EFFECT OF SOME PESTICIDES ON LATERAL GENE TRANSFER IN BACTERIAL AND SOME BACTERIAL FEATURES

Rehab Edarous M. E. Salem[1] and M. M. A. Elashtokhy[2]
[1]Plant production Department, Fac. Agric., Zagazig Univ., Egypt.[2]Genetics Department, Fac. Agric., Zagazig Univ., Egypt.

Abstract

Herbicides and insecticides have been widely and intensively used in agriculture areas worldwide to enhance crop yield. However many pesticides cause serious environmental problems. In addition to the pesticides may also have some effects on the treated agricultural crops and soil cultivate with plants. The present study aimed to determine the effects of two pesticides belonging to different groups of chemicals, namely Gesparim as (herbicide) and Lambda-cyhalothrin as (insecticide) at recommended rates, on two species of bacteria. The effects of pesticides on total viable bacteria, mutagenicity and gene transfer between bacteria were investigated in laboratory experiments.

The results showed that, total survival percentage (S%) bacteria number was decreased and inhibited as compared with control when treated by herbicide Gesaprim. At concentrate 3000 **µg/ml** S% was greatly affective reached 81 – 89 %. The corresponding picture was different in the case of the insecticide Lambda-cyhalothrin where, total survival bacteria number was normal as compared with control during incubation. At concentrate of 3000 **µg/ml** Lambda - cyhalothrin it gave low effect on S% which reached 91 – 97 %. This means that the effect of all rates of the pesticides is suitable for the growth of bacteria to some extent. Prophage F116 induction from different *P. aureginosa* at concentration 500 – 3000 **µg/ml** explained that the Gesaprim have a mutagenic activity this is higher as compared with Lambda-cyhalothrin at concentration of 1500 – 3000 **µg/ml**. Moreover, protein SDS-PAGE analysis of *P. aeruginosia* strain PAo1 after treatment with the highest three concentrations of each pesticide 2000, 2500 and 3000 µg/ml as compared with the control revealed the differences in their banding pattern which was the highest with Gesaprim. As well as possible genes can be transferred by conjugation. Conjugation frequencies ranged from 1.16 $x10^{-3}$ to 9.4 x 10^{-4} and 1.04 $x10^{-3}$ to 9.0 $x10^{-4}$ for each of Lambda - cyhalothrin and Gesaprim respectively.

Key works: Pesticides, Lateral Gene Transfer, SDS-PAGE.

INTRODUCTION

In order to enhance the agricultural crop yields, pesticides have been used to control weeds, insects and fungi in a wide range of application. Several million tons of organic and inorganic chemicals with antimicrobial and insecticidal properties are added annually into

soil and their environment (**Vishnu *et al.* 2015**). Pesticides have become an essential part of modern agriculture. Pesticides are used several times during one season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment. The influence of pesticides on soil bacteria is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides. Over-application of pesticides had severed effects on soil ecology that may lead to alterations in or the erosion of beneficial or plant probiotic soil microflora **(Anu and Gosal, 2011).**

Chemical control is quick, more effective time and labor saving. On the other hand without using chemical control , it was generally regarded as less cost-effective as compared with chemical management that mainly due to the fact that all non- chemical weed control methods required repeated treatments and may be labor intensive and costly. Chemical weed control is suggested by many researchers (**Spitters *et al.* 1998)**.

Since bacterial metabolism is very versatile, strains with the specific capability to remove xenobiotic compounds could be used for the bioremediation of polluted sites and waters **(Desai *et al.* 2010).**

Some isolates were able to degrade pesticides, and the formation of degradation products (γ-pentachlorocyclohexane (γ-PCCH), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD)) were observed in liquid culture confirmed their degradation capability **(Lovecka *et al.* 2015)**.

Bacteria may acquire genetic information from either related or phylogenetically distinct populations in the community by 'lateral gene transfer', or xenobiotic catabolic mobile genetic elements (MGEs), like plasmids **(Springael and Top, 2004)**. It has been widely accepted that pollutant degrading enzymes have evolved from isozymes (enzymes that differ in amino acid sequence but catalyze the same chemical reaction) in response to industrial production and environmental release of xenobiotics **(Wackett, 2004 and Singer, 2006)**.

