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Functional Redundancy Diversity of Gram Positive Bacteria as Response to Pesticide (Malathion) Exposure in Soil

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Pesticide (Malathion) has been used extensively in Saudi Arabia when there was outbreak of the Rift Valley fever in the south region in September 2000 for elimination of the host (mosquito) and it is still in use. We studied the effect of this pesticide on treated soil. This study started in 2009 in Riyadh Saudi Arabia. Samples were collected from different locations of Riyadh area. The amounts of CO_2 from soil microbial community were determined using compuflow 8650 and shown difference between the treated and untreated soil. The mean proportion of carbon dioxide of the treated was statistically and significantly lower than the untreated (3240.3 vs. 5492.6 ppm) respectively. Regression analysis revealed significant reduction of carbon dioxide equal to (b = -48.92 ppm /d) for the treated soil while for the untreated soil, the slope of regression line was stable and not significant (b = 0.37 ppm /d). BIOLOG GP2 micro plates were used to determine 95 different carbon sources substrate utilization patterns of microbial communities.

Keywords: Pesticide, Rift Valley fever, Mosquito, Soil, Microbial community.

Use of pesticide is growing day by day and thus enhances the environmental pollution and hazards. Bioremediation of pesticides is a relatively new technology, which is going through intense study as of recent decades (Adhikari, 2010). Microorganism play an extensive role in the decomposition of many organic matters and is in symbiotic relationship with plants and animals (Shair *et al*; 2010). Two bacteria identified as *Pseudomonas putida* and Acinetobacter-rhizosphaerae able to rapidly degrade the organophosphate (OP) fenamiphos (FEN) were isolated (Chanika *et al*; 2010). Efficiencies of local bacterial isolates in malathion degradation were investigated. Five bacterial isolates obtained from agricultural waste water were selected due to their ability to grow in minimal salt media, supplied with 250ppm malathion as sole source of carbon and phosphorus (Mohameda *et al*; 2010). Profenofos, a well-known organophosphate pesticide, has been in agricultural use and bioremediation of Soil contaminated with this compound was examined using soil treated with 200 µg/g profenofos, which resulted in a higher degradation rate than control soils without inoculation (Malghani *et al*; 2009).The external agricultural inputs such as mineral fertilisers, organic amendments, microbial inoculants, and pesticides are applied with the ultimate goal of maximizing productivity and economic returns, while side effects on soil

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organisms are often neglected. A summarized current understanding of how agricultural inputs affect the amounts, activity, and diversity of soil organisms was reported. Natural populations that are exposed to pesticides in their environment may at the same time be exposed to natural stressors like parasites and predators, which may interact with pesticide exposure. This may not only impact target pest species but also a wide variety of non-target species (Jansen *et al*; 2011). The use of organophosphate pesticides is an integral part of commercial farming activities and these substances have been implicated as a major source of environmental contamination and may impact on a range of non-target fauna. Oxidative stress (OS) has been reported as a possible mechanism of malathion toxicity in humans (Pamela *et al*; 2010). Biologic monitoring has been widely used to assess exposures, susceptibility, and effects of chlorpyrifos and malathion(Dana *et al*; 2006). The lack of technical support and an inadequate understanding of the potential risks that pesticides may pose to human health and the environment are the main causes of the indiscriminate application of pesticides, which is frequently accompanied by incorrect practices (Rico *et al*; 2011). Concerning over the effects of anthropogenic activities on soil quality has fuelled efforts to identify and measure those factors that affect soil quality. The metabolic diversity of microbial communities is fundamental for the multiple soil functions mediated by microorganisms. Community level physiological profiles (CLPPs) based on sole carbon source oxidation have been used as a fast and reproducible tool to study soil microbial functional diversity because the utilization of available carbon is the key factor governing microbial growth in soil.

