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Using Halophiles (*Bacillus licheniformis*) to Treat Spinetoram (pesticide) Contamination

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Abstract

The present study aimed to identify *Bacillus licheniformis* in Iraqi environments and test their ability to degrade the pesticides usually used in agricultural fields. Three temperatures (25, 30, 35 ºC) and pH values (5,7,9) were chose for this study, while three concentrations of above mentioned pesticide (600, 1200, 1800) ppm were used. 94% and 91% were achieved at pH values 9 and 7 at 35ºC and 600 ppm.

Keywords: Halophilic bacteria, Spinetoram pesticide, Biodegradation.

استخدام البكتريا المحبة للملوحة *Bacillus Licheniformis* لمعالجة التلوث بمبيد الـ(spinetoram)

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الخلاصة:

هدفت الدراسة الى تشخيص النوع البكتيري *Bacillus licheniformis* واختبار قابليته على التفكيك الحيوي للمبيدات الحشرية التي تستخدم في المناطق الزراعية. اختيرت ثلاث درجات حرارة (25، 30، 35) °م وثلاثة قيم من الpH (5 ، 7 ، 9) لهذا الغرض ,واستخدم ثلاثة تراكيز من المبيد (600 ، 1200 ، 1800) جزء في المليون. كانت النتائج عند درجة حرارة 35 °م ودالة حامضية 9 و 7 هي الأفضل وكانت نسبة الإزالة 94٪ و 91٪ على الترتيب عند التركيز 600 جزء في المليون من مبيد السبينترام.

Introduction

Pesticides can be define as any substance or mixture of substances, which used to control damaging pests such as insects, organisms cause disease to plants and weeds, include many living organisms for example, nematodes, arthropods other than insects, and vertebrates that endanger our food supply, comfort, or health. The term pesticide shows chemical substances that change biological processes of living organisms deemed pests, whether these are insects, fungi, or mold, weeds or noxious plants. Pesticides are widely used in most areas of crop production to reduce infestations by pests and thus protect crops from reduction of product quality and potential yield losses. Spinetoram (insecticide) produced a number of detrimental effects on survival in sub-chronic toxicity experiments carried out on mice, rats, and dogs, but

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declines and/or increase in body weight, and/or food intake have been noticed. It causes anemia in many examined organs of animals (mice, rats and dogs) with the development of histiocytic clusters in various tissues and organs (thymus, lymph nodes, spleen, and bone morrow) [1]. In a study on the extent of pesticides remaining in the soil, results showed that pesticides polluted the water and that agricultural chemicals such as fertilizers and pesticides are carried by the drain water which empties into streams and rivers finally falling into the oceans. Fish and other marine life are affected due to the higher concentration of these contaminants [2]. In a previous study, bacteria was used to breakdown insecticide resulting in the breakdown of the pesticide into its secondary components within a period of 14 days [3].

The use of water for irrigation leads to the push of pesticides deep into the ground (ground water) and may even reach the rivers. 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 broken-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].

Halophilic are 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%. *Bacillus* is an aerobic, rod shaped, positive to gram stain and endospore forming bacteria, a strain that is isolated from drains (*Bacillus licheniformis*) and has the ability to adapt in hard conditions such as high salinity [6].

The most widespread species found in environment, which is aerobic, rod shaped, positive to gram stain and endospore forming follow the genus *Bacillus*. Due to their capacity to produce spores and survive in range of different environmental conditions, species of this genus readily adapt to a variety of environments [7].

Material and Methods

Sample Collection area:

Samples were taken from three drainages, which have been given symbols A, B, and C belonging 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.

Halophiles Media:

The media used for the isolation and the cultivation of halophilic bacteria used in this study is halophilic agar M590 by Al-Muttairi and Al-Mayaly, [8].

Laboratory Experiment:

Sets of Erlenmeyer flasks 250ml containing 200 ml of the medium were prepared. The pH was adjusted to 7 and autoclaved at 121˚C for 15 min. Later sets of spinetoram with three concentrations (600, 1200, 1800 ppm) were added to flasks. *Bacillus licheniformis* that was isolated and incubated after activation in nutrient broth for five days were incubated in a shaker incubator for 21 days. The bacterial growth was measured by recording the values of optical density by a spectrophotometer every two days [9].

