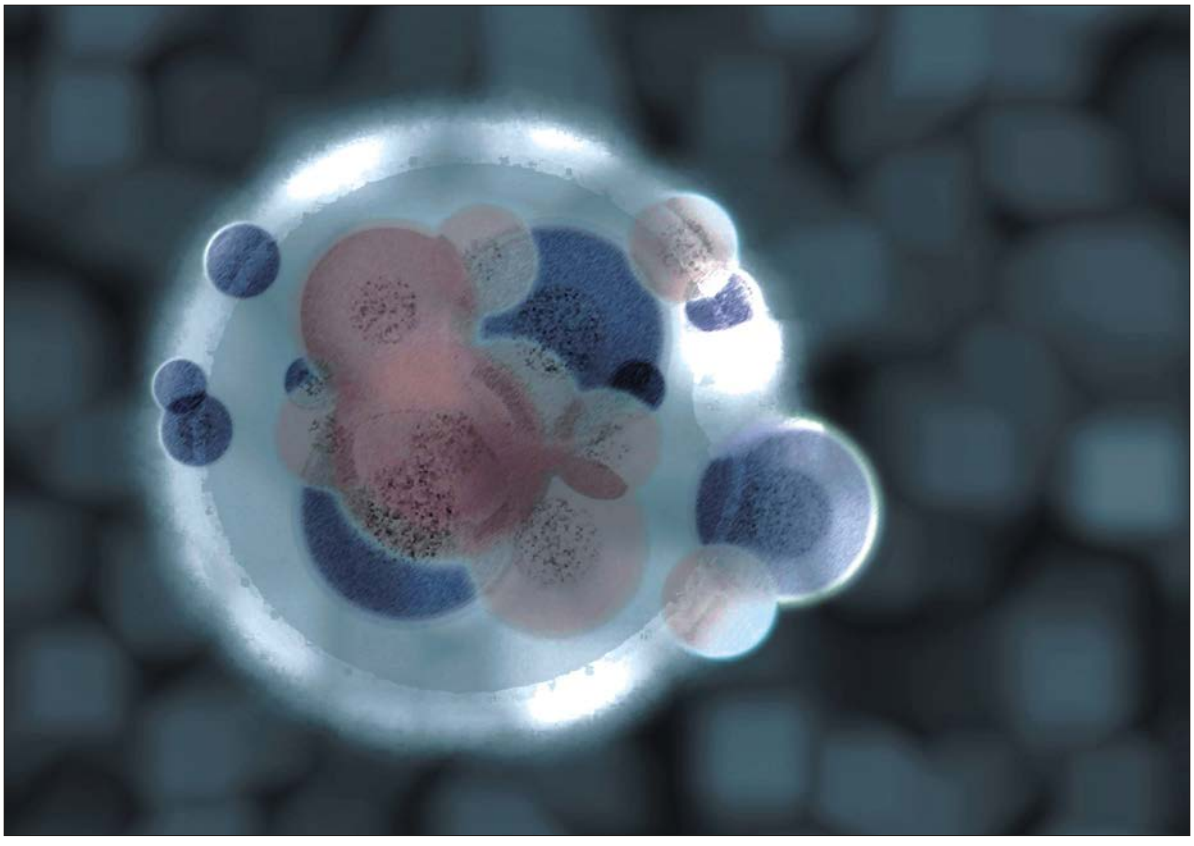

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Ability of Insecticidal Formulations to Support Growth of Bacteria and the Absence of Their Mutagenic Activity in the Ames Salmonella Test

Ismail Saadoun, Saleh Taye, Ahmed Elbetieha and Wajih M. Owais
Department of Applied Biology, Jordan University of Science and Technology,
Irbid-22110, Jordan

