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Using Halophiles (*Bacillus pumilus*) to treat spinetoram (pesticide) Contamination

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Abstract

Pesticides can be define as any substance or mixture of substances, which used to control damaging pests such as insects, When pesticides applied, the impacts begin to appear on human, plants, or animals, these toxic compounds (pesticides) have been engaged in various disorders and diseases including cancer In this study, new strain of bacillus species were identification in Iraqi environments and test their ability to degrade the pesticides that was used usually in agricultural fields. The samples taken from agricultures areas in Al-Zubaydiya, Wasit Governorate and cultured on agar, three temperatures 25, 30, and 35 °C were chosen. Also, three pH values of 5, 7, and 9 for this purpose, three concentrations of spinetoram pesticide 600, 1200, and 1800 ppm were used. The results at a temperature of 35 ° and pH 9 and 7 were the optimum conditions with removal ratios of 99 %, 97 % or 94 % respectively at 600 ppm of spinetoram.

Keywords: Halophilic bacteria, spinetoram pesticide, biodegradation.

1. Introduction

Environmental pollution caused by anthropogenic activity has reached all types of ecosystems, soils, marine and fresh water and air, which affected by the dispersion and spread of contaminants [1]. Pesticides are one of the chemical compound groups [2], the majority of the 3 million annual pesticide poisoning victims are in developing nations, up to 20,000 people die as a result [3]. The metabolic way of pesticides is dependent on environmental factors (oxygen, moisture, temperature, pH, and other). Microbial groups or plant species, pesticide features (solubility, hydrophilicity, chemical structure) chemical and biological reactions. The primary method of detoxification is by far enzymatic transformation, which is mostly the product of biotic processes mediated by plants and microbes [4].

Microbiological, chemical, and photo-degradation processes impact pesticide persistence. A single pesticide may be break-down by all three mechanisms. Pesticide persistence may be impacted by microbial activity, temperature, soil and water pH, distribution between foliage and soil, and other soil properties [5]. Spinetoram (XDE-175) is a multicomponent tetracyclic macrolide developed for the control of Lepidoptera larvae, Leaf miners, and Thrips on a variety of crops. It consists of two closely related active ingredients, XDE-175-J and XDE-175-L, present in an approximate 3:1 ratio. Spinetoram is a fermentation product derived from the actinomycete bacterium *Saccharopolyspora spinosa* and is an analogue of the insecticide *Spinosad*. *Spinetoram* and *spinosad* are considered toxicologically equivalent. It acts by causing persistent activation of insect nicotinic acetylcholine receptors [6].

Halophilic microorganisms that thrive in hypersaline environments. Halophilic and halotolerant bacteria can thrive in up to 30 % NaCl, while non-halophilic microorganisms show optimal growth below 2 %. According to the salt concentrations that they require to flourish, halophilic bacteria can be divided into three categories: low halophiles, moderate halophiles and extreme halophiles. *Bacillus* is aerobic, gram positive, rod shaped and endospore forming bacteria, the strain that isolated from drains (*Bacillus pumilus*) has ability to adapted in hard conditions such as high salinity and that improved in this study [7]. The most widespread species found in environments, which is aerobic, gram positive, rod shaped and endospore forming bacteria belonging to the genus *Bacillus*. *Bacillus spp*. readily adapt to a variety of environments due to their capacity to produce spores and survive in a range of different environmental conditions [8].

2. Material and methods

2.1. Sample collecting area

Three samples were taken from three drainages, which have been given symbols A, B, and C. These samples belong to agricultural areas located in the district of Al-Zubaydia in Wasit Governorate, approximately 30 km from the Zubaidiah thermal power station. In December 2021, each sample cultured on halophilic media and nutrient agar at three replicates and incubated at 35-37 °C for five days (for halophilic media) and 72 hours for nutrient agar.

2.2. Halophiles media

The halophilic media used for the isolation and the cultivation of halophilic bacteria used in this study is Halophilic Agar M590 [9]. The halophilic media used as broth in experiment work was prepared from the same contents of the halophilic media without agar.