High Performance Liquid Chromatography (HPLC):

HPLC system was used to detect spinetoram concentrations in samples.

Results and Discussion

Identification of Bacteria

Bacillus licheniformis were isolated, purified and selected based on their ability to grow in a culture medium which was diagnosed according to morphological characters. The bacterial isolate (*B. licheniformis*) was identified based on the nucleotide sequence of the 16S rRNA gene (PCR technology) and documented in gene bank (NCBI) under website (https://www.ncbi.nlm.nih.gov/nuccore/ON024904).

Measuring Environmental Factors of Drainages:

The environmental factors measured when taking samples from the drainages (named A, B, C) were water and air temperature, pH and salinity (Table 1).

Table 1: Measured environmental factors in sample collecting area

No. of drainage	Temperature 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 was measured by thermometer and pH values were measured by pH meter. Salinity of drainage was accounted depending on electrical conductivity values and later depending on the Equation 1. The results were recorded as part per thousand (ppt):

$$\boldsymbol{Salinity\ (ppt)} = \frac{\boldsymbol{EC\ (\mu S/cm) - 14.78}}{\boldsymbol{1589.08}} \quad \text{….. Eq. (1) [10]}$$

Concentrations used in current study were 600, 1200, and 1800 ppm which were measured according to the Equation 2.

$$\boldsymbol{C_1 \times V_1 = C_2 \times V_2} \text{ ….. Eq. (2)}$$

The spinetoram content was detected by using HPLC and the percentage of spinetoram biodegradation which was measured according to the Equations 3 and 4) [11]:

$$\boldsymbol{Percentage\ of\ Biodegradation} = \frac{\boldsymbol{Conc.of\ Standard - Conc.of\ Sample}}{\boldsymbol{Conc.of\ Standard}} \boldsymbol{X\ 100} \text{ ….. Eq. (3)}$$

Or

$$\boldsymbol{Percentage\ of\ Biodegradation} = \frac{\boldsymbol{Beak\ Area\ of\ Standard - Beak\ Area\ of\ Sample}}{\boldsymbol{Beak\ Area\ of\ Standard}} \boldsymbol{X\ 100}$$

.Eq. (4)

Impact of Temperature and PH:

Temperature, for example, affects pesticide bioavailability and biodegradation. The changes in the bacterial growth of the tested bacterial strain in HM media containing spinetoram were affected by various incubation temperatures and pH. The growth increase of the tested bacteria was detected by measuring the optical densities during the experiment which were kept for 21 days of incubation at pH 5, 7 and 9, and then the bacterial strain that could grow at many degrees of temperature (25, 30 and 35◦C) was examined. An increase in biomass of bacterial at 600 nm was recorded compared to control [12].

Incubation Period of Bacteria:

Effects of 21 days incubation time, pH = 9, 7 and 5, and incubation at 35, 30, 25℃ at three concentrations (600, 1200, 1800 ppm) on the biomass of the chosen bacterial strain at various intervals were studied. Measuring bacterial growth as a function of time using spectrophotometry at 600 nm showed a clear increase in bacterial growth as the incubation period went on (biomass). Better results were shown at pH 9 and 7, 35℃, and at 21-day incubation period.

Optical Density Results:

The mean results of optical density recorded are shown in the figures below. Figure 1(a) represents optical density mean values for 600 ppm of spinetoram, Figure 1(b) represents optical density mean values for 1200 ppm of spinetoram, while Figure 1(c) represents optical density mean values for 1800 ppm of spinetoram.