Abstract: The mutagenic activities of two insecticides [Vapcocidine-20 FL (Fenvalerate) and Cypermethrin-10 FL (Cypermethrin)] that are commonly used in Jordan were tested by Ames test. None of the insecticides was mutagenic to *Salmonella typhimurium* his⁻ tester strain TA 1530 and TA 1537. Treatment of both tester strains with these insecticides indicates the presence of toxicity and absence of mutagenicity. The potential of these insecticides to support growth of six different bacterial species was tested by following their growth for 10 days as evaluated by measuring the Optical Density (OD) at 540 nm. Data indicated that the two insecticides supported the growth of all isolates with turbidity reading ranged between 0.01 and 0.4 during 10 days incubation. *Staphylcoccus cohnii* showed a percentage increase of turbidity of more than 100% after two days of incubation. However *Acinetobacter lowffi* exhibited weak growth on Cypermethrin-10% and almost no growth on Vapcocidin 20.

Key words: Ames test, growth, insecticides, *Salmonella typhimurium*

INTRODUCTION

In recent years, there has been growing scientific and public awareness of potential carcinogenic and mutagenic hazards associated with the introduction of thousands of new chemicals into the environment including pesticides and herbicides that have been widely used in the world for a long time. Insecticides are the most numerous and most valuable pesticides. Clinical, occupational or environmental exposures to these agents cause serious health risks to mammals including mutagenicity, carcinogenicity, teratogenicity (Mccann and Ames, 1977; Berteau *et al*., 1989; Book *et al*., 1991; Prendergast *et al*., 1989; Russel *et al*., 1987) and effects on fertility (Potashnik and Inbar 1987) which become potential hazards to human health. Investigation of the safety of the pesticides and herbicides and their related compounds should be done systematically by sensitive and reliable tests for detecting their harmful effects. Ames test (Mccann and Ames, 1977) is probably the most widely used method.

Hundreds of chemicals have been tested with a high degree of correlation between mutagenicity and results of animal bioassays (Ames *et al*., 1975). Pesticides such as DBCP (L,2 - dibromochloropropane), a nematocide and 2,3 - dibromo-proposal have been shown to include oligo- or even azoospermia in factory workers exposed to the product (Potashnik and Inbar 1987). Several reports mentioned infertility with oligo-and azoospermia in workers exposed to kepones (Cannon *et al*., 1978). Exposure to methylisocyamide causes an increase in spontaneous abortion.

These novel compounds are continually encountered as natural habitat pollutants. The metabolic diversity and adaptability of microorganisms are exploited to restore the contaminated environments by degradation or transformation of wide range of organic (i.e., insecticides) and inorganic contaminants (Kalyuzhnyi, 2000). Of these microorganisms are the bacteria which are considered to represent the predominant agents of hydrocarbon degraders in the environment (Roling *et al*., 2002).

In the present research, the ability of two different insecticides [Vapcocidine-20 FL (Fenvalerate) and Cypermethrin-10 FL (Cypermethrin)] (Table 1) that are commonly used in Jordan to interact with DNA of *Salmonella typhimurium* and thus its potential to be mutagenic was tested. Also, the ability of these insecticides cypermethrin-10% and Vapcocidin 20 to support the growth of six different bacterial species was evaluated.

Corresponding Author: Ismail Saadoun, Department of Applied Biological Sciences, Jordan University of Science and Technology, P.O. Box 3030, Irbid- 22110, Jordan Tel: 962-2-7201000 Fax: 962-2-7095014

Table 1: Different insecticides, their composition, properties and uses to control pests (Agricultural Products Manufacturing Company (VAPCO)/Amman-Jordan)