Most of genes responsible for catabolic degradation are located on the chromosomes, but in a few cases these genes are found in plasmids or transposons. Mobile genetic elements such as plasmids and transposons have been shown to encode enzymes responsible for the degradation of several pesticides **(Hussain *et al.* 2009)**.

Lambda-cyhalothrin is an insecticide registered by the U.S. Environmental Protection Agency (EPA) in 1988. Lambda-cyhalothrin belongs to a group of chemicals called pyrethroids. Pyrethroids are manmade chemicals that are similar to the natural insecticides pyrethrins. **(Hornsby *et al.* 1995).**

Gesaprim 80 WP (6 chloro –N- (1 – methylethyel)-1,3,5 triazine -2,4-diamine . Therefore, the present work aimed to 1- Study the effect of pesticides on the ability of bacteria to survival 2- Study the effect of pesticides on mutagenic activity 3– Study the effect of pesticides on transfer their genetic materials.

MATERIAL AND METHODS

This investigation was carried out at the Microbial Genetic Lab., Genetics Dept., Fac. Agric., Zagazig Univ.

Pesticides and Reagents

Commercial pesticides (Lambda-cyhalothrin as insecticide and Gesaprim as herbicide) were purchased from Novartis Co. (Egypt). The pesticides were selected based on the use pattern of pest control in Egypt. Other chemicals were used throughout the experimental studies were analytical grade and purchased from Al-Gomhoria Company. for medical and chemical supplies, Zagazig, Egypt.

Growth Media

The nutrient agar (NA) and nutrient broth (NB) media were used. Soft agar (0.8% w/v agar) was prepared in distilled water and kept at 45°C on water bath. Phosphate buffer was prepared from 1/15M potassium phosphate (KH2PO4) and 1/15M disodium phosphate (Na2HPO4. 2H2O). Streptomycin (12 mg/ml) was added as sterilized solutions by filtration through 0.2 µm filter membrane to the media after autoclaving.

Bacterial Strains

Bacterial strains of *Pseudomonas aeruginosia* (PAol and MAM2) were obtained from Prof. Dr. M. Day, University of Wales, Cardiff, UK, by Prof. Dr. M. K. Amin, University of Zagazig, Faculty of Agric. Egypt, *Bacillus thuringensis subsp. var. kurstaki* (HD1) obtained from Microbial Molecular Biology Laboratory, Agricultural Genetic Engineering Research Institute, Giza, Egypt, Bacterial strains *Pseudomonas aeruginosia* are lysogen with F116 phage and resistance to streptomycin.

Prophage Induction from Lysogenic Strains

The lysogen cells were inoculated into 10 ml of Nutrient broth (NB), then placed in a 150 rpm shaker incubator LAB- LINE overnight, at 30°C followed by centrifugation REMI Model .R 32 A at 5000 rpm for 30 min. The supernatant was removed, a few drops of chloroforme were added and passed through a sterile filter membrane (0.2 µm, Whatman) and titred **(Louis and Ognien, 2013)**.

Phage Titration

Serial hundred-fold dilutions of phage were prepared in phosphate buffer (PH 7.0). Phage titer was determined by mixing

equal volumes (0.1 ml) of a phage dilution with host cells (growing overnight in NB at 30°C), adding soft agar and pouring immediately onto an NA plate **(Gulig *et al.* 2002)**. Plates were incubated at 30°C for 24h. Plaque-forming unit (pfu/ml) was recorded.

Survival Percentage of Bacterial Strains

The overnight bacterial cultural was prepared. Two ml of each individual strain (PAo1, MAM2 and HD1) were added to each individual volume from pesticides. After incubation at 30°C for Overnight, The tested dilutions were prepared; 0.1 ml of each dilution was spreaded onto NA plates. The plates were incubated at 30°C for 24h. The colonies were counted and the survival was calculated as colony forming units (cfu /ml). Survival percentage (S%) was calculated by dividing cfu/ml of each treatment on cfu/ml of control and killing percentage (K%) = 100- S% **(Abdul-Raouf *et al.* 1993)**.