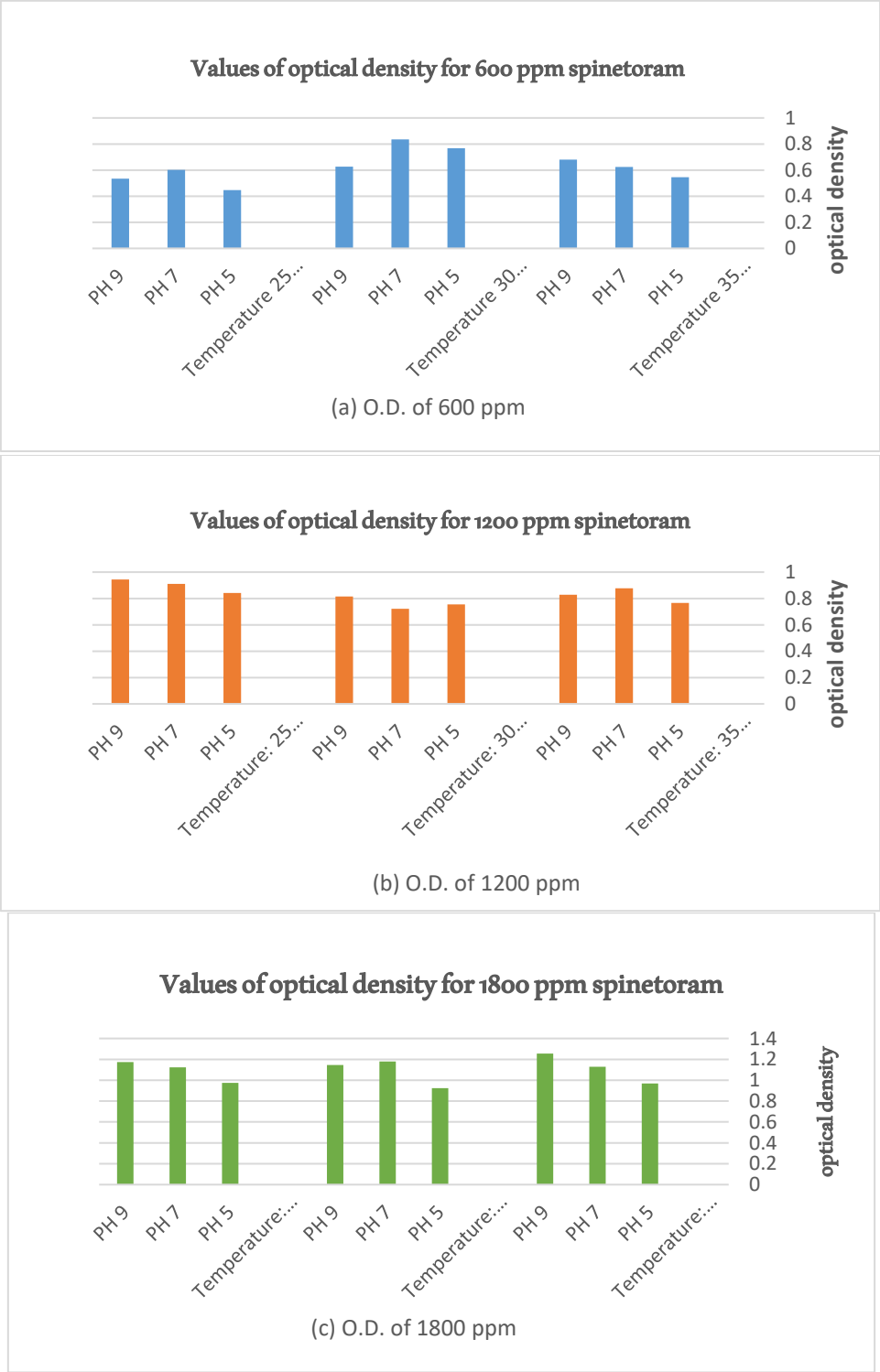

Figure (1 a, b, c,): Values of optical density**.**

In the first figure (600 ppm concentration), the highest temperature reading was 30°C and pH = 7, and the temperature was 35°C and pH = 9. Significant differences in *p*-value = ≤0.05 were noticed in the results at 35° C temperature. As for the second figure (1200 ppm concentration), the highest value was recorded at 25° C and pH 9, and at 35° C and pH 7. According to the statistical analysis, no significant differences were noticed in this concentration. In the third figure at 1800 ppm, the highest values were recorded at 35°C and pH 9 and at 30°C and pH 7. Significant differences were recorded at 35°C.