Insecticide/Trade name	Cypermethrin-10 FL	Vapcocidine-20 FL
Common name	Cypermethrin	Fenvalerate
Trade name	Cypermethrin-10%	Vapcocidin 20
Chemical Formula	$C_{22}H_{19}C_{l2}NO_3$	$C_{25}H_{22}ClNO_3$
Composition/L	Cypermethrin 100 g (RS)-Alpha-cyano-3-phenoxybenzyl (1RS)-*cis, trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate (IUPAC)	Fenvalerate 200 g (RS)-Alpha-Cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methyl-butyrate (IUPAC)
Used to control	Army worm, Boll worm, Catterpillars, White fly, Cabbage moth, Codling moth, Grape berry moth	Boll worms, Pink boll worms, Bud and leaf worms, Cut worms, Thrips, White fly, Beetles, Leaf miner, Leaf worms, Army worm, Boll worm, Tuber moth, Fruit moth, Codling moth, Grape berry moth, Aphids, Borer, Weevils.
Toxicity	Acute Oral: LD_{50}-247 mg kg^{-1} (Male Rat) Acute Oral: LD_{50}-309 mg kg^{-1} (Female Rat) Acute dermal: LD_{50}->2460 mg kg^{-1} (Rabbit)	Acute Oral: LD_{50}-451 mg kg^{-1} (Rat) Acute dermal: LD_{50}-2500 mg kg^{-1} (Rabbit)
Ecotoxicity	Low toxicity to birds and fish Avian dietary LC_{50} - Mallard Duck and Bobwhite Quail: LC_{50} > 20,000 ppm	Toxic, fish and bees, and low toxicity to birds.96-hour LC_{50}'s 0.64 ppb (bluegill fish), 0.81 ppb (Channel catfish) and 6.2 ppb (rainbow trout)
Boiling point	170-1495°C	ND
Formulation	Emulsifiable concentrate	Emulsifiable concentrate

MATERIALS AND METHODS

Chemicals: Table 1 shows two different insecticides that have been provided by Veterinary and Agricultural Products Manufacturing Company (VAPCO)/Amman-Jordan. Different solution concentrations of the insecticides for mutagenicity test were sterilized by filtration through 0.45 unit membranes (Millipore Corp. MA, USA). Distilled water (solvent) was used as the negative control.

Bacterial strains: *Salmonella typhimurium* his^- strains TA 1530 and TA 1537 were obtained from Dr. Bruce Ames [Department of Biochemistry, University of California, Berkeley-94720, CA (USA)].

Mutagenesis assay: Reversion tests to his^+ were performed sing the plate test as described by Ames *et al.* (1975). Different concentrations (ppb) of the tested samples were added along with 0.1 mL of *S. typhimuriun* TA 1530 and TA 1537 strains to 2.5 mL top agar containing 0.6% NaCl, 0.05 mM D-biotin and 0.05 mM L-histidine. The mixture was poured onto Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) and incubated at 37°C for 48 h. Control plates containing only the bacterial suspension were used to account for spontaneous reversion (negative control). The activity of the two insecticides was evaluated using the positive control sodium azide (10^{-4} M). All tests were done in triplicate.

Collection of soil samples: Collection and processing of soil samples was described by Saadoun (2002).

Isolation of bacteria: Sub samples of 1 g were suspended in 100 mL of sterile distilled water, agitated in a shaker incubator (120 rev for 45 min), serially diluted to 10^{-6}, then a volume of 0.1 mL was spread over the surface of nutrient agar (NA) plates, incubated at 28°C for 48 h.

Characterization and identification of bacteria: The morphological features such as colour, size, form, margin and elevation of each colony were determined. Gram stain test was performed for each isolate. The bacterial stains were identified on the basis of Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994).

Biochemical tests: The following biochemical tests were used in the identification of bacterial isolates: gelatin liquefaction; citrate utilization; oxidase; catalase; indole formation; glucose fermentation, nitrate reduction and triple sugar iron test (Cappuccino and Sherman, 1996).