2.3. Laboratory experiment

Sets of Erlenmeyer flasks 250 ml containing 200 ml of the liquid prepared media, the pH was adjusted to 7 and autoclaved at 121 °C for 15 minutes. Then sets of spinetoram with three concentrations at 600, 1200, and 1800 ppm were added to flasks. The addition of *Bacillus pumilus* that isolated and incubated after activated in nutrient broth for five days and flasks incubated at shaker incubator for 21 days and the growth was measured by recorded values of optical density by spectrophotometer each two days [10].

2.4. The HPLC

The HPLC system was used to detect spinetoram concentrations in samples.

3. Result and discussion

3.1. Identification of bacteria

Bacillus bacteria were isolated from the drains were diagnosed by PCR technique (16s RNA), as the results showed that the diagnosed bacteria are a new strain of *Bacillus pumilus*, and it was registered in the National Central for Biological Information (NCBI). Moreover, (table 1) lists the specific primer of 16s rRNA of the gene.

Table 1: The specific primer of 16s rRNA of the gene.

Primer	Sequence	Temp. °C	GC (%)	Product size
Forward	5'-AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair
Reverse	5'-GGTTACCTTGTTACGACTT- 3’	49.4	42.1	

3.2. The environmental factors of drainages

Environmental factors were measured when taking samples from the drainages that are labeled as A, B, and C are water and air temperature, pH and salinity as shown in (table 2).

Table 2: Measured environmental factors in sample collecting area.

No. of Drainage	Temperature of (C°)		pH	EC (µS/cm)	Salinity (ppt)
	Air	Water			
A	23	15	7.76	10000	6
B		13	7.92	12000	7.5
C		12	7.78	15000	9

Temperature measured by thermometer, PH values measured by PH meter and salinity of drainage was accounted depending on electrical conductivity values and later depending on the equation, the results were recorded as part per thousand (ppt):

Salinity (ppt) = (EC (µS/cm)-14.78)/(1589.08) [11]

The concentrations that used in the study were 600, 1200, and 1800 ppm were measured as the equation below.

$$C1 \times V1 = C2 \times V2$$

However, the spinetoram residues concentration in samples were measured in according to [12] by the following equation:

Spinetoram concentration (ppm)= (Conc. of standard × peak area of sample)/(peak area of standard)

Where Conc. of Standard = (120 µg/L) 120000 ppm, and peck area of stander = 17132.9

Furthermore, the spinetoram content was detected by using HPLC and the percentage of spinetoram biodegradation was measured according to the following equations [13]

Percentage ratio of biodegradation = (Conc. of standard- Conc .of sample)/(Conc. of standard) × 100

or

Percentage ratio of biodegradation = (Peak area of standard-Peak area of sample)/(Peak area of standard) × 100

3.3. Degradation kinetics of spinetoram

The HPLC results showed a decrease in the concentration of spinetoram, as shown in (table 3). An analysis of a standard solution of pesticide was done, where the concentration of the standard solution was 100 ppm, and the peak area was 951.9 per 2.69 minute. The standard figure of spinetoram was explained in (figure 1) before degradation. The best results of biological removal by bacteria were at 35 °C and pH 9 at concentrations of 600, 1200, and 1800 ppm. Moreover, the removal percentages were 99.7 %, 71.4 %, and 16.4

% respectively. The least degradation results were in temperature 25 °C and the acidic pH of the concentrations 600, 1200, 1800 ppm, where they were as follows: 78.2 %, 22.8 %, and 1.2 % respectively. It was concluded that the high temperature and pH lead to an increase in the efficiency of bacteria to degrade the pesticide and that agreed with study and conclude effect of PH on degradation and explain the degradation in alkaline media better than acidic media [14-15].

Table 3: Peak areas, removal percent and remain concentration of samples.