HPLC Analysis of Biodegraded Spinetoram:

For the degradation efficacy test, the bacteria was inoculated into halophilic media with spinetoram at three concentrations (600, 1200, 1800 ppm), the concentration of pesticide was (120000 ppm) and the same concentration that was being used by farmers. The pesticide was added to 200 ml of halophilic media at three different temperatures and pH values. These samples were analyzed with HPLC system [13].

An analysis of a standard solution of pesticide at the 100 ppm concentration of the standard solution. The peak area was 951.9 per 2.69 minute. The comparison of spinetoram was done according to the concentrations used in study which is explained in Figures (2A, B and C). The peak area of used concentrations before degradation appeared as following: 5710.9 at 600 ppm, 11421.9 at 1200 ppm and 17132.9 at 1800 ppm.

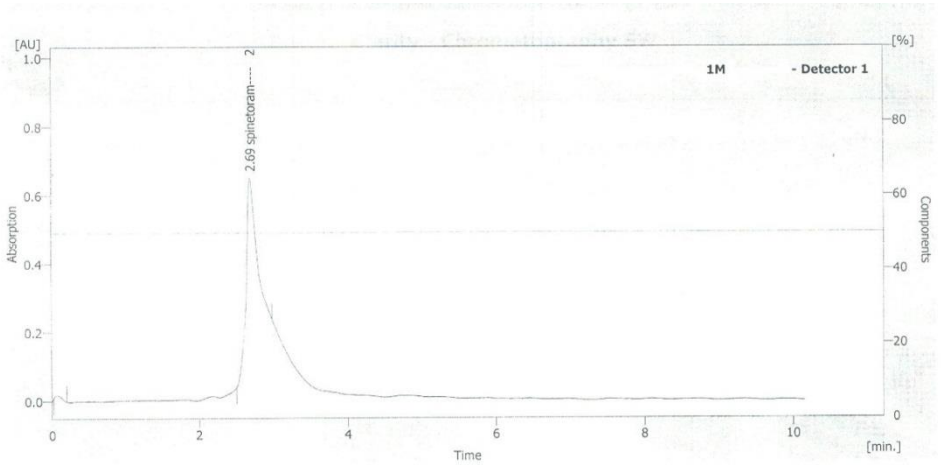

Figure 2: A- Peak area for 600 ppm of spinetoram

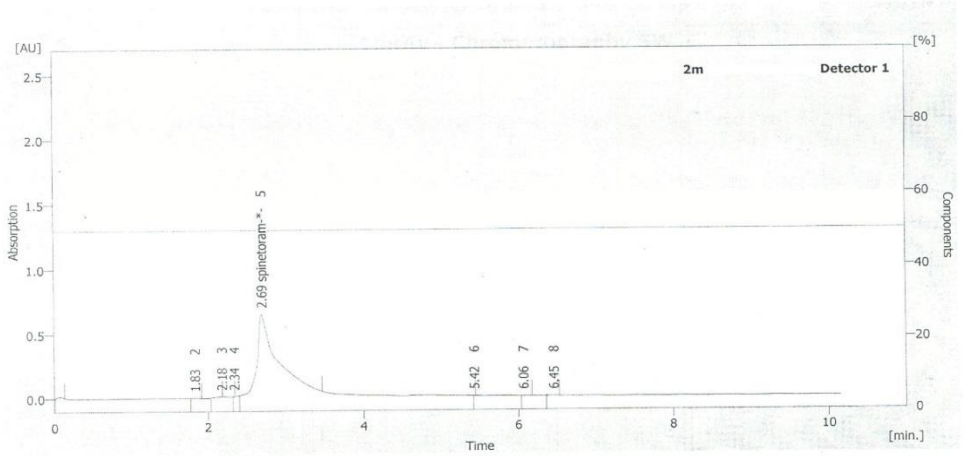

Figure 2: B- Peak area for 1200 ppm of spinetoram

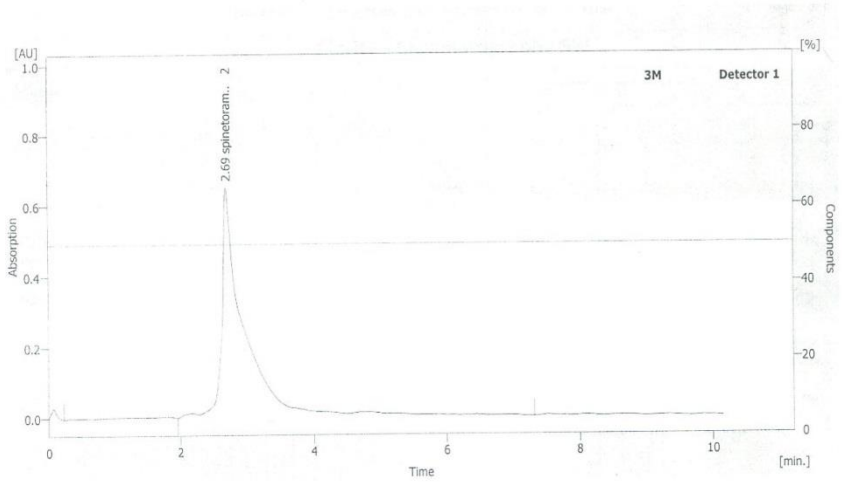

Figure 2: C- Peak area for 1800 ppm of spinetoram

The results of the HPLC analysis mentioned in Table 2 explain the concentrations of spinetoram, peak area of samples, percentage of removal and remaining concentration for each sample.

