

## **BIODETERIORATION OF CYPERMETHRIN BY LOCAL ISOLATE ISOLATED FROM EGYPTIAN PETROLEUM CONTAMINATED SOIL**

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### **ABSTRACT:**

Cypermethrin is a widespread pyrethroid pesticide and is an environmental pollutant because of its toxicity and persistence. Biodegradation of such chemicals by microorganisms may provide a cost-effective method for their detoxification. Sixty-two cypermethrin degrading bacteria were isolated from Egyptian contaminated soil and water samples. The most potent bacterial strain was identified as *Ochrobactrum intermedium* SP9 based on 16S rRNA gene sequence analysis, as well as several morphological, physiological and biochemical characteristics examinations on classical level. The maximum biodegradation of cypermethrin by bacterial strain SP9 was achieved at 35°C and pH 7.0, and the degradation rate reached 69.1% within 8 days under the optimal conditions. Hence, the *Ochrobactrum intermedium* SP9 could potentially be used in the future for bioremediation of cypermethrin contaminated soil.

**Keywords:** Cypermethrin, *Ochrobactrum intermedium*, Biodegradation, pesticide, contaminated soil.

## 1. Introduction

One of the most strategic problems in our societies is the dangerous accumulation of hazardous wastes compounds in the environment. Many different manufacturers and companies produce hazardous wastes such as pesticides, dyes and heavy metals, etc. Some of these hazardous compounds were accumulated inside the animal and plant tissues, then subsequently transfer to human bodies through ways as eating, drinking and inhalation causing different diseases for human, such as cancer, tumors, respiratory illness, inflammation and other fever, etc (**Masindi and Muedi 2018**).

Pesticides are a large and varied group of substances that are specifically designed to kill biological organisms including weeds, insects, and rodents. However, the extensive use of pesticides may result in their accumulation in the agricultural products (**Tayade et al., 2013**). The problem of the environmental contamination by pesticides goes beyond the locality where it was used. The agricultural pesticides that are exhaustively applied to the land surface travel long distances and can move downward until reaching the water table at detectable concentrations, reaching aquatic environments at significantly longer distances. Therefore, the fate of pesticides has been often uncertain; they can contaminate other areas that are distant from where they were originally used. Thus, decontaminating pesticide-polluted areas was a very complex task (**Gavrilescu, 2005**). It is interesting to note that insecticides frequently applied in agriculture, mostly belong to the chlorinated hydrocarbon, organophosphate, carbamate and synthetic pyrethroid groups, but their comparative residual effects on microorganisms under a particular soil conditions have rarely been reported (**Jayaraj et al., 2016**).

Synthetic pyrethroid insecticides were introduced into widespread use for the control of insect pests and disease vectors more than three decades ago. In addition to their value in controlling agricultural pests, pyrethroids are at the forefront of efforts to combat malaria and other mosquito-borne diseases (**Nieradko-Iwanicka and Borzêcki, 2008**), and are also common ingredients of household insecticide and companion animal ecto-parasite control products reported (**Elsheikha et al., 2011**).

Cypermethrin [(±) a-cyano-3-phenoxybenzyl (±)-cis-tran3 (2, 2dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate] is a photostable synthetic pyrethroid insecticide. It was widely used for control of pests in cotton and vegetable crops, for the treatment of cattle and other livestock and for indoor and outdoor pest control (**Lin et al., 2011**). It acts on central nervous system of insects by producing hyper excitable state by interacting with sodium channels (**Nieradko Iwanicka and Borzecki, 2008**). However, it was also highly toxic to fish and aquatic invertebrates which classified as a possible human carcinogen. It adversely affects the CNS and causes allergic skin reactions and eye irritation in humans (**Tallur et al., 2008**). Widespread occurrence of cypermethrin has been reported in downstream water bodies and sediments due to runoff and erosion from agricultural fields and nonagricultural applications. Cypermethrin persistence in environment varies from 14.6 to 76.2 days (half-life) depending on physicochemical properties of soil (**Weston et al., 2009**).

