

Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com

**Research Article** 

# EFFICIENCY OF *Staphylococcus aureus* IN THE DEGRADATION OF AN ORGANO PHOSPHOROUS PESTICIDE, MALATHION

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DOI: 10.7897/2277-4572.02686

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Received on: 13/10/13 Revised on: 20/11/13 Accepted on: 25/11/13

#### ABSTRACT

Pesticides and insecticides are substances or mixture of substances, intended for preventing, destroying, repelling or mitigating pests and insects, mainly in the agricultural field. However, it causes several health hazards and environmental impacts. Hence it is crucial to degrade the pesticides to prevent the harmful effects on environment as well as human health. Among the various methods used for the degradation of pesticides, biodegradation is the most widely used. The aim of the present study was to analyse the efficiency of the strain *Staphylococcus aureus* in the degradation of the organophosphorous pesticide Malathion. This was carried out both in minimal medium containing pesticide and in artificially contaminated soil sample. The samples were subjected to degradation for ten days. In the minimal medium, the percentage degradation was around 59.76 %. In the soil, the percentage degradation was around 50 % for Malathion. Hence the pesticide was effectively degraded by *Staphylococcus aureus* species proving the efficiency of biodegradation in the removal of pesticides.

Keywords: pesticides, insecticides, organophosphorous pesticide, malathion, Staphylococcus aureus, minimal medium, biodegradation

## INTRODUCTION

Pesticides are a class of biocide. These are generally a chemical or biological agent. Target pests can include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, spread disease or are vectors for disease. For many decades we are using many kinds of pesticides for agricultural purpose<sup>1</sup>. Malathion (Diethyl 2-[(dimethoxyphosphorothioyl) sulfanyl] butanedioate) is a nonsystemic, wide-spectrum organophosphorous insecticide. It is the first organophorous insecticide with high selective toxicity. Malathion degradation products include dimethyl phosphate, dimethyldithiophosphate, dimethylthiophosphate, isomalathion and malaoxon<sup>2</sup>. Malathion kills insects by preventing their nervous system from working properly. When healthy nerves send signals to each other, a special chemical messenger travels from one nerve to another to continue the message. The nerve signal stops when acetylcholinesterase (AChE) is released into the space between the nerves. Malathion binds to the enzyme and prevents the nerve signal from stopping. This causes the nerves to signal each other without stopping. The constant nerve signals make it so the insects can't move or breathe normally and they die. Malathion is used to combat the Mediterranean fruit fly and West Nile Virus and to kill the nuisance mosquitoes. In agriculture, it is suited for the control of sucking and chewing insects on fruits and vegetables. Low doses of malathion (0.5 % preparations) is used to treat the pediculosis and scabies. Although there are benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals. Malathion itself is of low toxicity, it mainly concentrates in peel and may not readily removed by washing in water alone but easily enter the body through ingestion, inhalation and absorption through the skin results in its metabolism to malaoxon which is substantially more toxic. But it is clear from the body quickly, in three to five days<sup>3</sup>. Malathion has been found to have both acute and chronic effects in living

organisms. Acute exposure to extremely high levels of Malathion will cause skin and eye irritation, cramps, nausea, diarrhoea, excessive sweating, seizures and even death. It has also been found to have the chronic effects of reproductive, mutagenic and carcinogenic effects<sup>4-6</sup>. Due to these health hazards degradation of pesticides in soil taking a large space of interest. Recently the use of microbes such as bacteria and fungi for effective detoxify, degrades and removal of toxic compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments. In earlier study the biodegradation and detoxification of malathion using *bacillus* sp have also received considerable attention.<sup>7-8</sup> Hence in the present study is to examine the efficiency of *Staphylococcus aureus* in the degradation.

## MATERIALS AND METHODS

#### Pesticide

The marketed pesticide Koshaathion (Malathion EC 50) was obtained from Karuppaiah fertilizers and co., Pudukkottai.

## Organism

The organism *Staphylococcus aureus* NCIM 2079 was obtained from National Collection of Industrial Microorganisms, Pune, India. They were maintained at 4°C as slant cultures of sterile nutrient agar for a maximum of 1 month in the Department of Pharmaceutical Technology, BIT campus, Tiruchirappalli, India.