Growth on insecticides: Colonies of the different bacterial isolates were transferred to Erlenmeyer flasks containing 50 mL of nutrient broth then incubated at 28°C for 3 days with shaking at 120 rev min^{-1}. After incubation the broth was centrifuged (10000 rpm for 15 min), the supernatant was discarded and the pellet was collected and washed for 3 times under aseptic conditions. The final washed pellet was suspended in 5 mL of sterile normal saline. One milliliter of each bacterial suspension was then transferred to two Erlenmeyer flasks, each containing mineral salts (MS) broth (Per liter: $FeSO_4$ 1 mg, $MgSO_4.7H_2O$ 200 mg, Na_2HPO_4 210 mg, NaH_2PO_4 90 mg, $CuSO_4.5H_2O$ 5 μg, H_3Bo_3 10 μg, $MnSO_4.5H_2O$ 10 μg,

Table 2: His$^+$ revertants/plate induced by various concentrations (ppb) of insecticides using TA 1530 and TA 1537 strains

	Concentration (ppb)					
Insecticide	0.2	1	10	100	1000	1500
TA 1530						
Vapcocidine-20	9.00±1.00	7.66±0.57	6.00±1.00	7.00±0.00	4.33±0.57	4.33±0.57
Cypermethrin 10%	17.66±0.57	8.00±1.00	8.00±2.00	7.00±.00	6.00±0.00	6.33±0.57
TA 1537						
Vapcocidine-20	16.00±0.57	16.66±1.52	12.33±0.57	12.66±0.57	8.00±1.00	8.66±0.57
Cypermethrin 10%	51.00±1.00	24.00±1.00	26.33±0.57	19.00±3.00	17.33±0.57	16.66±0.57

*Data represent means of 3 plates Positive controls were performed using 10^{-4} M Sodium Azide, number of revertants was too numerous to count (TNTC). Negative control showed 22±0.57 and 27±1.00 revertants for TA 1530 and TA 1537 strains, respectively

$ZnSO_4.7H_2O$ 70 μg, MoO_3 10 μg, $CoSO_4$ 10 μg, KCl 40 mg, $CaCl_2$ 15 mg, NH_4Cl 500 mg and $NaNO_3$ 2 mg) (Leadbetter and Foster 1958) supplemented with 0.1% of either Cypermethrin-10% or Vapcocidin 20 (sterilized by filtration). Flasks were incubated in a shaker incubator (120 rev min^{-1} and 28°C) and the OD measurements were carried out at 2 days interval for 10 days in a plastic cuvette and using UV-visible spectrphotometer (Jenway 6405) at 540 nm. All of the OD measurements were carried out against a negative control that was used as a blank flask with no inoculum for each insecticide.

Growth on benzoic acid: To test if the recovered bacteria can grow on other chemicals other than the tested insecticides, colonies of the different bacterial isolates were transferred to a MS agar plates supplemented with 0.1% (w/v) benzoic acid. Plates were incubated at 28°C for 3 days. Growth response of the different bacterial isolates on benzoic acid was arbitrary scaled as weak (+), moderate (++) and strong (+++).

RESULTS AND DISCUSSION

In the last few decades the agricultural technology aiming to enhance the quantity and quality of the agricultural products has been dramatically going forward and a huge number of synthetic chemical compounds or modified natural compounds has been in use to serve the purpose of that technology. But some of these compounds may accumulate in the environment as pollutants that have toxic effects on the living organisms.

In this research, four insecticides were evaluated to interact with DNA of *Salmonella typhimurium* by testing their mutagenic activity in the Ames Salmonella test. Also, the ability of two insecticides (Cypermethrin-10% and Vapcocidin 20) to support the growth of six different bacterial species were tested, knowing that microorganisms, which biodegrade the various components of hydrocarbons such as aromatic hydrocarbons, including naphthalene, monoaromatic hydrocarbons, or aliphatic hydrocarbons such as the n-alkanes, are readily isolated from the environment, particularly from petroleum-contaminated sites.