Mutagenic Activity of pesticides

All assays were carried out in triplicates. The pfu/mL and cfu/mL were calculated, fold increase (F.I.) was calculated by dividing pfu/ml of each concentration on pfu/ml of control, Mutagenic index (M.I.) was detected according to **Heinemann, (1971)** and were carried out under complete sterile conditions.

SDS-PAGE analysis

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of **(Weber and Osborn, 2006)**. Protein SDS-PAGE analysis of *P. aeruginosia* strain PAo1 was made after treatment with the two tested pesticides and compared with the control.

Gene transfer by Transduction

Transduction by lysate:

Recipient cells were grown in NB overnight, washed 2-3 times by phosphate buffer (pH 7.0) and then resuspended. Viable counts of the recipient strains were made. Equal volumes (0.1 ml) of phage lysate and recipient cell suspensions were mixed (multiplicity of infection MOI +10: 1). The mixture was kept for 15-30 min at room temperature, to allow phage adsorption. Serial of dilutions have been prepared and placed onto selective media. Number of transductants was recorded and frequency of transduction per recipient was calculated **(Russell *et al.* 1972).**

Gene transfer by conjugation:

All donor and recipient isolates were inoculated in liquid media and incubated for 24 h. Equal volumes (1 ml) of donor and recipient were added on surface of complete media plates and incubated for 24 h. The growth was washed by 10 ml phosphate buffer and removed by spreader to sterile flasks. Serial dilutions were prepared, 0.1 ml

spreaded on selective media for donor, recipient and transconjugants **(Amin *et al.* 2008).**

RESULTS AND DISCUSSION

1– Effect of the tested pesticides on survival Percentages of *Pseudomonas aureginosa* Strains and *Bacillus thuringensis* (HD1) in case of treatment by different concentrations after 24h

The results showed that treatment insecticide with Lambda-cyhalothrin the survival percentages of the two tested strains were similar at recommended dose (Table 1). At 3000 µg/ml S% reached 91- 97 % (cfu/ml = 3.65 $x10^9$ - 2.94 $x10^9$), while at 2500 ug/ml. S% were 88 – 92 %(cfu/ml = 3.45 $x10^9$ - 2.83 $x10^9$). The corresponding picture was different on Gesaprim herbicide at recommended rates (Table 2). At the 3000 ug/ml. S% reached 81-89 % (cfu/ml = 2.53 $x10^9$ - 3.25 $x10^9$), while at 2500 ug/ml. S% reached 83-90 % (cfu/ml = 2.61 $x10^9$ - 3.31 $x10^9$).

Data showed that Lambda-cyhalothrin had a low effect on bacterial number cells, while Gesaprim had a higher effect on decrease bacterial number cells. Nevertheless, the bacterial number determination indicate that populations of bacteria capable of metabolizing and degradation the Lambda-cyhalothrin insecticide. Decreases in the number of total viable bacteria occurred during the incubation periods after treated with the tested pesticides. These agreements with **Abo – Amer (2012)**. Indicated that *Pseudomonas*, is the most efficient bacterial genus for the degradation of toxic compounds. The ability of these bacteria to degrade these compounds, is related to the contact time with the compound, the environmental conditions in which they develop and their physiological versatility. **Lavkin *et al.* (2005)**. Showed that bacteria mainly of genus Al *caligenes*, *Pseudomonas*, *Flavobacterium* are the potent degraders of pesticides. Studies showed that these microorganisms produce various extracellular enzymes that cause the degradation of different types of complex organic compounds.

2 - Mutagenic Activity of pesticides on Prophage Induction.