#### Degradation studies in minimal medium

Degradation of pesticides was initially carried out by the addition of pesticides to the M9 Minimal Medium. This was conducted in order to know the efficiency of the strain in degrading the pesticides. Addition of carbon source and nitrogen source to the Minimal Medium, Glycerol was added as an external Carbon source and yeast extract was added as an external nitrogen source. This was studied in order to know the difference in the degradation of pesticides with and without carbon and nitrogen source in the medium. 5000 ppm (0.5 ml/100 ml) was added and yeast extract was added up to 500 ppm (0.05 mg/100 ml).

#### Supply of pesticides to minimal medium

The medium was prepared in four 500 ml conical flasks which were named as M1, M2, M3 and M4 among which M1 contained Minimal Medium with only 600 ppm (6 ml/100 ml) of Malathion) as the sole Carbon source, M2 contained Medium with 600 ppm Malathion and 5000 ppm Glycerol, M3 contained medium with 600 ppm Malathion and 500 ppm Yeast extract whereas M4 contained medium with 600 ppm Malathion, 5000 ppm Glycerol and 500 ppm Yeast extract. The sample composition of pesticide, Carbon and nitrogen source was used for Profenofos in conical flasks named as M5, M6, M7 and M8. To the conical flasks containing M1, M2, M3, M4, M5, M6, M7 and M8, inoculated with Staphylococcus aureus. Then these flasks were incubated in an incubator. These flasks were checked at regular intervals for the optical density of the medium which was used to find the degradation of the pesticides.

## Study of degradation of pesticides

The optical density of the media M1, M2, M3 and M4 containing the pesticide Malathion with inoculum, carbon source, nitrogen source and Carbon and nitrogen source were determined at 215 nm on the 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> days of incubation. Similarly for the flasks containing M5, M6, M7 and M8 with Profenofos, carbon and nitrogen source, the optical density was measured at 254 nm on the 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> days of incubation<sup>7,9</sup>.

## Degradation studies in the soil sample Collection of soil sample

The soil sample was collected from an agricultural field near Anna University, BIT campus. The soil sample was sieved through an 850  $\mu$  sieve to get uniformly sized particles. Then the collected sample was dried for a week in hot air oven at above 60°C to sterilize the soil and make it free of microbes.

## Addition of pesticides

The dried sample was artificially contaminated with marketed pesticides Malathion. 500 g of dried soil sample was weighed from which 50 g was taken separately. To this 50 g, 6 ml (3 ml of Malathion in 3 ml of vehicle) of Malathion EC-50 was added and dried in air. Then this dried contaminated soil was mixed with the remaining 450 g of soil and mixed thoroughly. This 500 g of contaminated soil was then dried at room temperature for a week. The pesticide was added in this manner to 1500 g of soil<sup>10-12</sup>.

## Inoculation of bacteria into the soil

Inoculated broth 5 ml was added to the dried contaminated soil. The optical density of the broth was found to be 2.56 with a maximum absorbance at 321.5 nm. The inoculated soil samples were then incubated in an incubator.

## Study of degradation of pesticides

The incubated soil samples were collected at regular intervals to study the amount of pesticide degraded during the particular interval. The samples were checked for NPK, heavy metals, pH, concentration of pesticide and the functional groups present. The concentrations of the pesticides were analyzed using High Pressure Liquid Chromatography (HPLC) by filtering 10 g of soil in 15 ml of acetone through a filter paper and the functional groups were determined using Fourier Transform Infrared Spectrophotometric (FTIR) analysis. For these analyses the samples were given in Archbishop Casimir Instrumentation Centre, St. Joseph's college, Trichy. The heavy metal contents were analyzed using AAS in the agricultural laboratory, Mannarpuram, Trichy and the NPK, pH etc were analyzed in the soil testing laboratory, Mannarpuram, Trichy<sup>13-14</sup>.

## **RESULTS AND DISCUSSIONS**

## Degradation studies in minimal medium

The degradation of the pesticide Malathion and was studied in minimal medium with and without carbon source. The initial Optical Density of the inoculum was 2.56 at 321.5 nm. Absorbance values were recorded at 215 nm for Malathion which corresponds to their  $\lambda_{max}$ . From the above absorbance values, it was noted that the absorbance values got reduced with increasing interval of time in directly indicating a decrease in concentration. However in some cases, no readings were observed. These could have risen due to contamination. The percentage degradation of the pesticides in these intervals was calculated using the formula

> % Degradation =  $[(A_0 \sim A_n)/A_0] \times 100\%$ Where,  $A_0$  – Initial Absorption of the inoculum (2.56 abs);  $A_n$  – Absorption on the n<sup>th</sup> day on incubation For Malathion (M1) on day 1, % Degradation=  $[(2.56 \sim 2.298)/2.56] \times 100\% = 10.54\%$

The percentage degradation values obtained for Malathion at various intervals are given in the Table 1. The percentage degradation values given in the table indicate the continuous degradation of pesticides in the medium resulting in decreased absorbance and increased degradation. Maximum degradation was observed in medium M3 containing an additional carbon source glycerol.

