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Bioremediation of Pesticides by Bacteria in Jazan Area

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ABSTRACT

Diazinone & malathion bioremediation, in vitro study, was done in a liquid medium by soil isolated bacterial strains. Malathion and diazinone residue concentrations were measured at interval times until 14 days after incubation. Results indicated that three isolates namely, F1, F3 and D3 showed high ability of degrading both diazinone & malathion with degradation ratio 35.8 , 56.1 and 29.8 % respectively, for malathion and 33.6, 49.7 and 24.5 % respectively, for diazinone, after 14 days of incubation. The best temperature for biodegradation was 30^{o}C for all isolates. While the best pH values for degradation were 7.0 for both Bacillus & Staphylococcus isolates and 7.5 for Pseudomonus . Also this study improved that the degradation percentages was better in case of the absence of other carbon source in the medium.

INTRODUCTION

Jazan is considered as the main agricultural area in KSA, (food basket), it has a large planted area (2,088,608 acres), with many economical crops such as vegetables (cucumber, okra, tomato, pepper, aborigine and watermelon), corn, fruit trees (mango, figs, lemon and guava) and palms. And as a followed habit for wide area farming, to achieve higher productivity and higher expected return, chemical insecticides are used for eliminating insect pests. And because of dramatic increase for using insecticides, several problems have emerged ranging in severity, such environmental contamination and animal & human poisoning. Some authors such Al-Hatim *et al.,* 2015 analyzed 44 well and 3 dams' samples for the presence of 15 pesticides used in Jazan area. They found that the most detected compounds were diazinon, fenitrothion and cyfluthrin with avarage conc. of 0.098, 0.104 and 0.394 µg / L, respectively. Also, Abd El-Ghany *et al.,* 2015 studied the pesticides-fungi interaction in Jazan, they found that *Trichoderma harizianum* showed biodegradable efficacy of malathion.

Biodegradation by bacteria is an example of general approach for soil and water decontamination. So, the main purpose of this research, based on the results of previously mentioned researches, is to found soil-isolated bacterial strains have degradable activity of two selected insecticides namely, malathion and diazinone.

MATERIALS AND METHODS

Isolation and purification of bacteria from soil and water.

Pesticides contaminated soil and water samples were collected from different localities in Jazan area. The samples were collected from 5-15 cm depth of agricultural area, and then were serially diluted in distilled sterile water. LB agar medium was prepared and autoclaved, and then inoculated with 0.5 ml from each diluted sample, which spread over the surface of LB petri plate. The plates were incubated at 30oC for 48h. Microbial colonies obtained were sub -cultured for purification. These procedures were repeated until identical colonies in shape and morphological characteristics were observed. (Lederberg and Lederberg, 1952). The identification of the isolates was carried out according to Bergey's Manual of determinative Bacteriology.

Selection of malathion and diazinone degrading bacteria.

Eleven single colonies of pure cultures were picked and initially tested for ability of degrading malathion and diazinone. 0.3 ml of an 18 hr culture, grown in liquid media was inoculated into 10 ml MSM containing the pesticide in concentration of 100 µg/ml. then incubated for 48 hr at 28oC in rotary shaker. Flasks were covered by aluminum foil in order to prevent optical degradation of pesticides. The ability of insecticide degradation are inferred by measuring the optical density of the media (turbidity as indicator of bacterial growth). Yadav *et al*., 2015.

Material and preparations.

The organophosphate insecticides used in this study were obtained from Al-Raghy Agricultural Company Ltd., KSA. All chemicals, media and reagents obtained from Merc (Darmstadt, Germany). Calibration curves for each selected pesticide concentrations were prepared and quantified by GC-MS (HP G1800A system, capillary column HP5 (30 m x 0.25 mm) Hewlett-Packard, Germany).

Microbial degradation studies.

Selected bacterial isolates, degrading bacteria, were inoculated to pure liquid media containing pesticide (0.1 µg / 1 ml medium) in test tubes, shaken for 30 min., then incubated at 33±2oC. Then has been inoculated in a sterile culture medium. Samples were taken at successive intervals after incubation at zero,3h, 1,2,4,8,12 and 14 days compared with control samples,(without inoculum), at each interval. Zero time is the initial concentration before incubation. Kumari, *et al.,* (2012).