Microbial activity in soil also plays vital role in determining the fate and behavior of cypermethrin in soil. In the natural environment, hydrolysis of the ester linkage is the principal degradation route and leads to the formation of 3-phenoxybenzoic acid (PBA) and cyclopropane carboxylic acid (DCVA) derivatives (Chen *et al.*, 2012). Soil bacteria possess diverse catabolic pathways to degrade pesticides, and this potential can be exploited to design bioremediation strategy to reduce pollution load in contaminated environments. Several bacteria such as *Ochrobactrum lupin*, *Pseudomonas spp.*, *Streptomyces spp.*, *Bacillus spp.* and *Serratia spp.* have been isolated from contaminated soil and activated sludge system, and also capable of degrading cypermethrin (Tiwary and Dubey 2016). Degradation pathway of cypermethrin involves initial ester hydrolysis to yield 3-phenoxybenzoic acid (3-PBA) which can be converted either to 4-hydroxy-3-phenoxybenzoic acid (Tiwary and Dubey, 2016), or protocatechuate and phenol (Tallur *et al.*, 2008). Microbes isolated from contaminated agricultural soils may possess auxiliary traits such as N-fixation, mineral solubilization, or plant growth regulator production along with biodegradation capacity (Akbar *et al.*, 2015).

This work aims for (i) isolation and purification of bacterial strains from different soil and water samples and selection of the most potent bacterial strains for biodegradation of Cypermethrin insecticides. (ii) Identification of the most potent bacterial strain by conventional methods, vitek, and molecular methods. (iii) Study the effect of various environmental parameters on biodegradation of cypermethrin insecticides. Finally, evaluation the availability of bacterial strain for bioremediation of cypermethrin contaminated soil.

## 2. Materials and methods

### 2.1. Chemicals and media composition.

Cypermethrin (95% purity) obtained from (NCIC) El-Nasr-Intermediate Chemicals Company (The Egyptian Armed Forces). This obtained from Ali Akbar group, Pakistan. A stock solution of cypermethrin (95% purity) was prepared by dissolving (1,052 ml) Cypermethrin in (100ml) of methanol solution to obtain (10,000 ppm) and this stock solution was added to desirable concentration in medium as the sole carbon source.

Mineral Salts Medium (MSM) (g/L): 1.0  $\text{NH}_4\text{NO}_3$ , 1.0  $\text{NaCl}$ , 1.5  $\text{K}_2\text{HPO}_4$ , 0.5  $\text{KH}_2\text{PO}_4$ , 0.2  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1L dis. Water, pH 7.0. For solid plate, 1.5% (w/v) agar was added. The media were sterilized by autoclaving at 121°C for 20 min before use.

### 2.2. Isolation, purification and identification of Cypermethrin by utilizing microorganisms.

Three samples were collected from contaminated soil of Misr gas stations at Aga and met ghamer cities, Al-Dakahlia governorate, Egypt, for isolation of the Cypermethrin-degrading bacteria. The samples were collected at summer season of 2017 where the temperature range was 38-40°C, and the samples were taken from a

distance of 20 cm after taking out nearly 5 cm of the soil superficial. The samples were retained in sterile polyethylene bags, closed strongly and stored at 4°C for further study

The procedure used for the isolation of Cypermethrin utilizing microorganisms was carried out by preparation of the previously mineral salt medium, and the selected insecticide Cypermethrin were added separately as a sole source of carbon and energy. This medium was first cooked and sterilized at 121°C and 1.5 atmospheric pressure for 20 min. Then this medium was then allowed to cool to about 40°C and then supplemented with selected Cypermethrin at different concentrations as a sole source for carbon and energy. One ml from previous enrichment medium was transferred and dispensed in sterilized Petri dishes left for solidification on each Petri-dish and then spread over the surface of the media by using a sterile glass spreader. After inoculation, the Petri-dish was incubated at 35°C for seven days (**Chen *et al.*, 1959**).

The procedure for the purification of the bacterial isolates under study was carried out by the streak plate method All bacterial colonies of different forms and colors were picked up and reinoculation again onto plates containing the same isolation medium which showing separate growth on solid media. At the end of incubation period, only the growth, which appeared as a separate colony on solid media was picked up and reinoculation again for several consecutive times to ensure its purity. Then the separated colonies were preserved in slants for further work. Purity was checked up microscopically and morphologically (**Johnson *et al.*, 1959**).

### **2.3. Screening for the most potent bacterial isolates concerning the selected Cypermethrin biodegradation activity.**

Screening for potential isolates was carried out by analyzing the growth range of isolate on gradient plate and the concentration of Cypermethrin degradation potential. Gradient plates were prepared by adding A base layer of 15 ml mineral salt medium and setting it in wedge Shape at 30°C. Another 15 ml of agar containing 50 mg/L Cypermethrin was poured on set base to make Cypermethrin gradient across the plate surface (**Murugesan *et al.*, 2010**).