Temperature (°C)	Concentration of pesticide (ppm)	pH	Peak area (mAU.s)	% Removal	Remain conc. (ppm)
35	600	9	43.36	99	4.5
		7	161.72	97	16.9
		5	320.41	94	33.6
	1200	9	4883.35	57	513
		7	5632.38	50	591.7
		5	7098.58	37	745.8
	1800	9	14307.13	16	1503.1
		7	14708.69	14	1545.3
		5	14983.18	12	1574.1
30	600	9	408.26	92	42.8
		7	724.55	87	76.1
		5	1515.92	73	159.2
	1200	9	8672.54	24	911.1
		7	9121.57	20	958.3
		5	10048.71	12	1055.7
	1800	9	15189.54	11	1595.8
		7	15429.47	10	1620.9
		5	15543.11	9	1816.5
25	600	9	2013.43	64	211.5
		7	2730.55	52	286.8
		5	3725.25	34	391.3
	1200	9	10190.47	10	1070.6
		7	10940.17	4	1149.4
		5	11224.71	1.7	1179.3
	1800	9	16510.76	3.6	1734.6
		7	16609.69	3	1745
		5	16917.85	1	1777.3

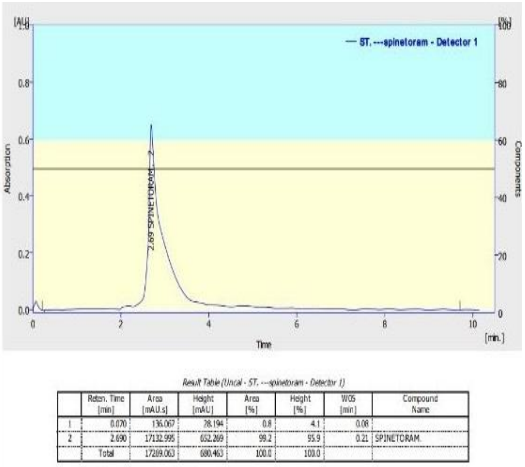


Figure 1: Peak area of spinetoram as standard.

The highest observed with 600 ppm in 35 °C with pH 7, and 9. While the lowest degradation were at 25 °C and PH 5, as shown in figures 2 and 3 for concentration 600 ppm. Figure 4 and 5 for concentration 1200 ppm, and figures 6 A and 7 for a concentration of 1800 ppm. The results of the peak area, residual concentration and removal percentage were also shown in (table 2).

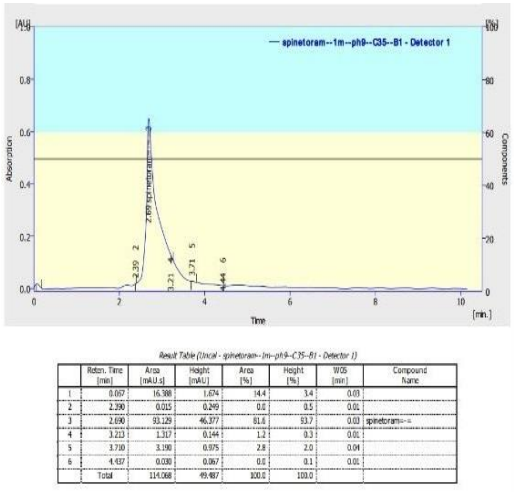
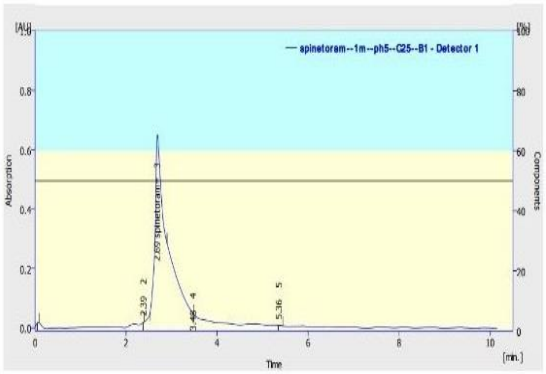


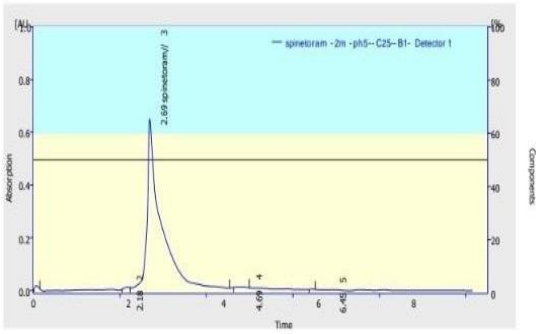
Figure 2: Peak area of 600 ppm, 35 °C, and pH 9.