## Degradation studies in the soil sample

The degradation studies were carried out using HPLC, FT-IR, AAS and the soil was also analysed for many other parameters like pH, NPK, texture etc, the results of which are given as follows. From the heavy metal analysis, it was found that the pesticide degradation had no tremendous change on the concentration of the metals. However, minor changes observed only in the case of iron and Manganese. The heavy metal concentration on the third and the sixth days were found to be unchanged indicating that the pesticide requires a long time to induce change in heavy metals present in the soil shown in Table 2.

## **Soil Parameters**

The soil was analyzed for parameters like Texture, NPK, Calcium Carbonate, pH and Electrical Conductivity. The following table gives the standard values of the parameters shown in Table 3. In the Table 4 indicates the change in various soil parameters during the period of degradation. The Calcium Carbonate levels were found to remain in the medium level whereas the electrical conductivity values were found to be increased within the medium level. The pH values were found to be decreased as compared with the blank soil.

#### Table 1: Percentage degradation of Malathion in Minimal medium at 215 nm

Medium Components	Per	rcentage Deg	radation (%)	)
	Day 1	Day 3	Day 6	Day 9
Minimal medium + Malathion (M1)	10.54	29.29	32.81	47.96
Minimal medium + Malathion + Glycerol (M2)	25.54	44.80	49.49	63.00
Minimal medium + Malathion + Yeast extract (M3)	10.00	51.69	56.09	59.76
Minimal medium + Malathion + Glycerol + Yeast extract (M4)	14.92	33.76	nil	47.15

S. No.	Name of the Heavy metal	Before degradation	After 10 days of degradation
1	Total Zinc (ppm)	2.64	2.62
2	Total Copper (ppm)	1.09	1.10
3	Total Iron (ppm)	18.64	19.65
4	Total Manganese (ppm)	4.59	4.86
5	Total Chromium (ppm)	0.59	0.45
6	Total Nickel (ppm)	0.05	0.02
7	Total Cobalt (ppm)	0.32	0.28
8	Total Cadmium (ppm)	0.13	0.13

Table 2: Heavy Metal Analysis using AAS

Heavy metal composition before and after degradation

## Table 3: Parameters of blank soil

S. No.	Parameters	Values
1.	Texture	Sand
2.	Calcium Carbonate	Medium
3.	Electrical Conductivity	Harmless (0.24 dS/m)
4.	pH	Neutral (8.1)
5.	Nitrogen	Low (53.2 kg/acre)
6.	Phosphorous	Low (2 kg/acre)
7.	Potassium	High (212 kg/acre)

Table 4: Malathion contami	nated soil
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Parameters	Values			
	Day 0	Day 3	Day 6	Day 10
Texture	Sand	Sand	Sand	Sand
Calcium Carbonate	High	Medium	Medium	Medium
Electrical Conductivity	Harmless (0.33)	Harmless (0.53)	Harmless (0.50)	Harmless (0.52)
pH	Neutral (7.9)	Neutral (6.7)	Neutral (6.9)	Neutral (7.1)
Nitrogen	Low (54.6)	Low (77.0)	Low (91.0)	Low (109.2)
Phosphorous	Medium (8.5)	High (14.5)	High (14.5)	High (12.0)
Potassium	High (260)	High (214.0)	High (211)	High (236)
	Texture Calcium Carbonate Electrical Conductivity pH Nitrogen Phosphorous	Day 0TextureSandCalcium CarbonateHighElectrical ConductivityHarmless (0.33)pHNeutral (7.9)NitrogenLow (54.6)PhosphorousMedium (8.5)	Day 0Day 3TextureSandCalcium CarbonateHighMediumElectrical ConductivityHarmless (0.33)pHNeutral (7.9)NitrogenLow (54.6)Low (54.6)Low (77.0)PhosphorousMedium (8.5)High (14.5)	Day 0Day 3Day 6TextureSandSandCalcium CarbonateHighMediumElectrical ConductivityHarmless (0.33)Harmless (0.53)pHNeutral (7.9)Neutral (6.7)NitrogenLow (54.6)Low (77.0)PhosphorousMedium (8.5)High (14.5)