Isolates were streaked along the pesticide gradient and bacterial growth range along the gradient was recorded after incubation. Degradation potential of isolates was determined by the growing of these bacterial strain in mineral salt medium containing different concentrations of Cypermethrin from (100mg/L to 400mg/L) for 8days. After incubation of residual Cypermethrin concentration, the selection of the most potent bacterial isolate depending on concerning the biodegradation of Cypermethrin insecticide can be resistant to the high concentration of the selected insecticides.

### **2.4. Characterization and identification of cypermethrin degrading bacteria.**

A cypermethrin degrading isolate designated as SP9 was showed the highest degradation rate and selected for further study. The purified SP9 was identified on the basis of its morphological characteristics, biochemical tests and 16S rRNA gene sequence analysis. The isolate SP9 was confirmed by 16S rRNA gene sequence. The DNA was extracted and purified using the Qiagen genomic DNA buffer set. PCR

amplification was performed as described by Mirnejad et al (Mirnejad *et al.*, 2012). The 16S rRNA sequencing was performed by Beijing Liuhe Huada Genomic Company (Beijing, China). The sequences with the highest 16S rDNA partial sequence similarity were selected and compared by CLUSTAL W. Phylogenetic and molecular evolutionary analyses were conducted by MEGA 4.0 software with the Kimura 2-parameter model and the neighbor joining algorithm (Haws *et al.*, 2011). Confidence estimates of branching order were determined by bootstrap resampling analysis with 1000 replicates.

## 2.5. Inocula preparation.

Unless otherwise stated, the inoculants for this experiment were bacteria cultured in a 130 mL serum bottle containing 120 mL of PSB medium in a light incubator at 35°C and 7500 lux. At the exponential phase (about 2–3 days), the cell pellets were harvested via centrifugation (5000×g, 10 min), washed 3 times with 50 mL of KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (0.15mol/L, pH 7.0), and then suspended in the same phosphate buffer as the inoculants. In order to avoid the effects of hydrolysis and photolysis, each treatment was set in triplicates with non-inoculated samples as control under the same conditions and analyzed in the same manner (Holmstrup *et al.*, 2010). Samples for residual pesticide concentration analysis were collected from the cultures at regular intervals.

## 2.6. Optimal conditions for degrading cypermethrin by *Ochrobactrum intermedium* SP9.

To determine the optimal conditions for degrading cypermethrin *Ochrobactrum intermedium* SP9 the single-factor test was designed in this study under different conditions. To confirm the effect of temperatures on the degradation, the media were placed in illuminating incubators at (10, 20, 25, 30, 35 and 40°C), respectively. To determine the effect of cypermethrin concentrations on the degradation, MSM media were supplemented with cypermethrin ranging in concentration from (10 mg/L to 350 mg/L). The media were prepared at different pH values from (3.0 to 11.0) buffers for the measurement of the effect of pH values on the degradation (Chowdhury *et al.*, 2008).

The media were prepared using different types of nitrogen sources to confirm the effect of nitrogen sources on cypermethrin insecticide degradation such as (NaNO<sub>3</sub>, NH<sub>4</sub>Cl<sub>2</sub>, Urea, Peptone, Aspartic acid, and Glutamic acid).

Finally, the media were prepared using different types of carbon sources to confirm the effect of different types and concentrations of carbon sources on cypermethrin insecticide degradation such as (Glucose, Maltose, Glycerol, Starch, and Lactose). All experiments were conducted in triplicates. The non-inoculated controls throughout the study were implemented at the same condition in order to exclude the abiotic degradation effect.