Result Table (Uncal - spinetoram--1m--ph5--C25--B1 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.057	1.768	1.075	0.0	0.2	0.03	
2	2.390	0.015	0.249	0.0	0.0	0.01	
3	2.690	4192.064	496.134	99.9	99.6	0.14	spinetoram-
4	3.480	0.244	0.033	0.0	0.0	0.01	
5	5.363	2.580	0.689	0.1	0.1	0.04	
	Total	4196.671	498.178	100.0	100.0		

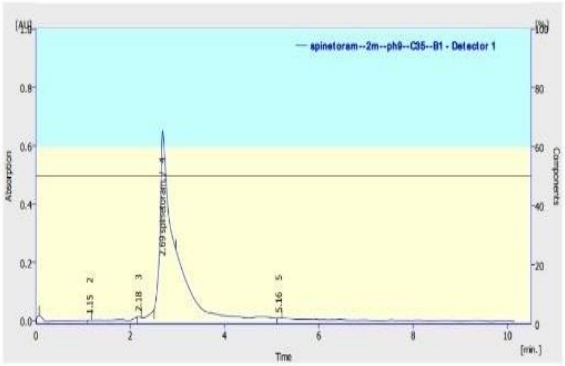
Figure 3: Peak area of 600 ppm, 25 °C, and pH 5.



Result Table (Uncal - spinetoram--2m--ph5--C25--B1 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	CompoundName
1	0.067	65.133	12.067	0.5	1.8	0.10	
2	2.183	63.891	8.192	0.5	1.2	0.14	
3	2.690	11295.591	644.336	98.8	96.4	0.20	spinetoram//
4	4.693	36.257	2.382	0.3	0.4	0.26	
5	6.453	6.196	1.348	0.0	0.2	0.07	
	Total	11367.067	668.327	100.0	100.0		

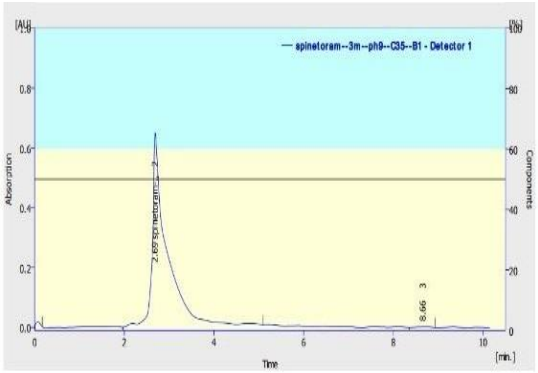
Figure 5: Peak area of 1200 ppm, 25 °C, and pH 5.



Result Table (Uncal - spinetoram--2m--ph9--C35--B1 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.063	7.734	1.341	0.1	0.3	0.02	
2	1.150	1.444	0.608	0.0	0.1	0.03	
3	2.183	3.886	1.113	0.1	0.2	0.06	
4	2.690	5163.369	529.094	99.7	99.3	0.16	spinetoram_/
5	5.160	2.736	0.584	0.1	0.1	0.09	
	Total	5179.169	532.739	100.0	100.0		

