation*, 106, 53-59.

[23] Karpouzas, D.G. and Walker, A. (2000). Factors influencing the ability of *Pseudomonas putida* strains epI and epII to degrade the organophosphate in soil. *Soil biology and Biochemistry*.32: 1753–1762.

[24] Pankaj, A. S., Gangola, S., Khati, P., Kumar, G., and Srivastava, A. (2016). Novel pathway of cypermethrin biodegradation in a Bacillus sp. strain SG2 isolated from cypermethrin-contaminated agriculture field. *3 Biotech*, 6(1).

[25] Anastassiades. M., Lehotay, S.J., Stajnbaher, D., and Schenck, F. J. (2003). Fast and easy multi residue method employing extraction/partitioning and dispersive solid phase extraction for the determination of pesticide residues in produce. *J AOAC Int* 86:412–431.

[26] Abioye,O. P., Akinsola, R. O., Aransiola, S. A., Damisa, D. and Auta, S. H. (2013). Biodegradation of crude oil by *Saccharomyces cerevisiae* isolated from fermented Zobo(Locally fermented beverage in Nigeria).*Pak .J. bio.scien.*16(24):2058-2061.

[27] Garoiaza, H., Berrabaha, M., Elidrissia, A., Hammoutia, B., and Rı´os, A. (2012). Analysis of cypermethrin residues and its main degradation products in soil and formulation samples by gas chromatography-electron impact-mass spectrometry in the selective ion monitoring mode, *International Journal of Environmental Analytical Chemistry*, 92:12, 1378-1388, DOI: 10.1080/03067319.2011.581365.

[28] Orji, F. A., Ibiene, A. A. and Dike, E. N. (2012). Laboratory scale bioremediation of petroleum hydrocarbon – polluted mangrove swamps in the Niger Delta using cow dung. Malays. *J. Microbiol.*, 8, 219-228.

[29] Jilani, S. (2013). Comparative assessment of growth and biodegradation potential of soil isolate in the presence of pesticides. *Saudi journal of biological sciences*, 20(3), 257-264.

[30] Janssen, P. H., Yates, P. S., Grinton, B. E., Taylor, P. M. and Sait, M. (2002). Improved cultureability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Appl. Environ. Microbiol*. 68:2391–2396.

[31] Naveenkumar, S., Manoharan, N., Ganesan, S., Manivannan, S. and Velsamy, G. (2010). Isolation, screening in vitri mutational assessment of indigenous soil bacteria for enhanced capability in petroleum degradation .*International journal of environmental sciences*, vol. 1(4): 0976 – 4402.

[32] Lin-Vien, D., Colthup, N. B., Fateley, W. G. and Grasselli, J. G. (1991). *The handbook of Infrared and Raman Frequencies of Organic Molecules*; Academic Press: London.

[33] Ricci, R. W., Ditzler, M., and Nestor, L. P. (1994). Discovering the Beer-Lambert Law. J. Chem. Educ, 71(11), 983.

[34] Kaur, P., Sharma, A., and Parihar, L. (2015). In vitro study of mycoremediation of cypermethrin-contaminated soils in different regions of Punjab. *Ann Micobiol*. doi:10.1007/s13213-015-1033-1.

[35] Xiao, Y., Chen, S., Gao, Y., Hu, W., Hu, M., and Zhong, G. (2015). Isolation of a novel beta-cypermethrindegradings train Bacillus subtilis BSF01 and its biodegradation pathway. *Appl. Microbiol. Biotechnol*. 99,2849–2859.doi: 10.1007/s00253-014-6164-y.

[36] Chen, S, Hu, M, Liu, J, Zhong, G, Yang, L, Rizwan-ul-haq, M, and Han, H. (2011). Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel Ochrobactrum lupine DG-S-01. *Journal of Hazardous Materials*. , 187(1), 433-440.

[37] Laffin, B., Chavez, M., Pine, M. (2010). The pyrethroid metabolites 3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol do not exhibit estrogenic activity in theMCF-7 human breast carcinoma cell line or Sprague-Dawley rats. *Toxicology* 267:39–44.