### 3. Results and discussion.

#### 3.1. Isolation and characterization of cypermethrin degrading bacterium.

After repeated enrichment and purification processes, we obtained many strains of organisms with different colony from the activated sludge and polluted samples. But the degradation experiments showed that, the isolate (SP9) was possessed the relatively higher degradation, capacity of degrading cypermethrin (350 mg/L) after incubation for 8 days at pH 7.0 and temperature 35°C. The isolate (SP9) was utilized cypermethrin as its sole carbon and energy source in MSM (Zhao *et al.*, 2013). Thus strain (SP9) was selected and then preserved for further detail investigations. The isolate (SP9) was identified morphologically as a gram-negative, rod shape and the shape of the (SP9) colony grown for 10 days on MSM agar plate, was reddish-brown, smooth, circular, wet, nontransparent, glistening, and with entire margins. SEM observations showed that the cells are ovoid to rod shaped, sometimes even longer, measuring about 1 mm in size and are motile by means of lophotrichus flagella. The physiological and biochemical characteristics of (SP9) were shown in Table (1) which. The bacterial isolate was appeared as rod shape, negative for Gram reaction, non-spore forming, the oxidase test positive, motile by lophotrichus flagella and can be grown under aerobic condition but can't be grown under strict anaerobic condition. Additionally, 3% KOH positive and cytochrome oxidase positive. The production of tryptophanase was positive, while Arginine Dihydrolase, Urease, Gelatinase and  $\beta$ -Galactosidase were negative. The bacterial cell cannot accumulate the poly  $\beta$  hydroxybutyric acid (PHB) and can't produce any pigments. The bacterial isolate could degrade hydrogen peroxide ( $H_2O_2$ ) by producing Catalase enzyme. The bacterial isolate under study can't grow in presence of NaCl 8.5% and can be grown at 25oC to 40oC and have the ability of nitrate reduction. This result was agreed with (Schleifer, and Bell, 2009). Bergey's manual of systematic bacteriology. And also agreed with (Saxena *et al.*, 2015). Laboratory Manual of Microbiology, Biochemistry and Molecular Biology.

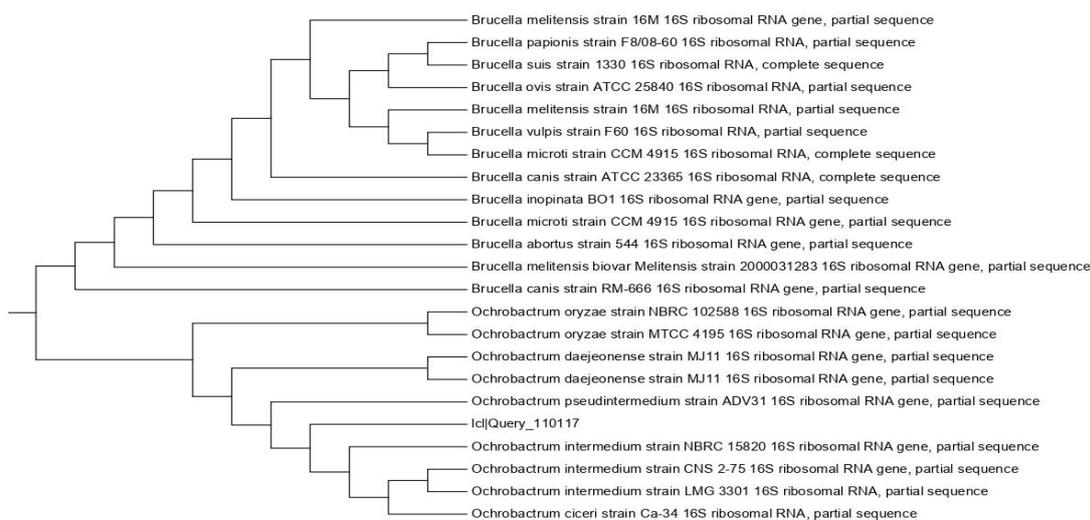
**Table (1).** Physiological and biochemical characteristics for bacterial isolate.