Degradation of tested pesticides were measured using "gas chromatography method" (GLC) after 14 days From the beginning of the treatment. Khani,M. *et al* (2016).

Effect of environmental conditions on microbial degradation.

1. Effect of incubation temperature.

To study the effect of incubation temperature on bacterial efficacy of biodegradation, culture media containing the insecticides were incubated at different temperatures, 25, 28, 30 and 35^{o}C. % degradation was determined after 14 days of incubation.

2. Effect of initial pH.

Effect of initial pH on degradation was tested by adjusting the tested media at different pH values, 6.5, 7.0, 7.5 and 8.0 then incubated. % degradation was determined after 14 days of incubation.

3. Effect of addition other carbon sources.

To study the efficacy of bacterial isolates for pesticide degradation with addition of another carbon source in medium, an experiment was conducted using pesticide containing media with addition of glucose or

starch (5 %). After incubation, % of degradation was determined and compared.

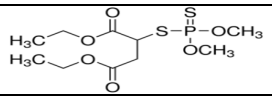
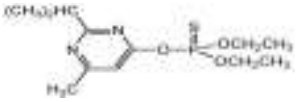
Statistical analysis:

T-test was used for analyzing the obtained data statistically to define the significance levels with the basis outlined by Snedecor and Cochran (1967).

RESULTS

Bioremediation is the process of application of biological means (including bacteria, algae, fungi, etc.) to degrade a material. In this research bioremediation by bacterial isolates of malathion & diazinone, was tested. Characterization of malathion & diazinone were illustrated in Table (1), both insecticides are broad spectrum organophosphorus insecticides. They are neurotoxicants to the central and peripheral nervous system of animals. Their products introduced in a variety of formulations, which can have implications for product efficacy and exposure to human and other no-target organisms. Thabit and El-Naggar (2013) found that malathion was degraded by soil isolated bacteria.

Table 1. Characterization of Malathion & diazinone insecticides.

Compound	Formula	Structure	Chemical class & function
Malathion	$C_{10}H_{19}O_6PS_2$		Organophosphorus; insecticide
Diazinone	$C_{12}H_{21}N_2O_3PS$		Organophosphorus; insecticide

For the biodegradation, bacterial isolates from both soil and water were obtained according to soil isolation methods. Data in Table (2) illustrated the characteristics of each isolate. The isolates belong to the genera Pseudomonas, Bacillus and Staphylococcus. The identification of the isolates were done according to morphological & biochemical characters (Bergey's Manual of determinative Bacteriology).

Table 2. Isolation and purification of bacteria from soil and water.

Genus	Gram staining	Morphology	Culture code
Pseudomonus spp.	ve-	Short rod	F1*
Pseudomonus spp.	ve-	Short rod	F2*
Bacillus spp.	ve+	Rods	F3*
Bacillus spp.	ve+	Rods	F4*
Bacillus spp.	ve+	Rods	F**
Pseudomonus spp.	ve-	Short rod	D1*
Pseudomonus spp.	ve-	Short rod	D2*
Staphylococcus spp.	ve+	Coccoid	D3*
Bacillus spp.	ve+	coccobacilli	D4*
Bacillus spp.	ve+	Rods	D5*
Bacillus spp.	ve+	Rods	D6*

***Soil isolates ** Well water isolates**
-Results are an average of three replicates,
F- isolates from Fayfaa Gov. D- isolate from Dayer bni-malek Gov.

The selection of malathion and diazinone degrading bacteria was done according to the ability of the isolate to grow in the presence of pesticide in the growth medium. The isolates F1, F3 and D3 had the best growth in the presence of both pesticides, measured as turbidity, by spectrophotometer. Microbial degradation results by the three isolates illustrated in Table (3), after incubation for 14 days. F3 showed that the best degradation percentage for both pesticides being 56.1 and 49.7 %. Followed by F1 isolate with degradation percentages 35.8 & 33.6 % malathion and diazinone degradation, respectively. Finally, comes the isolate D3 with percentages 29.8 and 24.5 % degradation, as presented in Table (3) & illustrated by Figures 1, 2, 3 & 4.

Table 3. Microbial degradation percentages by the three isolates F1, F3 and D3.