Ames test: The mutagenicity of two pesticides [Vapcocidine-20 FL (Fenvalerate) and Cypermethrin-10 FL (Cypermethrin)] (Table 1) was tested on tester strains of *Salmonella typhimurium* TA 1530 and TA 1537 (Table 2). Six concentrations of each insecticide ranging between 0.2 ppb and 1500 ppb were tested. None of the concentrations of each insecticide with the tester strain TA 1530 showed a significant his$^+$ revertants above the background level. However, the activity of the different concentrations of these insecticides on the tester strain TA 1537 in some cases showed a statistical slight increase in his$^+$ revertants above the background level. Treated tester strains (TA1530 and TA1537) with 10^{-4} M azide exhibited a strong mutagenic activity, which is too many times, more than that of the negative control. Treatment of both tester strains TA1530 and TA1537 with these insecticides indicates the presence of toxicity and absence of mutagenicity. However, azide is highly mutagenic in the same tester strains under the same conditions.

Identification of bacterial isolates: Bacterial isolates being recovered on nutrient agar (NA) plates were subjected to phenotypic and biochemical identification. The results of these tests revealed the presence of the following bacterial species: *Pseudomonas putida*, *P. maltophilia*, *Pseudomonas mallaie*, *Acinetobacter lowffi*, *Arthrobacter oxydans and Staphylcoccus cohnii* (Table 3).

Potential of Cypermethrin-10% and Vapcocidin 20 to support the growth of the recovered bacteria: The different bacterial isolates were tested for their ability to grow on Cypermethrin-10% and Vapcocidin 20. The OD experiment shows the ability of most of the isolates to use Cypermethrin-10% (Fig. 1) and Vapcocidin 20 (Fig. 2) as the sole source of carbon. The growth on Cypermethrin-10% was in the order: *Staphylcoccus cohnii*, *Pseudomonas maltophilia*, *Arthrobacter oxydans*, *Pseudomonas mallaie* and *Pseudomonas putida*. However, the growth on Vapcocidin 20 was in the order: *Pseudomonas maltophilia*, *Arthrobacter oxydans*,

Table 3: Morphological and physiological properties of the different bacterial isolates and their potential to grow on benzoic acid

	Biochemical and cultural criteria*									
Isolate	Gram Reaction	Shape	Oxidase	Citrate	MR/VP	TSI	Nitrate Reduction	Motility	Bacterial Species	Growth on benzoic acid
A1-4a	-	Bacilli	-	-	-/-	K/Noc**	+	+	*Pseudomonas maltophilia*	++
A1-4c	-	Bacilli	+	-	-/-	K/Noc	+	+	*Pseudomonas mallaie*	++
A1-5a	+	Bacilli	+	-	+/-	Noc/Noc	+	+	*Arthrobacter oxydans*	++
A1-5b	-	Bacilli	+	+	-/-	K/Noc	-	+	*Pseudomonas putida*	++
A3-4a	-	Bacilli	-	-	-/-	A/A	-	-	*Acinetobacter lowffi*	+
B2-4a	+	Cocci	-	-	-/-	Noc/Noc	-	-	*Staphylococcus cohnii*	++

*All isolates were catalase positive, indole and gelatin negative, **Noc. No change