Data in Tables 3 and 4 showed that the induction of prophage F116, was increased by using different concentrations of Lambda-cyhalothrin and Gesaprim. Four to five concentrations with all pesticide respectively, caused fold increases + (> 3) than these observed in control were detected.

At 3000 µg/ml concentration there was high fold increase in most lysogen strains in both tested pesticides. At low concentrations 500 - 1000 µg/ml, no fold increase in the induction mechanism in lambda-

cyhalothrin compared with Gesaprim. This might due to the toxic effect of Gesaprim on bacterial cells. This results agreed with **Heinemann, (1971)**, any compound give 3 fold increases in prophage induction than control should be mutagenic agent. Comparing this assay with other microbial system such as *Salmonella* Ames test, the matching for the two systems was 71%. However, the sensitivity of prophage assay in detecting Mutagenicity was 76% opposite with 58% for *Salmonella* assay **(Rossman *et al.* 1991).**

Moreover, the mutagenicity of many compounds might escape from Salmonella test, but gave positive mutagenic activity by using prophage assay. Thus, the prophage induction assay was proven to be more appropriate methodology than the Salmonella to test the mutagenicity **(Vargas *et al.* 2001).** This contrast in results between the two pesticides may be due to the differences in chemical structure and chemical group. This data agreement with **(Boldt and Jacobsen, 1998)**, when explained the difference of strains sensitivity according to isomerism of this enzyme. The bacteria are sensitive to herbicides with mode of action affecting the universal biological processes in living system. For example, herbicides from the sulfonylurea group as (Gesaprim) effect on the acetolactate synthase, which is involed in the synthesis of valine to fluorescent *Pseudomonades* were neutralized by introduction of these amino acids into the medium. Toxic effects of drugs relative to fluorescent *Pseudomonades* was neutralized by introductions these scarce amino acids into the medium **(Volodymyr *et al,* 2016)**.

SDS-PAGE analysis

Protein SDS-PAGE analysis of *P. aeruginosia* strain PAo1 after treatment with the highest three concentrations of each pesticide 2000, 2500 and 3000 µg/ml as compared with the control revealed that the three concentrations of each pesticides awarded the differences in their banding pattern as shown in (Fig. 1) compared with control as shown in (Fig. 2) which reached to fifteen bands. The protein contents of the treated cells showing four condensation major band represented from (≈ 24-57 kDa). The molecular weight band was the same at ≈ 57, 42, 31 and 24 KDa for *P. aeruginosia* strain PAo1 for each concentration from the two tested pesticides. However there was a disappearance of some bands between (≈ 36 and 72 kDa) as compared with the control (Table 5). For treatment with Gesaprim disappear the band at 72 KDa as compared with all of the control and Lambda-cyhalothrin. While bands disappear once in all samples at 36 KDa. On the other hand the appear once of new bands at (≈ 25 and 27 kDa) only with Lambda-cyhalothrin as compared with the control and Gesaprim was also appear new bands at (≈ 20 kDa) in all transactions

was not like in control. These results referred to in general uses of pesticides effect on bacteria strains and represent a stress on growth which it was at much higher degree with Gesaprim. This change in the protein profile banding pattern may be a way of microorganisms resistant to the presence of pesticides within microbial degradation system. The microorganisms capable of degrading pesticides can be used for bioremediation of other chemical compounds to whom any microbial degradation system is known **(Singh and Walker, 2006)**. However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes, but also a wide range of environmental parameters **(Aislabie and Lloyd-Jones, 1995)**. Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants **(Vischetti *et al.* 2002; Iranzo *et al.* 2001)**.

According to **Abo-Amer (2012)**, *Pseudomonas*, is the most efficient bacterial genus for the degradation of toxic compounds. In other report **(Vásquez and Reyes, 2002; Senthilkumar *et al.* 2011)**, evaluated three *Pseudomonas* species for the biodegradation of the herbicide aroclor 1242, showing that these bacteria have a great ability to degrade it, according to their degradation percentage, 99.8, 89.4 and 98.4 respectively.