Isolate	time	Malathion residue after incubation (mg/L)	Degradation %	Diazinone residue after incubation (mg/L)	Degradation %
F1*	2	95.2	4.8	97.2	2.8
	5	80.3	19.7	90.6	9.4
	10	73.5	26.5	75.5	24.5
	14	64.2	35.8	66.4	33.6
F3*	2	90.3	9.7	92.3	7.7
	5	73.6	26.4	76.6	23.4
	10	50.7	49.3	54.7	45.3
	14	43.9	56.1	50.3	49.7
D3*	2	93.8	6.2	95.8	4.2
	5	79.2	20.8	80.7	19.3
	10	78.7	21.3	79.7	20.3
	14	70.2	29.8	75.5	24.5

- After 14 days of incubation as the sole carbon source, corrected for recoveries in sterile controls.
- 0 time concentration: 100 mg / L. -Results are an average of three replicates.

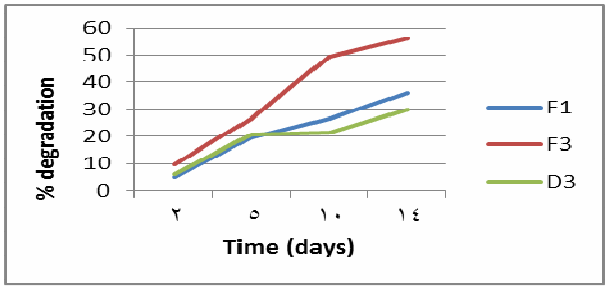


Fig. 1. % degradation of malathion by F1, F3 and D3 isolates after 14 days of incubation.

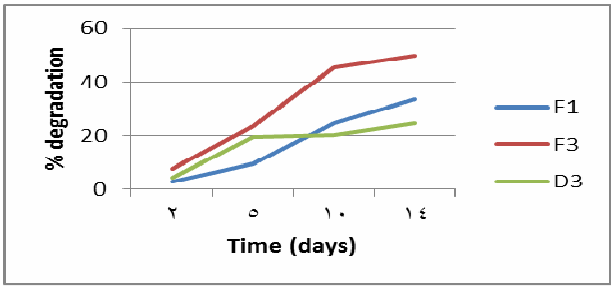


Fig. 2. % degradation of diazinone by F1, F3 and D3 isolates after 14 days of incubation.

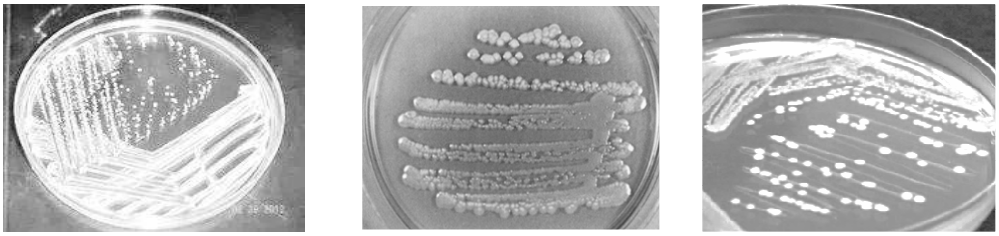


Fig. 3. Streak plates for F1 (Pseudomonus); F3 (Bacillus) and D3 (Staphylococcus).

An experiment was set in order to study the effect of some environmental conditions on the efficacy of degradation by the isolates. Factors studied were initial pH, temperature and presence of other carbon source in growth medium. As illustrated in Table (4), and Fig (4), the best pH value for the biodegradation by the isolates was 7.0 for F1 (Pseudomonas) and was 7.5 for F3 & D3 isolates, with % degradation 36.1, 55.9 and 30.0 % for malathion, respectively. And it was 33.9, 50.0 and 25.0 % for diazinone, respectively. While the best incubation temperature for the degradation was 30°C for all isolates. Results also represent that, the addition of other carbon source to the media has negative effect on degradation by bacteria even when the addition was simple carbon source such glucose or compound such starch. In the presence of glucose in the medium, the pesticide degradation is almost neglected. In case of starch supplemented soil, the degradation ratio is unstable; there are high variations in results with the three replicates.

Table 4. Effect of environmental conditions on microbial degradation after 14 days of incubation.