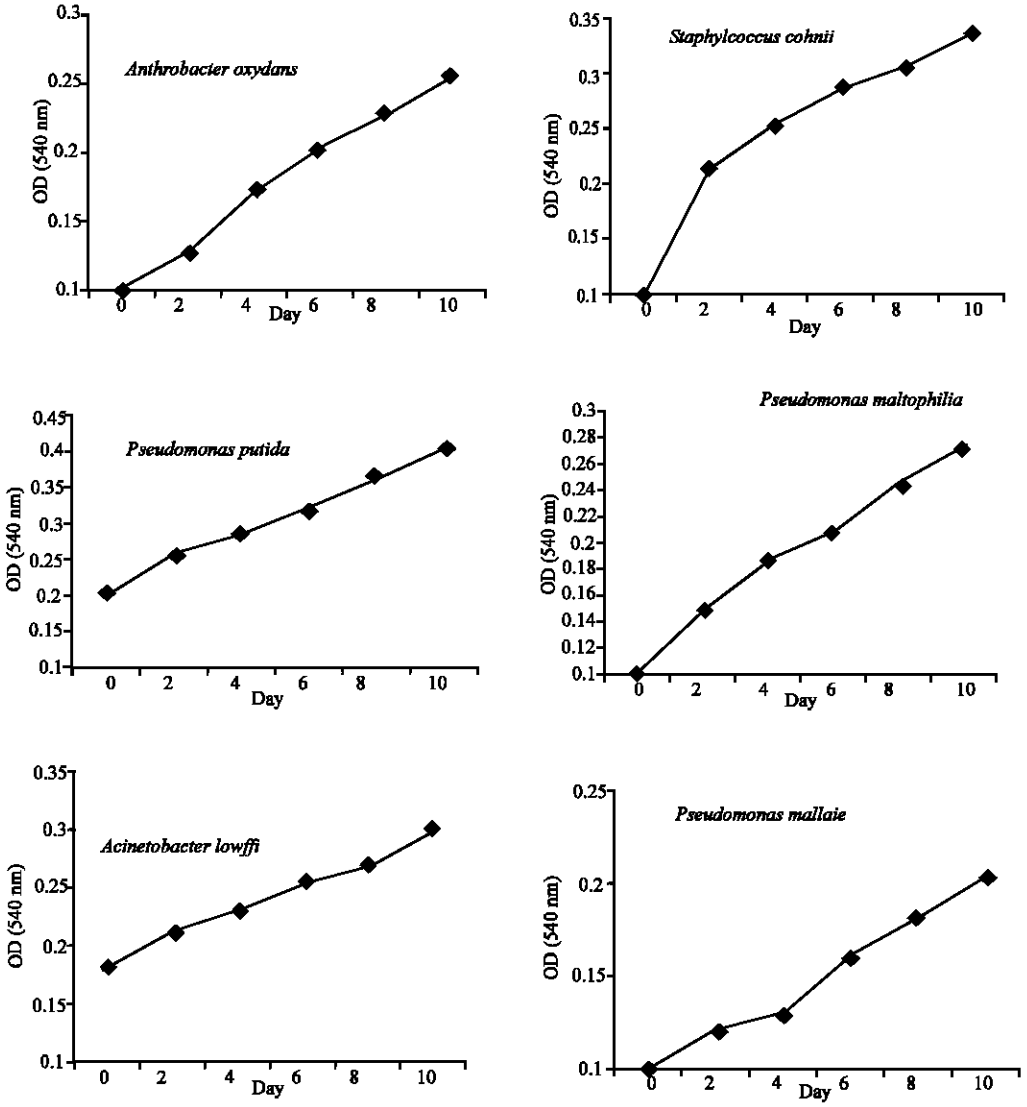

Fig. 1: The OD measurement at 540 nm for the six bacterial isolates grown on Cypermethrin-10% for 10 days

Staphylcoccus cohnii, *Pseudomonas mallaie* and *Pseudomonas putida*. *Acinetobacter lowffi* exhibited weak growth on both insecticides.

When the growth of bacterial isolates on both Cypermethrin-10% and Vapcocidin 20 was compared data revealed higher growth percentage on Cypermethrin-10% than on Vapcocidin. These results suggests that the insecticide Vapcocidin 20 may have higher toxicity than Cypermethrin-10% which led to the difference in the turbidity increase. Presence of toxicity and absence of