3 - Effect of pesticides on lateral gene transfer by conjugation:

3.1. Lateral gene transfer by conjugation

Gene transfer through conjugation can occur successfully among donor and recipient strains of *P. aeruginosa* using two antibiotic resistance genes, str^r and amp^r which grown on liquid culture containing the pesticide lambda-cyhalothrin (Table 6). Conjugation frequencies ranged from 1.16×10^{-3} to 9.4×10^{-4} for str^r and amp^r respectively, While *P. aeruginosa* which grown on liquid culture containing the pesticide Gesaprim, Conjugation frequencies ranged from 1.04×10^{-3} to 9.0×10^{-4} for str^r and amp^r respectively. Gene transfer can occur among different strains of *P. aeruginosa*. This refers to the ability of bacteria on the use of pesticides by degradation as a source of carbon. According to **Desai *et al.,* (2010)** bacterial metabolism is very versatile, and strains with the specific capability to remove xenobiotic compounds could be used for the bioremediation of polluted sites and waters.

3.2. Lateral gene transfer through transduction

The bacteriophages F116 have been allowed to propagate on original strain PA01 of *P.aeruginosa* bacteria, which grown on liquid culture containing the pesticide lambda-cyhalothrin and Gesaprim.

These prepared phages have been tested to assess their ability in horizontal gene transfer by transduction into MaM2 strain of the same species according to **Carlos *et al.* (2003)** (Table 7). Transduction frequency ranged from 2.4 $X10^{-2}$ up to 1.6 $X10^{-2}$ and from 2.2 $X10^{-2}$ up to 1.8 $x10^{-2}$ upon using MaM2 as recipient cells to the pesticides lambda-cyhalothrin and Gesaprim respectively. The results of this study clarified that lateral gene transfer can occur between bacterial In the presence of pesticides and that microorganisms are capable to grow in the presence of several commercial pesticides. Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source. Lateral genetic exchange might facilitate adoption of an optimal genetic profile for survival. Whereas transfer of plasmid pAM 81 between two species, *Streptococcus gordonii* and *Enterococcus Faecalis* was demonstrated in an ex vivo tooth model **(Christine *et al.* 2008).**

Finally it has been suggested that horizontal gene transfer is the essence of phlogeny, especially to primitive genomes with important evolutionary consequence **(Dagan and Martin, 2007)**. Distinct catabolic genes are either present on mobile genetic elements, such as transposons (a small segment of DNA that can move from one region of DNA to another) and plasmids (small molecules of circular, extrachromosomal DNA found in some bacteria), or the chromosome itself, which facilitates horizontal gene transfer and enhances the rapid microbial transformation of toxic xenobiotic compounds **(Sinha *et al.* 2009).** Eventually the individual cells best adapted to resisting or degrading the xenobiotic get selected and their populations increase in number as compared to others in the microbial community **(Top and Springaely, 2003 and Janssen *et al.* 2005).**

Senthilkumar *et al.* (2011) reports the *Pseudomonas aeruginosa Trichoderma viridae* ability in the endosulfan and methyl parathion pesticides degradation. Another experiments have been demonstrated the efficiency of the bacterium *Rhodococcus sp*. to degrade triazines to nitrate.

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Tables and figures

Table 1: Effect of Lambada-cyhalothrin on survival percentages of *P. aureginosa* lysogenic strains with phage F116 and *Bacillus thuringensis* (HD1) upon treatment to different concentration after 24h

	PAo1 F116			MAM2 F116			HD1		
Con. µg/ml	**cfu / mL x 10^9**	**Survival %**	**Killing %**	**cfu / mL x 10^9**	**Survival %**	**Killing %**	**cfu / mL x 10^9**	**Survival %**	**Killing %**
0	3.74	100	0	3.72	100	0	3.21	100	0
500	3.63	97	3	3.65	98	2	3.1	96	4
1000	3.59	95	5	3.61	97	3	2.98	92	8
1500	3.42	91	9	3.5	94	6	2.91	90	10
2000	3.44	91	9	3.52	94	6	2.85	88	12
2500	3.45	92	8	3.63	97	3	2.83	88	12
3000	3.65	97	3	3.6	96	4	2.94	91	9