Environmental factor		% degradation malathion			% degradation diazinone		
		F1	F3	D3	F1	F3	D3
pH value	6.5	28.3	39.3	21.1	28.6	33.9	20.0
	7.0	29.6	55.9	30.0	31.9	50.0	25.0
	7.5	36.1	42.5	25.3	33.9	44.7	23.1
	8.0	27.9	33.2	24.3	27.2	40.2	20.0
Temperature	25	29.8	50.3	20.9	30.6	45.5	22.1
	28	30.9	51.3	22.4	33.0	48.0	24.1
	30	35.9	55.7	30.0	33.6	49.7	24.5
	35	33.6	50.4	26.3	33.0	48.8	22.9
Glucose	(10 %)	2.3	8.6	3.9	3.6	6.7	2.1
Starch	(10 %)	-	-	-	-	-	-

Results are an average of three replicates.

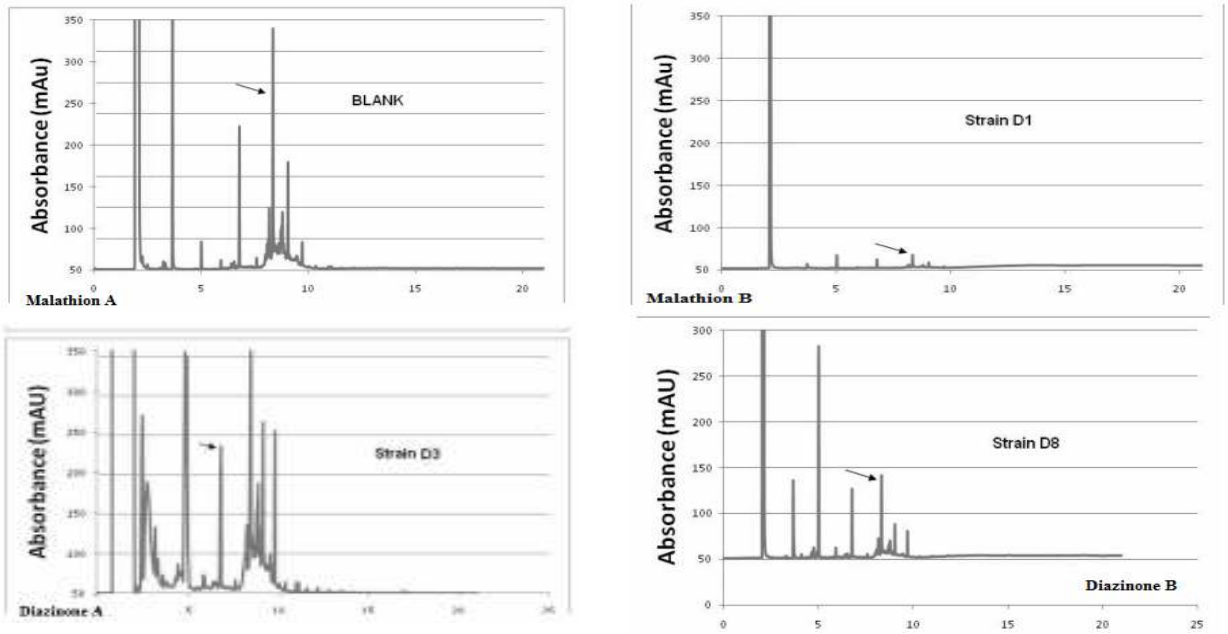


Fig. 2. Illustration of tested pesticides degradation after 14 days by GLC.

DISCUSSION

Malathion & diazinone are organophosphate insecticides used widespread to control many insect pests, Bourquin, (1977). These pesticides are used wide spread in Jazan area, against many insects and parasites. Al-Hatim *et al.,* 2015. And because of the wide application of these pesticides and the possibility of emersion of human side effects such poisoning after the transfer of residues to the body through the food chain. Therefore, there is an urgent need for ways to reduce the harmful effects of pesticide residues in the environment. Recently, biodegradation is a

safe way for environmental decontamination, which has been studied by man authors, Surekha, *et al*., 2008, Tgabit *et al*., 2013, Mahiuddin *et al*., 2014 and Khani *et al*., 2016. They isolated and identified bacterial isolates have high insecticides degradable activity, which belong to the genera, Bacillus, Pseudomonas, Enterobacter and Staphylococus. The soil in Fayfa governorate is sandy clay. The degree of salinity varies from light to medium, rich with calcium, potassium and magnesium. Such soil is rich also with a lot of microbial species, which are biologically active in such soil type. The genera isolated from this type of soil were Bacillus, Pseudomonas and Staphylococus.