MATERIALS AND METHODS

Phosphate Buffered Saline

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g
Distilled water	1000 ml
Malathion :	
Malathion 57%	3.89 ml
Phosphate Buffered Saline	1000 ml

Kit:

Biolog Gram Negative (GN2) 96-well microplates (Biolog Inc., Hayward, CA)

The kit contain 10 microplates ,each microplates contains (in dried form) a complex, low-concentration, buffered nutrient medium ,a tetrazolium redox dye, and a carbon substrate, The substrate blank well contains the nutrient medium and dye but no substrate, BiologGram Positive (GP2) 96-well micro plates (Biolog Inc., Hayward, CA).The kit contain 10 microplates, each microplates contains (in dried form) a complex, low-concentration, buffered nutrient medium ,a tetrazolium redox dye, and a carbon substrate, The substrate blank well contains the nutrient medium and dye but no substrate, SOS-ChromoTest Kit (EBPI, Canada).

Growth medium for the SOS-Chromotesttm bacteria Strain, The SOS-Chromotesttm freeze-dried bacteria, 10% DMSO in saline; the SOS-Chromotesttm diluents, Blue chromogen solution, Dried alkaline phosphatase substrate, Stop solution 96 well micro plates.

Malathion 57% was obtained from Anmar, Riyadh, Saudi Arabia, 3.89 ml of Malathion was dissolved in 1L of PBS. This concentration was used based on the concentration used to kill insects that written on the pesticide bottle. Soil samples were collected from different region in Riyadh area between February to March 2010. Samples were taken randomly from the top 5 cm of soil, rocks and remains of plants were removed mixed and saved at 4°C. The spraying process was carried out for 10 weeks for all the experiment.

Sample preparation

Soil samples were processed by mixing 100 grams of soil sample that has been sprayed in 200 ml of phosphate buffered saline (PBS) and keeping them in orbital shaker incubator for 4 hours at 37°C. After that, Cells were concentrated by centrifugation (10,000 µg, 10 min), and the cell pellets were suspended in 1 ml of PBS. The control was prepared without spray.

Micro plate protocols

Biolog GN2 (gram-negative) and GP2 (gram-positive) (BIOLOG Inc., Hayward, USA)) microliter plates were used to analyze functional diversity through the substrate utilization patterns shown by soil microorganisms. Biolog GN2 or GP2 micro plate wells were inoculated with 100 µl of the

soil suspensions. The plates were incubated at 25°C in the dark. Each well in a plate was measured for color absorbance at 595 nm every 24, 48, 72, 96 and 120h, read it with a microplate reader (Ascent software version 2.6). 4 replicates were performed for GN2 and GP2.

CO_2 production

For measuring carbon dioxide, 500g from soil was put in 3000 ml baker and cover with cilingfilm, incubated at 37°C and read emitted carbon dioxide every day by make small hole in the ciling film and enter compuflow 8650 meter in and repeat this process every day for 10 weeks with the spray of Malathion each day with 10 replicates. The control was not sprayed with Malathion.

RESULTS

Spraying soil with malathion (treated soil) resulted in significant reduction of carbon dioxide. The carbon dioxide of the treated soil was statistically significantly lower than that of the untreated soil (3240.3 vs. 5492.6 ppm). Regression analysis revealed significant reduction of carbon dioxide equal to (b = -48.92 ppm /d) for the treated soil (Fig. 2). While for the untreated soil, the slope of regression line was stable and not significant (b = 0.37 ppm /d) as shown in (Fig. 1). The results that are shown in (Fig. 3) illustrates results of consumed carbon of the control and treated wells (polymers) for gram positive bacteria by days at 120-h. Differences in consuming activities of carbon