PAo1 F116: *P. aureginosa* strain PAo1 lysogenic with phage F116.
MAM2 F116: *P. aureginosa* strain MAM2 lysogenic with phage F116.
HD1: *Bacillus thuringensis subsp. var. kurstaki* (HD1)

Table 2: Effect of Gesaprim on survival percentages of *P. aureginosa* lysogenic strains with phage F116 and *Bacillus thuringensis* (HD1) upon treatment to different concentration after 24h

	PAo1 F116			MAM2 F116			HD1		
Con. µg/ml	**cfu / mL x 10^9**	**Survival %**	**Killing %**	**cfu / mL x 10^9**	**Survival %**	**Killing %**	**cfu / mL x 10^9**	**Survival %**	**Killing %**
0	3.64	100	0	3.45	100	0	3.12	100	0
500	3.53	96	4	3.32	96	4	2.95	94	6
1000	3.43	94	6	3.22	93	7	2.85	91	9
1500	3.39	93	7	3.1	89	11	2.73	87	13
2000	3.37	92	8	3	86	14	2.64	84	16
2500	3.31	90	10	2.87	83	17	2.61	83	17
3000	3.25	89	11	2.85	82	18	2.53	81	19

PAo1 F116: *P. aureginosa* strain PAo1 lysogenic with phage F116.
MAM2 F116: *P. aureginosa* strain MAM2 lysogenic with phage F116.
HD1: *Bacillus thuringensis subsp. var. kurstaki* (HD1)

Table 3: Effect of lambda-cyhalothrin on prophage F116 induction from different *P. aureginosa* lysogens at 24h

	PAo1 F116				MAM2 F116			
Con. µg/ml	**Pfu/ml x 10^{12}**	**F.I**	**I.P**	**M.I**	**Pfu/ml x 10^{12}**	**F.I**	**I.P**	**M.I**
0	0.42	1	0	-	0. 42	1	0	-
500	1.2	2.8	0.78	-	1.14	2.7	0.72	-
1000	1.23	2.9	0.81	-	1.2	2.8	0.78	-
1500	1.42	3.3	1	+	1.54	3.6	1.12	+
2000	1.76	4.2	1.43	+	1.98	4.7	1.56	+
2500	2	4.7	1.58	+	2.11	5	1.69	+
3000	2.14	5	1.72	+	2.21	5.2	1.79	+

PAo1 F116: *P. aureginosa* strain PAo1 lysogenic with phage F116.
MAM2 F116: *P. aureginosa* strain MAM2 lysogenic with phage F116.
F.I. Fold increase I.P. Induced phage M.I. Mutagenic Index
- = Fold increase < 3 + = Fold increase > 3

Table 4: Effect of Gesaprim on prophage F116 induction from different *P. aureginosa* at 24h

Con. µg/ml	PAo1 F116				MAM2 F116			
	Pfu/ml x 10^{12}	F.I	I.P	M.I	Pfu/ml x 10^{12}	F.I	I.P	M.I
0	0.39	1	0	-	0. 39	1	0	-
500	1.14	2.9	0.75	-	1.22	3.1	0.83	+
1000	1.64	4.2	1.25	+	1.38	3.5	0.99	+
1500	1.84	4.7	1.45	+	1.56	4	1.17	+
2000	2.1	5.7	1.71	+	2.32	5.9	1.93	+
2500	2.32	5.9	1.93	+	2.24	5.7	1.85	+
3000	2.41	6.1	2.02	+	2.22	5.6	1.83	+

PAo1 F116: *P. aureginosa* strain PAo1 lysogenic with phage F116.
MAM2 F116: *P. aureginosa* strain MAM2 lysogenic with phage F116.
F.I. Fold increase I.P. Induced phage M.I. Mutagenic Index
- = Fold increase < 3 + = Fold increase > 3