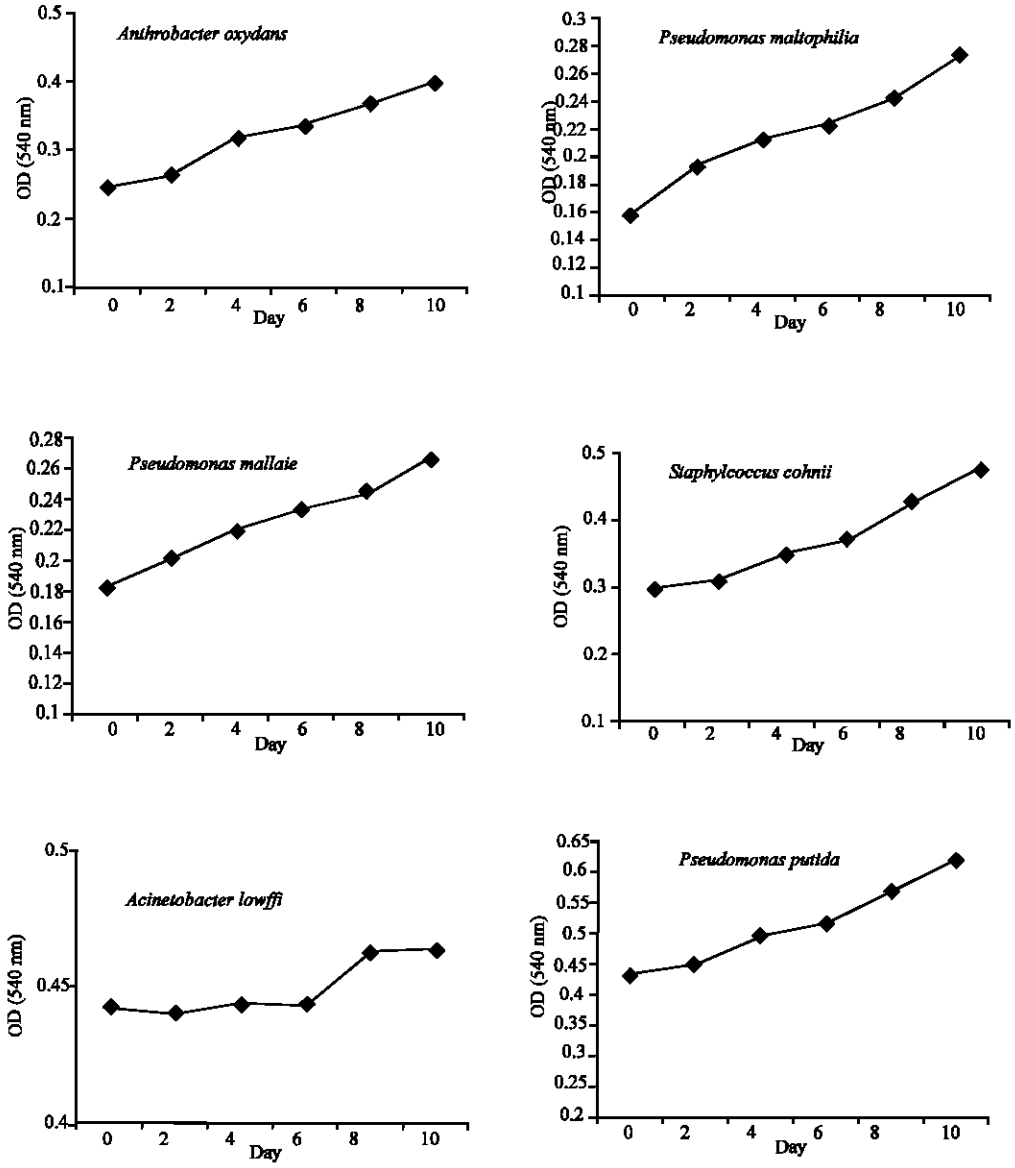

Fig. 2: The OD measurement at 540 nm for the six bacterial isolates grown on Vapcocidin 20 for 10 days

mutagenicity of Cypermethrin and Vapcocidin was indicated after treatment of the tester strains TA1530 and TA1537 with these insecticides.

It is known that the other components present in the insecticide formulation e.g. emulsifies such as polyethylene glycol, carboxymethyl cellulose or other detergents can sustain bacterial growth and the use of pure insecticides is more confirmative to prove that they have a potential to utilize such materials. However, the use of the commercial formulation in this study was intended to simulate the actual conditions that farmers are exposed to.