Table 1. Means and SD of Malathion effect treatment on gram bacteria by Apr-48 h

Groups	Gram negative bacteria		Gram positive bacteria		Groups effect		
	Treated Means	Control	Treated	Control	N	Means	SD
1-Polymers	0.3344	0.4173	0.3209	0.2414	24	0.3206	0.2741
2-Carbohydrate	0.7406	0.2824	0.1136	0.1572	138	0.2880	0.3420
3-Miscellaneous	0.4555	0.3116	0.1231	0.1692	44	0.2541	0.2195
4-Carboxylic acid	0.6234	0.7524	0.2656	0.1740	76	0.4908	0.5304
5-Amines/amides	0.3781	0.6035	0.3726	0.3000	16	0.4329	0.3855
6-Amino acid	0.4178	0.6363	0.2338	0.3250	62	0.4552	0.4841
7-Phosphate compound	0.8643	0.1972	0.1001	0.1415	20	0.2438	0.2853
		Gram negative bacteria		Gram positive bacteria			
Bacteria effect	N	190		190			
	Means	0.5348		0.1806			
	SD	0.4754		0.2034			
		Treated		Control			
Treated effect	N	190		190			
	Means	0.3732		0.3423			
	SD	0.4076		0.4047			
ANOVA							
S.O.V.	df	Mean Squares	F-Value	P-value	Sig.		
Bacteria (B)	1	4.250	34.602	0.000	****		
Treated (T)	1	0.108	0.882	0.348	NS		
groups (G)	6	0.234	1.909	0.079	NS		
B * T	1	0.0942	0.767	0.382	NS		
B * G	6	0.178	1.449	0.195	NS		
T * G	6	0.297	2.417	0.027	*		
B * T * G	6	0.429	3.491	0.002	**		
Error	352	0.123					

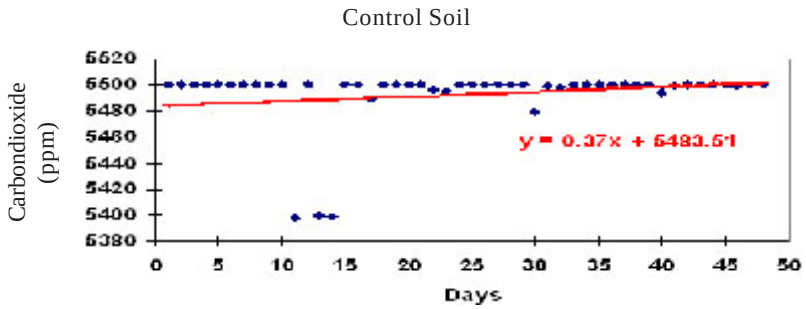

Fig. 1. Untreated soil with Malathion, the slope of regression line was stable and not significant (b=0.37 ppm /d)

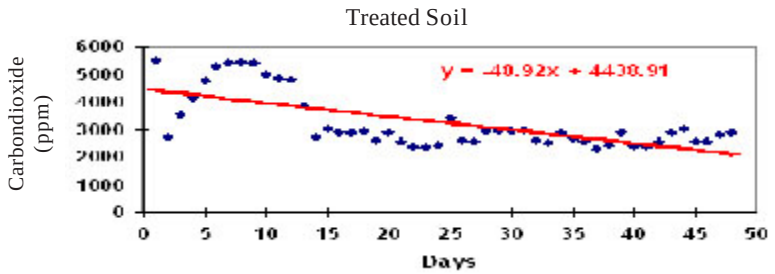

Fig. 2. Regression analysis revealed significant reduction of carbon dioxide equal to (b = 48.92 ppm /d) for the treated soil with Malathion

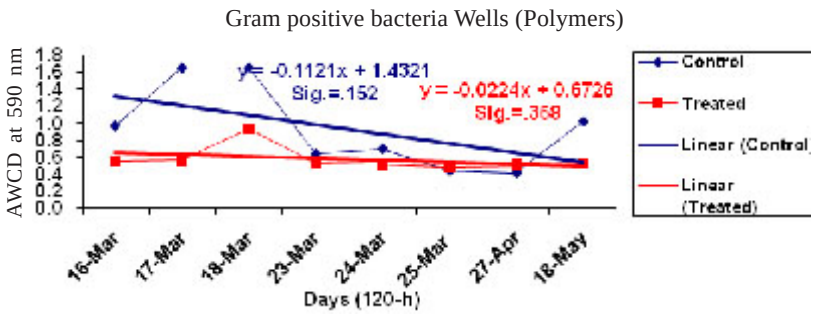

Fig. 3. Results of consumed carbon of control and treated wells (polymers) for gram positive bacteria by days at 120-h