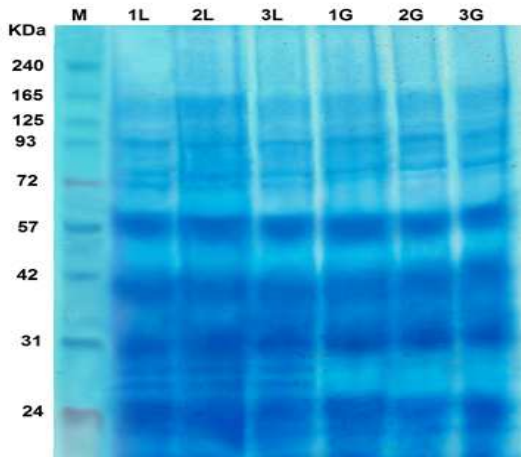

Fig. (1): SDS-polyacrylamide gel electrophoresis of protein in *P. aeruginosia* after treated with Lambda-cyhalothrin and Gesaprim.	**Fig. (2): SDS-polyacrylamide gel electrophoresis of protein in *P. aeruginosia* as a control.**
Lane M: PageRuler™ protein ladder, Lanes (1L:3L) represent *P. aeruginosia* treated with Lambda-cyhalothrin, with concentration 2000, 2500 and 3000 µm/ml. respectively, Lanes (1G:3G) represent *P. aeruginosia* treated with Gesaprim, with uses the same concentration.	**Lane M: PageRuler™ protein ladder, Lanes (C) represent *P. aeruginosia* as a control.**

Table 5: Analysis of molecular weight banding pattern after SDS- PAGE electrophoresis

No. of Band	M.W	Control	L1	L2	L3	G1	G2	G3
1	240	-	-	-	-	-	-	-
2	165	-	-	-	-	-	-	-
3	144	+	+	+	+	+	+	+
4	125	-	-	-	-	-	-	-
5	93	+	+	+	+	+	+	+
6	76	+	+	+	+	+	+	+
7	72	+	+	+	+	-	-	-
8	57	+	+	+	+	+	+	+
9	42	+	+	+	+	+	+	+
10	36	+	-	-	-	-	-	-
11	31	+	+	+	+	+	+	+
12	27	-	+	+	+	-	-	-
13	25	-	+	+	+	-	-	-
14	24	+	+	+	+	+	+	+
15	20	-	+	+	+	+	+	+

(1L:3L) represent *P. aeruginosia* treated with Lambda-cyhalothrin, with concentration 2000, 2500 and 3000 µm/ml. respectively, (1G:3G) represent *P. aeruginosia* treated with Gesaprim, with uses the same concentration.

Table 6: Conjugation in *Pseudomonas aeruginosa*

Pesticides	Donor	Recipient	Selective marker	No. of Transconjugants	Conjugation frequency
Lambda	MAM2 (str^r) $7.8 \pm 0.2 X10^6$	PAO1 (amp^r) $7.1 \pm 0.9 X10^6$	(str^r)	$8.3 \pm 0.2 X 10^3$	$1.16 X 10^{-3}$
	PAO1 (amp^r) $9.4 \pm 0.4 X10^6$	MAM2 (str^r) $7.5 \pm 0.6 X10^6$	(amp^r)	$7.1 \pm 0.3 X 10^3$	$9.4 X 10^{-4}$
Gesaprim	MAM2 (str^r) $8.2 \pm 0.4 X10^6$	PAO1 (amp^r) $6.9 \pm 0.8 X10^6$	(str^r)	$7.2 \pm 0.3 X 10^3$	$1.04 X 10^{-3}$
	PAO1 (amp^r) $8.7 \pm 0.5 X10^6$	MAM2 (str^r) $8.3 \pm 0.7 X10^6$	(amp^r)	$7.5 \pm 0.3 X 10^3$	$9.0 X 10^{-4}$

ampr : ampcillin resistance gene . strr : streptomycin resistance gene

Table 7. Lateral gene transfer by transduction.