Parameters of Malathion contaminated soil

Table 5: Blank soil			
Wave number (cm <sup>-1</sup> )	% T	Molecular Motion	Corresponding Functional group
3437.64	4.5	N-H stretch	Amide
1640.51	25.0	R <sub>2</sub> C=NR stretch	Imine
		N-H bend	Amide
		C=O stretch	Amide
		N-H bend	Amine
		C=C stretch(conjugated)	Alkene
		C=C stretch(isolated)	Alkene
1436.19	47.5	OH bend	Carboxylic acid
		C-F stretch	Alkyl halide
1234.06	53.0	OH bend	Carboxylic acid
		C-F stretch	Alkyl halide
1070.68	83.5	C=O stretch	Anhydride
		C-O-C stretch	Ether
		C=O stretch	Alcohol
		PH bend	Phosphine
		CF stretch	Alkyl halide
		CN stretch	Amine
976.62	58.0	S-O stretch	Sulfonate
		PH bend	Phosphine
		C=O stretch	Anhydride
896.00	46.0	PH bend	Phosphine
		S=O stretch	Sulfonate
745.32	34.0	CH bend (mono)	Aromatics
		CH bend (ortho)	Aromatics
		C-Cl stretch	Alkyl halide
	Γ	S-O stretch	Sulfonate
	<u>                                     </u>	PH bend	Phosphine
514.61	54.5	C-I stretch	Alkyl halides

Table 6: Malathion (liquid sample)			
Wave number (cm <sup>-1</sup> )	% T	Molecular motion	Corresponding functional group
3404.12	3.5	NH stretch (1/NH bond)	Amine
		N-H stretch	Amide
2918.45	43.2	C-H stretch	Alkanes
1644.92	27.3	R <sub>2</sub> C=NR stretch	Imine
		C=O stretch	Amide
		C=C stretch (isolated)	Alkenes
1557.65	30.0	N-H bend	Amine
		N-H bend (1)	Amide
		-NO <sub>2</sub> (aliphatic)	Nitro groups
1417.23	33.5	C-H(in plane bend)	Alkenes
		OH bend	Carboxylic acids
1254.39	54.9	C-O stretch	Alcohols
		C-O-C stretch (dialkyl)	Ether
		CO stretch	Carboxylic acid
		C-C(O)-C stretch	Esters
		C=O stretch	Anhydride
		CF stretch	Alkyl halide
		CN stretch(aryl)	Amine
1038.40	17.8	C-O stretch	Alcohols
		C-O-C stretch (dialkyl)	Ether
		C=O stretch	Anhydride
		PH bend	Phosphine
885.29	87.2	PH bend	Phosphine
761.47	45.0	CH bend (mono)	Aromatics
		CH bend (ortho)	Aromatics
		C-Cl stretch	Alkyl halide
		S-O stretch	Sulfonate
554.47	40.0	C-Cl stretch	Alkyl halide
		C-Br stretch	Alkyl halide
		C-I stretch	Alkyl halide

#### Table 6: Malathion (liquid sample)

## Table 7: Malathion contaminated soil (0<sup>th</sup> day of degradation)

Wave number (cm <sup>-1</sup> )	% T	Molecular motion	Corresponding functional group
3429.01	15.20	NH stretch (1/NH bond)	Amine
1620.60	47.50	NH bend	Amide
		NH bend	Amine
		C=C (conjugated)	Alkenes
1432.35	48.50	OH bend	Carboxylic acid
1361.04	62.0	CH bend	Alkene
		CF stretch	Alkyl halide
		-NO <sub>2</sub> (aliphatic)	Nitro
1136.11	88.80	C-O stretch	Alcohol
		C-O-C stretch	Ether
		C-C stretch	Ketone
		C-F stretch	Alkyl halide
981.56	63.50	C-H bend (mono-substituted)	Alkene
705.17	50.0	C-Cl stretch	Acid chloride
577.43	73.70	C-Cl stretch	Acid chloride
		C-Cl stretch	Alkyl halide
		C-Br stretch	Alkyl halide
		C-I stretch	Alkyl halide