Items	Results	Items	Results
Gram stain	+	Assimilation of adabic acid	-
Cell shape	Rod	Assimilation of phenylacetic acid	-
Motility	+	Assimilation of lactose	-
Endospore form	-	Assimilation of glucose	-
Colony shape	Circular	Assimilation of saccharose	-
Colony size	1mm	Assimilation of inositol	-
Aerobic growth	+	Assimilation of Manito	-
Strict anaerobic	-	Assimilation of sorbitol	-
pigment	-	Assimilation of melibiose	-
PHB accumulation	-	Assimilation of raffinose	-
Growth in Nacl 8.5%	-	Assimilation of rhamnose	-
Growth at temp. 45 °C	-	Assimilation of arabinose	-
KOH 3% test	+	Hydrolysis of 4-nitrophenyl-D-glucopyranoside	+
Catalase	+	Hydrolysis of bis-4-nitro phenyl phosphate	+
Oxidase	+	Hydrolysis of Acetamide	-
Urea's	-	Hydrolysis of Esculin	-
Lipase	+	Hydrolysis of L-proline	-
H <sub>2</sub> S production	+	Hydrolysis of 4-nitro anilide	-
Indol production	-	Hydrolysis of O-nitro phenyl beta-D galacto pyranosid	-
Cytochrome oxidase	+	Hydrolysis of p-nitro phenyl acetic acid	-
Arginine dihydrolase	-	Hydrolysis of 4-nitro phenyl beta D,galacto pyranoside hydrolysate	-
Gelatinase	-	Hydrolysis of 4-nitro phenyl beta D,xylo pyranoside	-
Lysine decarboxylase	-	Hydrolysis of colistn	-
Ornithine decarboxylase	-	Hydrolysis of esculin	-
Antibiotic resistance	-	Hydrolysis of acetamide	-
Assimilation of citric acid	+	Hydrolysis of nitro analide	-
Assimilation of glycerol	+		
Assimilation of maltose	+		
Assimilation of coumaric acid	-		

\* - = -ve test., + = +ve test

### 3.2. Confirmed the conventional identification by molecular identification based on 16S rRNA gene sequencing.

Molecular identification of bacterial isolates showed that the 16S rRNA gene sequences of the isolate (SP9) was identified as *Ochrobactrum intermedium*. The partial 16S rRNA gene sequences of the isolates were generated and detected as sharp bands at (1500bp) by agarose gel electrophoresis as compared with DNA ladder (marker) (Kumari *et al.*, 2015) The tree based on bacterial 16S rRNA gene sequences (Fig.1) Indicate that these isolates were grouped into Firmicutes (one isolate) and phyla; Gamma prototeobacteria (Shamsa and Akbar *et al.*, 2015).



**Figure(1)** Phylogenetic analysis of 16S rRNA sequences of the bacterial isolates with the sequences from NCBI.

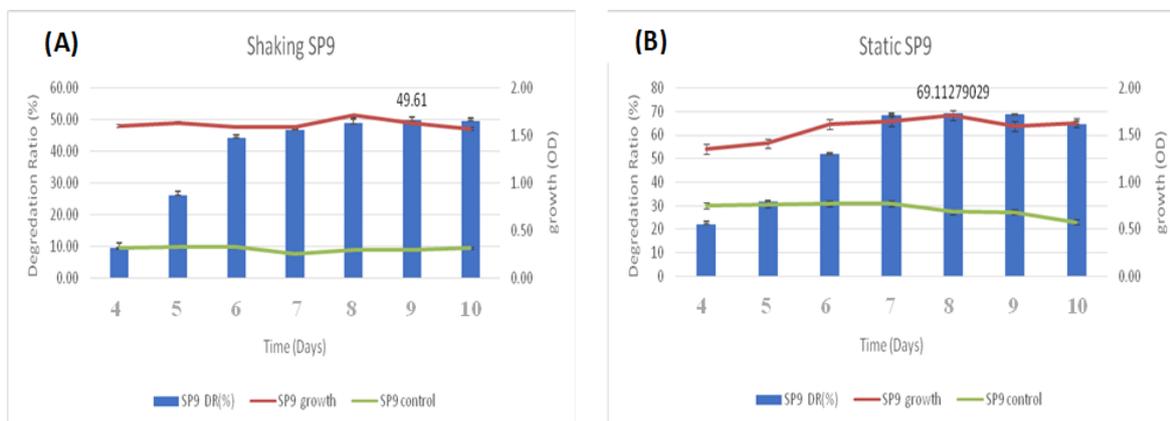
### 3.3. Study of physicochemical parameters

#### 3.3.1. Effect of incubation periods & incubation conditions on *Ochrobactrum intermedium*.

The *Ochrobactrum intermedium* strain was grown in mineral salt media supplemented with (3500ppm) of cypermethrin insecticides as a sole of carbon and energy source. *Ochrobactrum intermedium* strain was grown for 10 days on static and shaking conditions for determination of the best incubation period and incubation condition for biodegradation of cypermethrin (Xiao *et al.*, 2015).