Table 2: Peak area and removal percent of samples

Temperature ◦C	Concentration of pesticide (ppm)	pH	Peak area (mAU.s)	% Removal	Remain conc. (ppm)
35	600	9	93.129	98	9.7
		7	208.76	96	21.9
		5	336.43	93	35.3
	1200	9	5163.36	54	516.3
		7	6041.80	47	634.7
		5	7444.31	34	782.1
	1800	9	14351.20	16	1507.7
		7	14746.05	13	1549.2
		5	14996.26	12	1575.5
30	600	9	487.73	91	51.2
		7	838.07	85	88
		5	1640.57	71	172.3
	1200	9	9182.52	19	964.7
		7	10276.10	10	1079.6
		5	10685.22	10	1122.6
	1800	9	15310.58	10	1608.5
		7	15488.65	9	1627.2
		5	15638.62	8	1643
25	600	9	2381.86	58	250.2
		7	3164.78	44	332.5
		5	4192.06	26	440.4
	1200	9	10880.80	5	1143.1
		7	11193.06	2	1175.9
		5	11295.59	1	1186.7
	1800	9	16538.35	3	1737.5
		7	16779.42	2	1762.8
		5	17037.30	1	1789.9

The table above shows the dissociation of spinetoram pesticide in different concentrations at different temperatures and pH. The best obtained result was the dissolution of the pesticide into its secondary components at 35◦ C and pH 9 with 600 ppm concentration (Figure 3A).

And the lowest results obtained were at 25°C and pH 5 with 1800 ppm concentration (Figure 3B), which means that the higher the concentration of spinetoram and low pH value leads to less efficient bacteria in degrading the pesticide.