## Table 8: Malathion contaminated soil (3<sup>rd</sup> day of degradation)

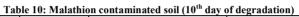
Wave Number	% T	Molecular Motion	Corresponding Functional Group
3433.09	53.44	N-H stretch	Amide
2926.67	79.08	O-H stretch	Carboxylic acid
1877.69	66.67	C=O stretch	Amides
1630.17	46.76	C=O stretch	Amides
		N-H stretch	Amines
		C=C stretch (isolated)	Alkenes
		C=C stretch(conjugated)	Alkenes
1036.89	3.23	P-H bend	Phosphine
778.61	37.73	C-F bend stretch	Alkyl halide
690.95	59.59	S-O stretch	Sulfonates
532.81	36.34	C-I stretch	Alkyl halide
		C-Br stretch	Alkyl halide

Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day)

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Wave Number	% T	Molecular Motion	Corresponding Functional Group
3433.91	53.20	N-H stretch	Amides
		N-H stretch(1/NH bond)	Amine
2925.39	80.34	C-H stretch	Aromatic
2857.27	89.31	O-H stretch	Carboxylic acid
		C-H stretch	Alkanes
1633.27	51.77	C=C stretch (isolated)	Alkenes
		C=C stretch (conjugated)	Alkenes
		N-H stretch	Amine
		C=O stretch	Amides
1036.41	3.23	P-H bend	Phosphine
		C-F bend stretch	Alkyl halide
		C-N stretch	Alkyl amines
		C-O stretch	Anhydride
		C-O-C stretch (dialkyl)	Ether
		C-O stretch (conjugated)	Alcohol
779.20	43.69	C-Cl stretch	Acid chloride
		S-O stretch	Sulfonates
691.54	66.19	C-Cl stretch	Alkyl chloride
		C-Cl stretch	Acid chloride
		C-H stretch	Aromatic
533.355	41.76	C-Br stretch	Alkyl halide

## Table 9: Malathion contaminated soil (6<sup>th</sup> day of degradation)



Wave number (cm <sup>-1</sup> )	% T	Molecular Motion	Corresponding Functional Group
3442.08	33.21	N-H stretch	Amides
		N-H stretch(1/NH bond)	Amine
2930.28	77.10	C-H stretch	Alkanes
		OH stretch	Carboxylic acid
1641.96	50.76	C=C stretch (isolated)	Alkenes
		C=O stretch	Amide
		R <sub>2</sub> C=N-R stretch	Imine
1430.50	49.63	OH bend	Carboxylic acid
1033.91	3.55	C-O stretch	Alcohol
		C-O-C stretch(dialkyl)	Ether
		C-O stretch	Anhydride
		C-N stretch(alkyl)	Amine
		C-F stretch	Alkyl halide
		PH bend	Phosphine
779.94	63.81	S-O stretch	Sulfonate
		C-Cl stretch	Alkyl halide
683.34	67.45	C-Cl stretch	Alkyl halide
		C-Cl stretch	Acid chloride
534.56	34.97	C-I stretch	Alkyl halide

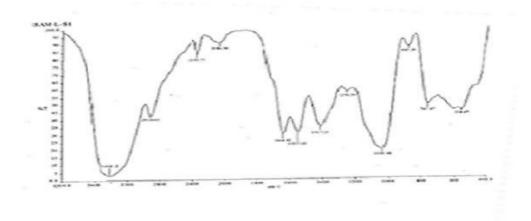


Figure 1: Functional groups present in Malathion

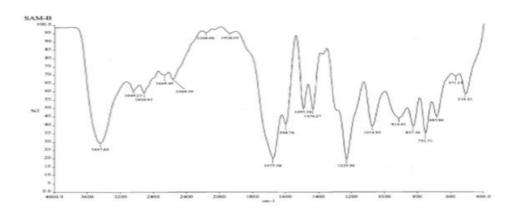


Figure 2: Functional groups present in Malathion contaminated soil (0<sup>th</sup> day)

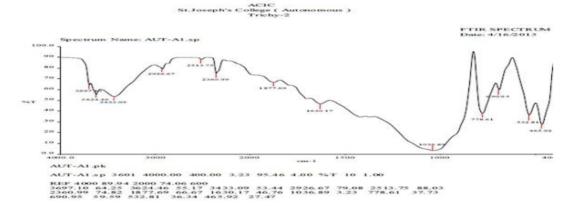


Figure 3: Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day)