Estimation of cypermethrin residue in culture and compare this result with control to show the best incubation period for biodegradation of cypermethrin by the most potent bacterial isolate *Ochrobactrum intermedium* (SP9). The highest biodegradation rate under static condition by using *Ochrobactrum intermedium* (SP9), after 8 days of incubation was (69.1%), while the highest biodegradation rate under shaking condition was (49.6%) after 9 days of incubation of *Ochrobactrum intermedium* (SP9). these results were shown in **Figure (2)**. This result was agreed with

(Castro and Tufenkji 2008 1959). who founded that, the optimal incubation period for cypermethrin biodegradation by soil microbe within 8 days under static condition.



**Figure (2).** Effect of incubation periods and incubation conditions (shaking (A) and static (B)) on biodegradation of cypermethrin using *Ochrobactrum intermedium* (SP9).

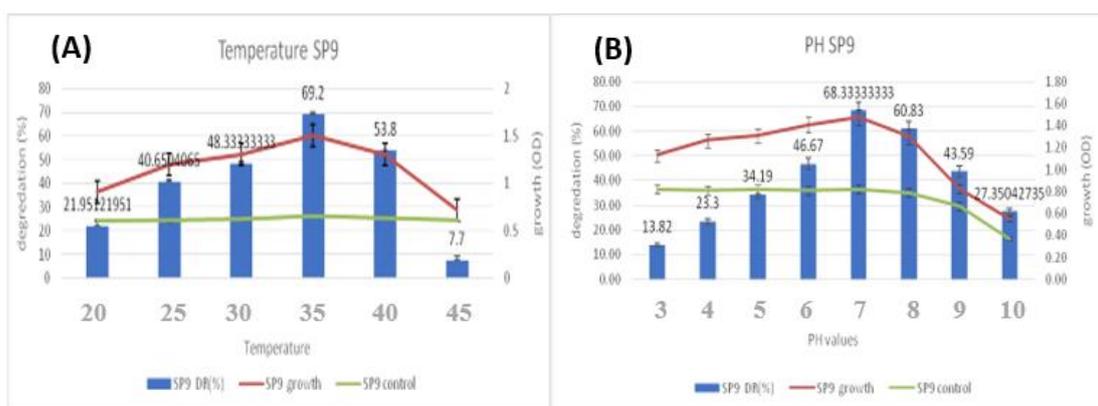
### 3.3.2. Effect of temperature on cypermethrin degradation by *Ochrobactrum intermedium*

Cypermethrin was degraded by *Ochrobactrum intermedium* SP9 during incubation temperatures range from (20°C to 45°C). The cypermethrin residues were detected after 8 days of incubation. The results showed that the degradation rate was 21.95% and 40.65% at 20°C and 25°C respectively. While at higher temperature, i.e. 30°C, 35°C and 40°C, the degradation rate was reached to 48.33%, 69.2% and 53.8% within 8 days, respectively, but the degradation rate was only 7.7% when incubated at temperature 45°C for 8 days (Figure 3.A). The best temperature for degradation was 35°C. Similar results were reported by **Lin et al., (2011)** who reported that, the temperature significantly influenced cypermethrin degradation by *Streptomyces sp.* strain HU-S-01. Our data also reveal that cypermethrin degradation occurred at 30–35°C indicating strain Sp9 preferred relatively high temperature condition. These results were consistent with previous findings of (**Chen et al., 2011a**). It is possible that some key enzyme(s) responsible for cypermethrin degradation have their optimum enzymatic activity over such range of temperature. In non- inoculated controls at different temperatures, abiotic degradation was negligible throughout these studies. (**Holmstrup et al., 2010**).

### 3.3.3. Effect of pH values on cypermethrin degradation in MSM medium by *Ochrobactrum intermedium*

The pH plays an important factor, which significantly effects the degrading ability of bacteria capable of degrading toxicities (**Howe et al., 2009**) and (**Rowland et al., 2011**). To determine the effect of pH value on degradation, MSM medium was