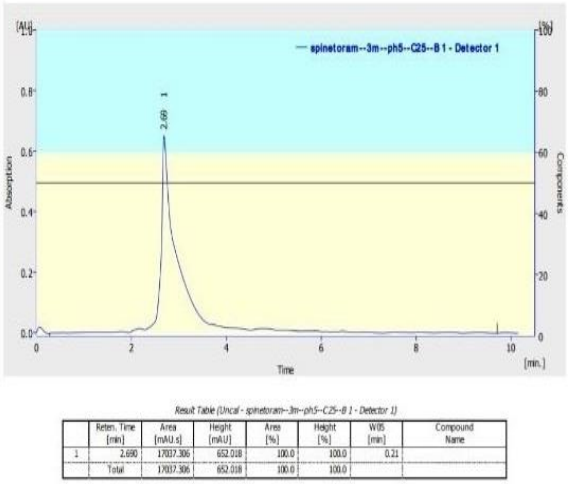
Figure 4: Peak area of 1200 ppm, 35 °C, and pH 9.



Result Table (Uncal - spinetoram--3m--ph9--C35--B1 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.057	80.807	13.366	0.6	2.0	0.10	
2	2.690	14351.202	647.813	99.1	97.6	0.21	spinetoram-_
3	8.663	54.374	2.569	0.4	0.4	0.28	
	Total	14486.383	663.748	100.0	100.0		

Figure 6: Peak area of 1800 ppm, 35 °C, and pH 9.



Result Table (Uncal - spinetoram--3m--ph5--C25--B 1 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.690	17037.306	652.018	100.0	100.0	0.21	
	Total	17037.306	652.018	100.0	100.0		

Figure 7: Peak area of 1800 ppm, 25 °C, and pH 5.

3.4. Optical Density results

Statically analysis of optical density results mentioned in tables 5 , 6, and 7, for 600 ppm, 1200 ppm and 1800 ppm respectively.

Table 5: Optical density results of bacteria (biomass) at 600 ppm.

pH	35 °C	30 °C	25 °C
pH 5	0.6006 ± 0.07	0.8458 ± 0.11	0.499 ± 0.09
pH 7	0.7355 ± 0.09	0.889 ± 0.09	0.9413 ± 0.10
pH 9	1.1705 ± 0.15	0.6096 ± 0.09	0.5561 ± 0.08
LSD Values	0.322 *	0.241 *	0.287 *

Table 6: Optical density results of bacteria (biomass) at 1200 ppm.

pH	35 °C	30 °C	25 °C
pH 5	0.6923 ± 0.08	0.8812 ± 0.12	0.8307 ± 0.10
pH 7	0.9555 ± 0.11	0.7572 ± 0.09	0.9814 ± 0.08
pH 9	1.3039 ± 0.14	0.8933 ± 0.10	0.9158 ± 0.12
LSD Values	0.322 *	0.147 NS	0.154 NS

Table 7: Optical density results of bacteria (biomass) at 1800 ppm.

pH	35 °C	30 °C	25 °C
pH 5	0.9567 ± 0.11	1.0052 ± 0.09	0.9667 ± 0.08
pH 7	1.1385 ± 0.14	1.2112 ± 0.12	1.0134 ± 0.12
pH 9	1.2931 ± 0.12	1.1529 ± 0.10	1.2514 ± 0.11
LSD Values	0.301 *	0.207 NS	0.308 NS

The mean results of optical density recorded in Figures 8, 9, and 10 with the optical density of 600, 1200 and 1800 ppm of pesticide spinetoram respectively.

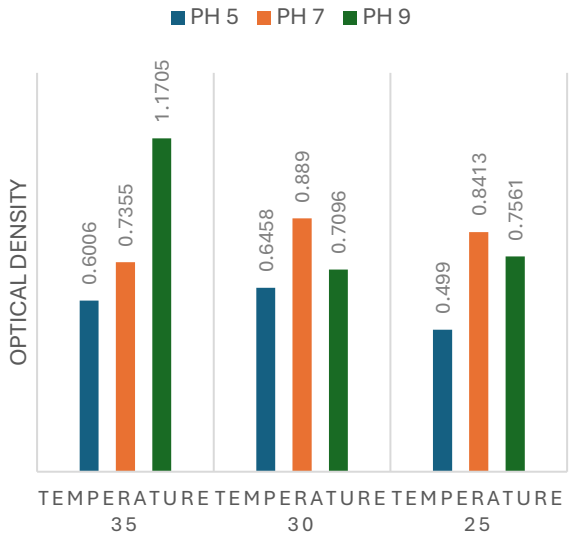


Figure 8: Optical density values (biomass) for bacteria where at 600 ppm.