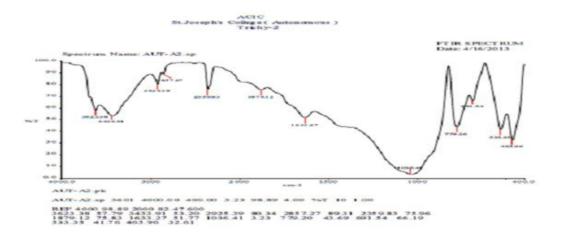
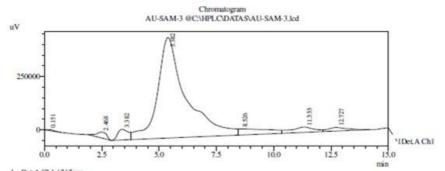


Figure 4: Functional groups present in Malathion contaminated soil ( $6^{th}$  day)

## HPLC ANALYSIS REPORT

	Sample Information
Acquired by	: Admin
Sample Name	: AU-SAM-3
Sample ID	
Vail#	1
Injection Volume	: 20 uL
Data Filename	: AU-SAM-3.lcd
Method Filename	: Ch.lem
Batch Filename	
Report Filename	: Default.lcr
Date Acquired	: 23-04-2013 PM 11:38:21
Data Processed	: 23-04-2013 PM 11:53:24



1 Det A Ch1/215nm

PeakTable

etector A C	h1 215nm	Реак Гаріс			
Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.151	107752	4188	0.223	0.675
2	2.468	840770	26575	1,739	4.282
3	3.382	1643039	49974	3,398	8.052
4	5.382	40477012	470099	83.712	75.741
5	8.526	2538055	28486	5.249	4.590
6	11.333	1737858	24784	3.594	3.993
7	12.727	1007945	16560	2.085	2.668
Total		48352431	620666	100.000	100,000

## Figure 5: Malathion concentration (3<sup>rd</sup> and 6<sup>th</sup> days of degradation)

## HPLC ANALYSIS REPORT

		Sample Information
Acquired by	: Admin	change internation
Sample Name	: AU-SAM-7	
Sample ID	1000	
Vail#	1	
Injection Volume	: 20 uL	
Data Filename	: AU-SAM-7.lcd	
Method Filename	: Ch.lem	
Batch Filename	:	
Report Filename	: Default.lcr	
Date Acquired	: 23-04-2013 PM 11:19:39	
Data Processed	: 23-04-2013 PM 11:34:41	

515829

4,78

Tota

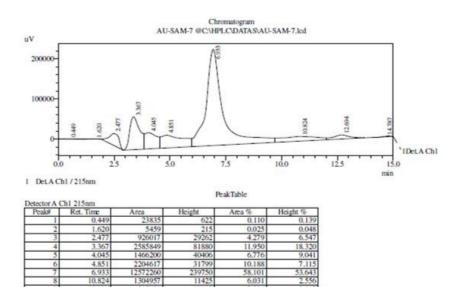


Figure 6: Malathion concentration (10<sup>th</sup> day of degradation)

10042

446937

6.031 2.384 0.156

100.000

247 344

100,000

The Nitrogen content was found to increase continuously with increasing period of degradation whereas the Phosphorous levels were in the rising phase on the  $3^{rd}$  and  $6^{th}$  days and got reduced on the  $10^{th}$  day of incubation. The initial increase was could be attributed to the addition of the organophosphorous pesticide, Malathion, and the later reduction in concentration could be attributed to the degrading effect of the species, *Staphylococcus aureus*, in degrading the pesticide. The concentration of Potassium was found to increase continuously.

#### **FT-IR** analysis

The FT-IR analysis was done for the determination of functional groups of blank soil, malathion and soil contaminated with the malathion in the various levels of degradation. The results are tabulated and pictured in Table 5 (Blank soil), Table 6 Malathion (liquid sample), Table 7 Malathion contaminated soil (0<sup>th</sup> day of degradation), Table 8 Malathion contaminated soil (3<sup>rd</sup> day of degradation), Table 9 Malathion contaminated soil (6<sup>th</sup> day of degradation), Table 10 Malathion contaminated soil (10<sup>th</sup> day of degradation), Table 10 Malathion contaminated soil (10<sup>th</sup> day of degradation) and Figure 1 represents functional groups present in Malathion, Figure 2 Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day), Figure 3 Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day), Figure 4 Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day), Figure 4 Functional groups present in Malathion contaminated soil (3<sup>rd</sup> day).