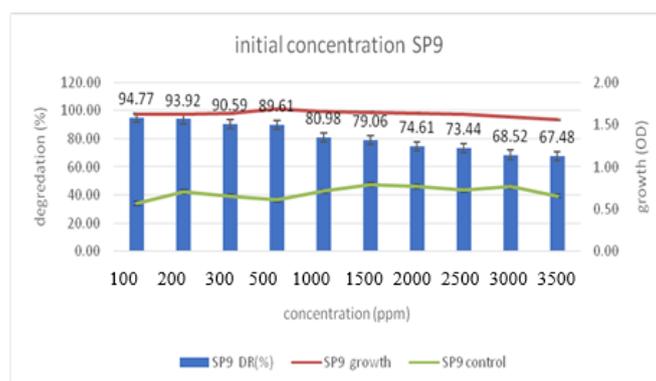
prepared with different pH buffers, fortified with 350 mg/L cypermethrin, and incubated at 35°C. Eight different pH values *viz.*, (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0) were tested in the optimization experiment. The result showed that the degradation rates were 13.82%, 23.3%, 34.19%, 46.67%, 68.3%, 60.83%, 43.59%, 27.35%, respectively (Figure 3.B). The optimal initial pH value for degradation was between 6.0 and 9.0. Results revealed that SP9 was able to degrade cypermethrin over a wide range of pH. Similar results were reported by Cycoń *et al.*, (2013) who reported that initial pH was significantly efficiently degraded by two *Serratia spp.*, that have rapid degradation rate of cypermethrin at high pH while it was relatively low at acidic pH. In non-inoculated controls at different pH conditions, abiotic degradation was negligible throughout the studies (Shen *et al.*, 2013).



**Figure (3).** Effect of incubation temperature (A) and pH values (B) on biodegradation of cypermethrin using *Ochrobactrum intermedium* (SP9) under static condition.

### 3.3.4. Effect of initial concentrations on cypermethrin degradation in MSM by *Ochrobactrum intermedium*

Cypermethrin degradation at different initial concentrations by strain *Ochrobactrum intermedium* (SP9) was investigated. The cypermethrin degradation rates were found to be (94.7%, 93.9%, 90.59%, 89.6%, 80.98%, 79.06%, 73.44%, 68.52%, and 67.48%) at concentrations range from (100, 200, 300, 500, 1000, 1500, 2000, 2500, 3000, and 3500 ppm), respectively (Figure 4). At low cypermethrin concentrations ranging from 100 to 1000 ppm, the degradation rate was reached above 80% within 8 days of incubation. However, at high concentration (1500 to 3500 ppm), the degradation rate was reached above 67% within 8 days. It might be because of the fact that microbial degradation starts slowly and requires an acclimation period before rapid degradation occurs at high concentration. Similar results were reported by Lin *et al.*, (2011) who reported that, the initial concentration of carfofuran was significantly efficiently degraded by *Pichia anomala* strain HQ-C-01 in contaminated soils. In non-inoculated controls at different initial concentrations, abiotic degradation was negligible throughout the studies.



**Figure (4).** The effect of initial concentrations on biodegradation of cypermethrin by isolate *Ochrobactrum intermedium* (SP9).

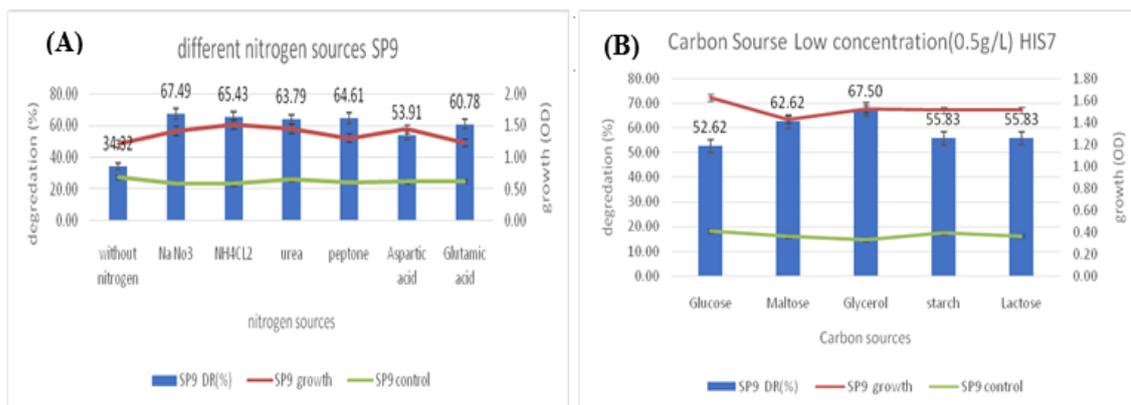
### 3.3.5. Effect of different nitrogen sources on cypermethrin degradation in MSM medium by *Ochrobactrum intermedium*