Pesticides	Phage	Donor	Recipient counts After24 h.	No. of Transductants	Transduction frequency
			MAM2 (str^r)		
Lambda	F116	PA01 (amp^r)	$1.5 X10^6$	$3.6 X10^4$	$2.4 X10^{-2}$
			$2.1 X10^6$	$3.2 X10^4$	$1.5 X10^{-2}$
			$2.6 X10^6$	$4.1 X10^4$	$1.6 X10^{-2}$
			MAM2 (str^r)		
Gesaprim	F116	PA01 (amp^r)	$1.3 X10^6$	$2.9 X10^4$	$2.2 X10^{-2}$
			$1.9 X10^6$	$3.0 X10^4$	$1.5 X10^{-2}$
			$2.1 X10^6$	$3.8 X10^4$	$1.8 X10^{-2}$

Recipient counts at Zero time 5.1 X 1010

تأثير بعض المبيدات على نقل الجينات الأفقي في البكتيريا وبعض الخصائص البكتيرية

رحاب عيداروس محمد السيد سالم[1]، محمد ممدوح عبدالتواب الأشطوخى[2]

1. قسم وقايه النبات- كليه الزراعه- جامعه الزقازيق. 2- قسم الوراثه- كليه الزراعه- جامعه الزقازيق

الملخص

مبيدات الحشائش والمبيدات الحشرية تستخدم على نطاق واسع وبشكل مكثف في الزراعة في جميع أنحاء العالم لتعزيز إنتاجية المحصول. ولكن العديد من هذه المبيدات تسبب مشاكل بيئية خطيرة. بالإضافة إلى بعض الآثار الضاره على المحاصيل الزراعية والتربه. لذا تهدف الدراسه الى تحديد الآثار المترتبة على اثنين من المبيدات التي تنتمي إلى مجموعات مختلفة من المواد الكيميائية، وهي الجوسبريم Gesparim (مبيدحشائش) ولمبادا Lambda (مبيد حشري) بالجرعات الموصى بها على نوعين من البكتيريا، وتمت دراسة تأثير المبيدات على مجموع البكتيريا القادره على البقاء والتأثير الطفرى للمبيد ونقل الجينات بين البكتيريا في التجارب المعملية. كما أن اختيار المبيدات استند إلى نمط استخدام مكافحة الآفات في مصر.

أظهرت النتائج أن إجمالي نسبة البقاء لعدد البكتيريا الحيه انخفض مقارنة مع الكنترول مع مبيد الحشائش الجوسبريم. عند تركيز 3000 ميكروجرام / مل كانت نسبة البقاء فعالة إلى حد كبير ووصلت الى 81-89٪. فى حين كانت الصورة المقابلة مختلفة مع مبيد الحشرات لمبادا حيث كانت عدد البكتيريا الحيه طبيعي مقارنة مع الكنترول. عند تركيز 3000 ميكروجرام / مل كانت نسبة البقاء بين 91-97٪ أى أن تأثير جرعات كل مبيد مناسبه لنمو البكتريا الى حد ما. انتاج فاجات F116 من بكتريا *P. aureginosa* أوضحت أن المبيد جوسبيريم كان له نشاط طفرى عند تركيز من 500-3000 ميكروجرام / مل وهذا أعلى بالمقارنة مع المبادا عند تركيز من 1500-3000 ميكروجرام / مل. الأكثر من ذلك تحليل حزم البروتين SDS-PAGE لسلالة PAo1 لبكتريا *P. aeruginosia* بعد المعامله بأعلى ثلاثة تركيزات من كل مبيد 2000و 2500 و 3000 ميكروجرام / مل مقارنة بالكنترول أظهرت الاختلافات في أنماط الحزم والتي كانت أعلى مع الجوسبريم ايضا. كذلك أمكن نقل الجينات من خلال الاقتران Conjugation. حيث تراوحت الـ Conjugation frequencies من $10x1.16^{-3}$ الى $10x9.4^{-4}$ ومن $10x1.04^{-3}$ الى $10x9.0^{-4}$ لكل من المبادا و الجوسبريم على التوالي.