The efficacy of degradation and degradation rate, by the isolates depends on the isolate, soil type, temperature, pH, carbon source and pesticide concentrations as mentioned by Shahgholi & Ahangar, 2014 and Yadav *et al.,* 2015.

Recommendations

This study is recommending for:

1- Using these bacterial isolates in commercial preparations in fields for degrading insecticides in order to not leakage in groundwater which is used by man as drinking water.

2- The soil which treated with degrading bacteria, better to not has high contents of simple carbohydrate sources in order to direct the bacteria for using and degrading the pesticide.

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REFERENCES

Abd El-Ghany, T.M. ; I.A. Masmali and M.Negm. (2015). Pesticide-fungi interaction and their impact on fungal population in agricultural soil. Inter. J. Mirobiol. & Apll. Sci. 1(4):18-22.

Al-Hatim,H.Y.; D.AlRajhi and A. Al-Rajab. (2015). Detection of pesticides residue in dams and well in Jazan area.American j. Environ. Sci. 11(5): 358-365.

Bourquin, A.W. (1977). Degradation of malathion by salt-marsh microorganisms.Apll. Environ. Microbial. 33(2): 356-362.

Khani,M.; A. Radbeh and Z. Mohammadi. (2016). Biodegradation of diazinone using gas chromatography method. Int. J. Adv. Biotechnol. Res. 7(4): 41-45.

Kumari,A.R.; G. Jeevan; M. Ashok and S.K.Vamsi. (2012). Malathion degradation by Bacillus spp. Isolated from soil. IOSR Journal of pharmacy. 2,4: 37-42.

Lederberg,J. and E.M. Lederberg (1952). Replica plating and indirect selection of bacterial mutants. J. Bacteriol., 63: 399-406.

Mahiuddin,M. ; A.N. Fakhruddin; A. Al-Mahin and M.A. Rahman (2014). Degradation of te organophosphorus insecticide diazinone by soil bacterial isolate. Int. J. Biotechnol. 3(1): 12-23.

Shahgholi,H. and A. G. Ahangar (2014). Factors controlling degradation of pesticides in the soil environment: A Review. Agric. Sci. dev.,3(8):273-278.

Snedecor, G.V. and W.G. Cochran (1967). Statistical methods 6th Ed. Iowa:Iowa state Univ. Press Ames.

Surekha, R. ; K.V. Lakshmi; R.J. Madhuri and S. Aruna (2008). Isolation and characterization of a chlorpyrifos-degrading bacteria from soil. Afr.J.Microbiol. Res. 2:26-31.

Thabit, T. M. and M.A. El-Naggar (2013). Malathion degradation by soil isolated bacteria and detection of degradation products by GC-MS. Int. J.Environ. Sci. 3(5):1467-1476.

Yadav, S. ; S. Kumar and H. S. Chaudhary (2015). Isolation and characterization of organophosphate pesticides degrading bacteria from contaminated soil. Online J. Biol. Sci., 15(1):113-125.

التحليل الحيوي للمبيدات الكيميائية بواسطة البكتيريا في منطقة جازان

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تم اجراء تجربة للتحليل الحيوي للمبيدات الكيميائية (الملاثيون والديازينون) بواسطة عزلات بكتيرية من التربة. وذلك في البيئات السائلة ثم قياس التركيز المتبقى من المبيدات على عدة فترات خلال 14 يوم من التحضين . وأظهرت النتائج وجود 3 عزلات هي F1, D3 , F3 لها قدرة عالية على التحليل الحيوي للمبيدين بنسبة 35,8 و 29,8 و 56,1 % للملاثيون على التوالي . ونسبة 33,6 و 49,7 و 24,5 % للديازينون بعد 14 يوم من التحضين . و كانت أفضل درجة حرارة للتحليلي الحيوي هي 30 درجة مئوية لجميع العزلات . بينما كانت أفضل درجة حموضة للتحليل هي 7,0 للعزلات Bacillus & staph و 7,5 للعزلة Pseudomonas . كذلك أثبتت الدراسة أن نسبة التحليل الحيوي كانت أفضل في حالة عدم وجود مصادر أخرى للكربون في البيئة المغذية مثل الجلوكوز والنشا .