The different nitrogen sources play an important factor, which significantly effects the degrading ability of bacteria capable of degrading toxicities. (Wen *et al.*, 2008). To determine the effect of different nitrogen sources on the degradation, MSM medium was prepared with different nitrogen sources, fortified with 350 mg/L cypermethrin, and incubated at 35°C-and pH 7.0. Different nitrogen sources such (Na NO<sub>3</sub>, NH<sub>4</sub>Cl<sub>2</sub>, urea, peptone, Aspartic acid, Glutamic acid) were tested in the optimization experiment. The results showed that the degradation rates were (34.32%, 67.49%, 65.43%, 63.79%, 64.61%, 53.91%, 60.78%,) respectively (Figure 5). The optimal nitrogen sources for the degradation of cypermethrin was Na NO<sub>3</sub> and NH<sub>4</sub>Cl<sub>2</sub>. Results revealed that SP9 was able to degrade cypermethrin over a wide range of different nitrogen sources. In non-inoculated controls at different nitrogen sources conditions, abiotic degradation was negligible throughout the studies (Castillo *et al.*, 2006; Wen *et al.*, 2008).

### 3.3.6. Effect of different concentrations of carbon source on cypermethrin degradation in MSM medium by *Ochrobactrum intermedium*.

The different concentrations of carbon sources also plays an important factor, which significantly effects the degrading ability of bacteria capable of degrading toxicities .To determine the effect of different concentration of carbon source on the degradation, MSM medium was prepared with high concentration of different carbon sources, fortified with 350 mg/L cypermethrin, and incubated at 35°C-and pH 7.0.Different carbon source such (Glucose 0.5g/L, Maltose0.5g/L, Glycerol 0.5g/L, starch0.5g/L, Lactose0.5g/L) were tested in the optimization experiment. The results showed that, the degradation rates were 52.62%, 62.62%, 67.50%, 55.83%, 55.83%, respectively (Figure 5). The low concentrations of different carbon sources were increased the degradation of cypermethrin. Results revealed that SP9 used low concentration of carbon source as source of energy to encourage degradation of cypermethrin and SP9 was able to degrade cypermethrin at high range of different low concentrations of carbon sources. In non-inoculated controls at different carbon sources

conditions, abiotic degradation was negligible throughout the studies (Gurjar and Hamde, 2018).



**Figure (5).** The effect of different nitrogen and carbon sources on biodegradation of cypermethrin by isolate *Ochrobactrum intermedium* (SP9).

## Conclusion

*Ochrobactrum intermedium* strain SP9 was isolated from petroleum contaminated soil. This isolate can utilize cypermethrin as sole source of carbon, nitrogen and energy. The optimal incubation condition was carried out under static condition for 8 days. The optimal temperature and pH value for biodegradation of cypermethrin by strain SP9 were 35°C and pH 7.0. The degradation rate was reached to 69.1% within 8 days under static condition. Our results indicated that, the strain *Ochrobactrum intermedium* SP9 could be an effective choice for the bioremediation of cypermethrin contaminated water and soil. However, further studies as interactions with environment, toxicological aspects, degradation enzymes, biochemical and genetic aspects are still needed before the application in actual field-scale bioremediation.

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### تكسير الثيبيرمثرين باستخدام عزلة محلية *Ochrabacrum intermedium* SP9 معزولة من تربة ملوثة بالبترول

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#### ملخص البحث:

يعتبر الثيبيرمثرين من المبيدات الحشرية العضوية الملوثة للبيئة نظراً لسميته وطول بقائه في التربة. استخدام الكائنات الدقيقة في التخلص من هذه الملوثات الكيميائية وتكسيرها يعتبر طريقة فعالة وغير مكلفة بالنسبة لإزالة سميتها. تم عزل 26 عزلة بكتيرية لها القدرة على تكسير الثيبيرمثرين من تربة ومياه ملوثة. كانت أكثر العزلات البكتيرية الفعالة هي *Ochrabacrum intermedium* SP9. وصلت نسبة تكسير الثيبيرمثرين باستخدام هذه العزلة إلى 69.1% عند درجة حرارة 35°م وتركيز الاس الهيدروجيني 7.0 في خلال ثمانية أيام تحت كل الظروف المثلى للعزلة ومن ثم يمكن استخدام هذه العزلة بفاعلية في المعالجة الحيوية للتربة الملوثة بالثيبيرمثرين.

**الكلمات المفتاحية:** الثيبيرمثرين، التكسير الحيوي، المبيدات الحشرية، التربة الملوثة