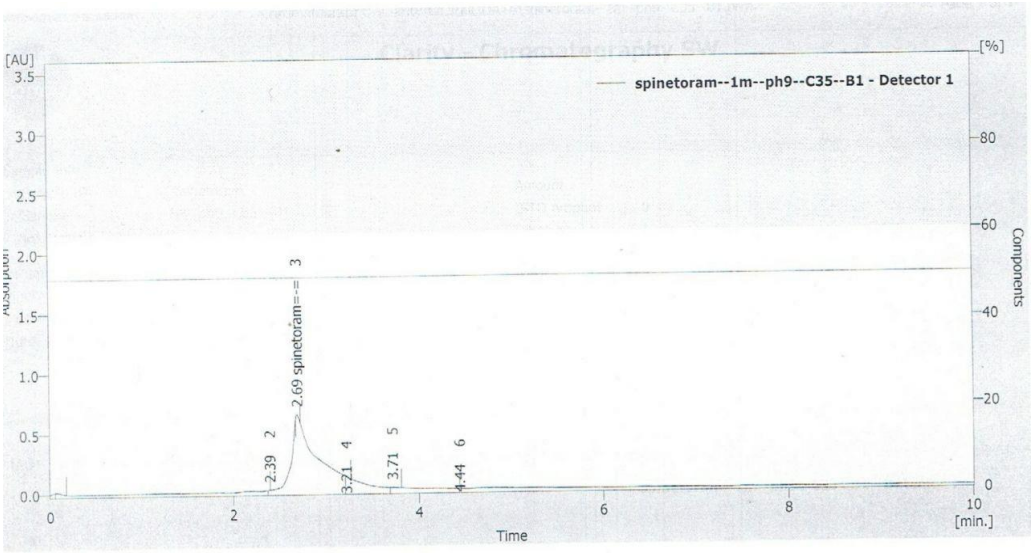

Figure 3: A- The highest removal of spinetoram

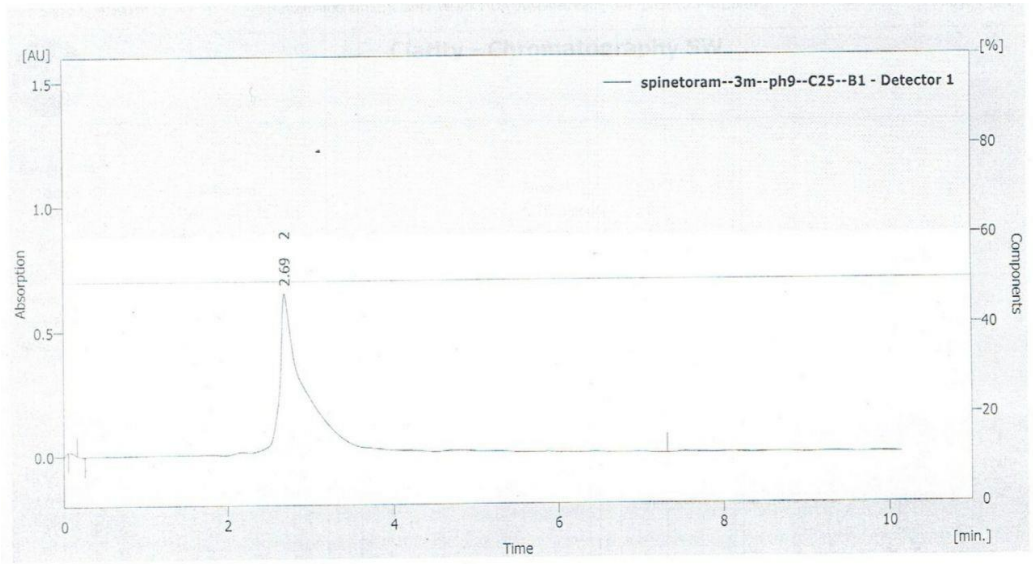

Figure 3: B- The lowest removal of spinetoram

Degradation rate increased with 600 ppm of spinetoram and reduced at 1800 ppm. Anwar *et al.* [14] showed that more efficiency in degradation was achieved at alkaline degree of pH. However, at acidic pH the degradation rate fell down to about 50%. While at neutral pH and at different concentrations and amounts of pesticide, the degradation rate was nearly 80%. Brajesh *et al.* [15] showed that the alkaline environments give more degradation than neutral and acidic because the stability of bonds increases in acidic environments. 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 of the current study agree with Khan *et al*. [16] and Mohapatra and Awasthi [18] who used *B. Licheniformis* to degrade insecticides, and showed 75 – 80 % percentage of

degradation of 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. In a comparative study conducted on an insecticide using bacillus bacteria 70% of the pesticide was degraded [19].

It can be concluded that *Bacillus licheniformis* has the ability to biodegrade the pesticide into its secondary metabolites. Higher temperature and acidity give best results and efficiency of halophilic bacteria in biodegradation of pesticide.

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