## Functional groups present in Malathion contaminated soil (10<sup>th</sup> day)

In the FT-IR analysis, the blank soil and marketed Malathion (Koshaathion) were used as standards. In the blank soil, functional groups like amide, amine, imine, carboxylic acids, alkyl halides, phosphine, and aromatics were detected. In case of Malathion, the above said groups were present along with nitro groups and ketone (for Profenofos only). On the zero<sup>th</sup> day of degradation of the Malathion contaminated soil, functional groups like amine, amide, nitro ether, ketone and alkyl halide groups were detected. Comparing the results of Zero<sup>th</sup> and Third days of degradation of Malathion, it was seen that phosphine (1036.89 cm<sup>-1</sup>) and sulphonates (690.95 cm<sup>-1</sup>) which were absent on the zero<sup>th</sup> day were present on the third day. Similarly nitro groups detected at 1361.04 cm<sup>-1</sup> on the zero<sup>th</sup> day were not present on the third of degradation. Amine groups were detected at 3429.01 cm<sup>-1</sup> with 15.20 % T on the zero<sup>th</sup> day was shifted to 1630.17 cm<sup>-1</sup> with 46.76 % T. On the 6<sup>th</sup> day of degradation of Malathion, sulphonate which was absent on the zero<sup>th</sup> day and was detected on 3<sup>rd</sup> day was again detected at 779.75 cm<sup>-1</sup> with 43.69 % T. On the 10<sup>th</sup> day of degradation, Imine, phosphine (1033.91 cm<sup>-1</sup> with 3.55 % T) and Sulphonates (779.94 cm<sup>-1</sup> with 63.81 % T) were detected which were absent on the zero<sup>th</sup> day.

#### **HPLC** analysis

The samples were analyzed for the concentration of Malathion during the degradation period. The readings were noted during the third and tenth days of degradation. The concentration of the compound was determined using the formula

Unknown Concentration= Concentration of its corresponding component of standard× peak area (sample)/peak area (standard)

Concentration of standard = 0.5 %, Concentration of sample on 0<sup>th</sup> day = 50 %, Peak area of standard = 1.5743 (mAU×min); the report of the HPLC analysis is given Figure 5 and 6. Malathion (3<sup>rd</sup> day): Retention time detected

= 5.38 minutes, Peak area of sample (after 3 days) = 83.712 (mAU×min), Concentration of sample after three days =  $0.5 \times 83.712/1.5743 = 26.58$  %

The HPLC peak for the pesticide was found to be the same during the third and sixth days indicating no effective degradation in the mean period of time. The retention time for Malathion is 5.20 minutes with Acetonitrile: Water (50:50) as the mobile phase. From the HPLC analysis, it was seen that Malathion could be effectively degraded by *Staphylococcus aureus*. The initial concentration of pesticide was 50 %, which was reduced to 26.58 %, which is comparative with the degradation of 30.93 % Malathion (0.5%) in the work done by B. Singh, R. Kaur and K. Singh. No significant differences were observed between the third and sixth day. After 10 days, no peaks were observed in the above mentioned retention times indicating a complete degradation of the pesticides. For a concentration of 100 %, the percentage degradation of Malathion was 46.84 %.

#### CONCLUSION

Biodegradation of pesticides has proved to provide a very simple and environment friendly method of degradation of pesticides. They provide several advantages over the conventional methods of degradation. In this study, the efficiency of Staphylococcus aureus species in the degradation of the organophosphate pesticide malathion was studied in the minimal medium supplemented with and without External nutrient sources. The results obtained clearly indicate that Staphylococcus aureus can effectively degrade the pesticide added. The work was also extended to artificially contaminated soil samples, in which the degradation was even more rapid than in the minimal Medium. The degradation was also confirmed by the decline in the phosphorous levels of the soil. The biodegradation was also advantageous in the way that it has little or no effect on the heavy metals concentration, which is effective for crop growth. Hence it could be concluded that the species Staphylococcus aureus acts as an excellent biological tool for the degradation of organophosphates. The environment and soil contain diversity of microorganisms, which could be used efficiently for biodegradation. This provides an economical and effective method of degradation.

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Source of support: Nil, Conflict of interest: None Declared



How to cite this article:

K. Akilandeswari, V. Sona. Efficiency of Staphylococcus aureus in the degradation of an organophosphate pesticide, Malathion. *J Pharm Sci Innov.* 2013; 2(6): 13-21.