To answer the question whether the observed maximum growth of the different bacterial test strains was in fact due to the target substrate and not due to solvents or other ingredients present in the tested insecticides, other concentrations (0.01%, 0.025% and 0.05%) of both insecticides were tested and results revealed no indication to support the growth of the bacterial isolates as indicated by a constant OD readings during the incubation period (data not shown).

Growth on benzoic acid: All of the tested bacteria exhibited a moderate growth (++) except *Acenitobacter*

lowffi that exhibit a weak growth (+). None of the isolates exhibited a strong growth (+++) (Table 3). Growth experiments on benzoic acid was performed to verify the ability of the isolates to utilize the degradation intermediates (benzoic acid) of aromatic hydrocarbons as a sole carbon source.

Further research is encouraged to focus on the molecular aspects of degradation capability of these bacterial isolates and looking further on the degradation pathways of these compounds.

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REFERENCES

Ames, B.N., J. Mccann and E. Yamasaki, 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian - microsome mutagenicity test. Mutation Res., 31: 347 -364.

Berteau, P. E., J.B. Knaak, D.C. Mengle and J.B. Schrider, 1989. Insecticide absorbtion from indoor surfaces. American Chemical Society, Washington DC, ACS Symposium Series, 382: 315-26.

Book, S.A., R.J. Jackson and A.M. Fan, 1991. Health risk assessment of arial application of malathion bait. Berkeley, California, Department of Health Services.

Cannon, S.B., J.M. Veasey, R.S. Jackson, V.W. Burse, C. Hayes, W.E. Straub, P.J. Landrigan and J.A. Liddle, 1978. Epidemic kepone poisoning in chemical workers. Am. J. Epidemol., 107: 529-537.

Cappuccino, J. G. and N. Sherman, 1996. Microbiology: A Laboratory Manual. The Benjamin/Cunmings Publishing Company, Inc. New York, pp: 129-182.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Stanely and S.T. Williams, 1994 In: Bergey's Manual of Systematic Bacteriology Holt, J.G. and N. R. Krieg, (Eds.), Baltimore, Md.: Williams and Wilkins, 1, pp: 141-199.

Kalyuzhnyi, S.V. 2000. The tandem of biocatalytical and engineering development. Biocatalysis, l9: 15-21.

Leadbetter, E.R. and J.W. Foster, 1958. Studies of some methane utilizing bacteria. Arch. Microbiol., 30: 91-118.

Mccann, J. and B.N. Ames, 1977. The Salmonella/microsome Mutagenicity Test: Predictive Value for Animal Carcinogenicity. In: Origins of Human Cancer Hiatt, H.H., J.D. Watson and J.A. Winsten (Eds.), pp: 1431-1450.

Potashnik, G. and Y. Inbar, 1987. Dibromochloropane (DBPC): An 8 years reevaluation of testicular functions and reproductive performance. Fert. and Ster., 47: 31-327.

Prendergast, T., B. Pech, R.J. Jackson, T. Slagel, K.W. Kizer and D.O. Lyman, 1989. End+/rin poisoning associated with taquito ingestion. California Morbidity and Mortality Weekly Report, 38: 345-47.

Roling, W.F., M. G . Milner, D.M. Jones, K. Lee, F. Daniel, R.J. Swannell and M. Head, 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Applied environ. Microbiol., 68: 5537-5548.

Russel, H.H., R.J. Jackson, D.P. Spath and S.A. Book, 1987. Chemical contamination of California drinking water. Western J. Med., 147: 615-22.

Saadoun, I., 2002. Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel. J. Basic Microbiol., 42: 420-428.

Vogel, H.J. and D.M. Bonner, 1956. Acetylornithinase of *E. coli*. partial purification and some properties. J. Biol. Chem., 128: 97-106.