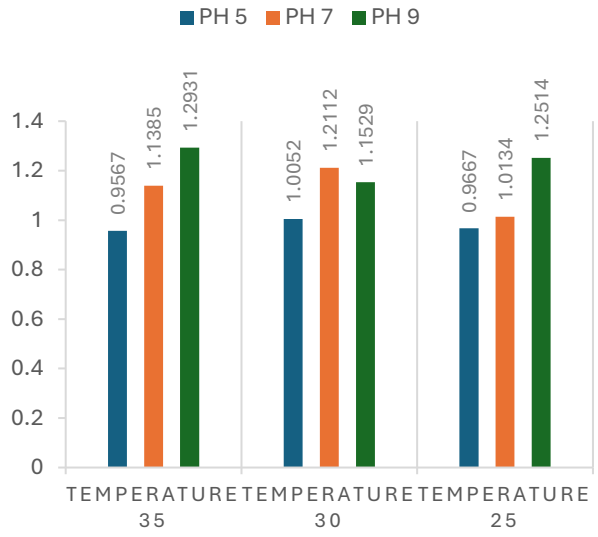


Figure 9: Optical density values (biomass) for bacteria where at 1800 ppm.

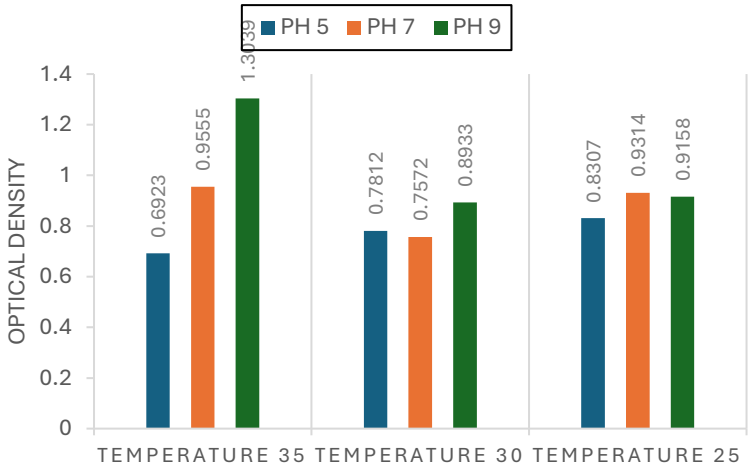


Figure 9: Optical density values (biomass) for bacteria where at 1200 ppm.

Figures 7, 8, and 9, illustrate that the best bacterial growth (the highest value of O.D.) was at 35 °C and pH 9. However, the lowest result was at 25 °C and pH 5. This explains the results of the biodegradation of the pesticide by bacillus pumilus used under study and agrees with (table 2) and the Figures 7 to 9 that show the best results of biodegradation by the bacteria for the three concentrations used in the study. It is remarkable to get new bacterial strains for effective degradation of insecticides. Several bacterial strains capable of degrading similar compounds have been isolated from pesticide contaminated environments [16, 17]. The results agreed with Khan, and Mohapatra [16, 18] which use *bacillus spp.* to degrade insecticides and show 75 – 80 % percentage

of degradation to insecticide by HPLC analysis. The slight difference in the percentage of bio-digestion is due to the efficiency of the bacteria, the type of strain isolated, and its adaptation to the environment.

4. Conclusion

In conclusion *Bacillus pumilus* can biodegrade the pesticide into its components, the higher temperature and alkaline environments, the greater efficiency of halophilic bacteria in biodegradation for the pesticide. The bacteria were recorded under the website (https://www.ncbi.nlm.nih.gov/nuccore/ON004119).

5. References

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