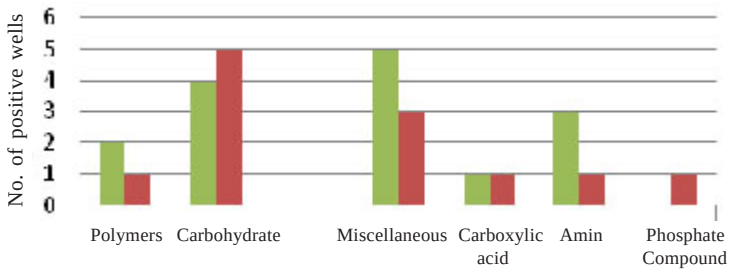

Fig. 4. Differences in activity of consuming the carbon sources between control and treated in 26 April

sources has been illustrate see (Fig. 4). Means and standard deviation (SD) of Malathion effect treatment on gram bacteria by Apr-48 h see (Table 2). For gram positive bacteria, there was significant reduction in carbon consummation over days within the experiment period consumed carbon of the control and treated wells. Consumed carbon of the treated well (carboxylic acid) was lower than that of control for all days of the experiment period. Results showed diversity between treated and untreated soils due to period of spray. Gram Negative bacteria were significantly less influenced by this organophosphate. Phosphate compound and miscellaneous wells were the most consumed carbon source. The carboxylic acid, amino acid, amines/amides and polymer wells were the least influenced.

DISCUSSION

A handful of soil may contain more than ten billion bacteria of thousands of different species, making soil the most complex ecosystem on earth. Most of these microbes are unknown, but novel approaches promise exciting progress in exploring their immense functional diversity. A study about screening bacteria from wet saline soil discovered one strain, designated as MHA, an obligate and moderate halophile, capable of hydrolyzing soluble starch and tributyrin and another strain, MHX, capable of hydrolyzing xylan by (Kondepudi *et al.*, 2011). Microorganisms especially in the species of *Acidithiobacillus ferrooxidans* are toxic metal toxicity resistant and reduce the toxicity of these minerals in nature (Mohseni, *et al.*, 2011). Community level physiological profiles (CLPPs) based on sole carbon source oxidation have been used as a fast and reproducible tool to study soil microbial functional diversity because the utilization of available carbon is the key factor governing microbial growth in soil. Although pesticides have been extensively used for controlling insects and disease pathogens of plants, little is known regarding the impacts of applying these pesticides on the microbial community and the dynamic biological system, and still is difficult to determine the composition of microbial communities in soil. We are also limited in the determination of microbial mediated reactions because present assays for determining the overall rate of entire metabolic processes as respiration or specific enzyme activities such as urease, protease and phosphomonoesterase activity that do not allow any identification of the microbial species directly involved in the measured processes.

Administered Malathion (100 or 200 mg/kg) orally once a day for four consecutive days to rat pups a significant decrease in the forelimb support latency and an inhibition of brain acetylcholinesterase (AChE) activity was reported and Repeated exposure of rat pups to the same doses of malathion caused a decrease in motor coordination, vestibular function and muscular strength/coordination and the investigator (Acker *et al*., 2011) has concluded that the brain activity of AChE is involved in the behavioral alterations caused by malathion in rat pups. The central problem posed by the link between microbial diversity and soil function is to understand the relations between diversity and community structure and between community structure and function.

The breakout of Refth Valley Fever in the southern region of Saudi Arabia in September 2000, needs an immediate act to limit and eradicate the hazard awaiting human and his live-stock resources, however an essential part of the hazard have been minimized through the use of insecticide (Malathon) and thousands of tons have been empted into the soil to kill the vector of the disease agent (mosquitos) and the process still going on till now. Its toxicity has been associated with the inhibition of acetylcholinesterase activity, leading to the interference with the transmission of nerve impulse, accumulation of acetylcholine at synaptic junctions, and subsequent induction of adverse health effects including headache, dizziness, nausea, vomiting, bradycardia.

The toxicity of organophosphates to a wide range of organisms necessitates the study of their degradation. A study designed to isolate an organophosphate-degrading bacterium and to detect the gene involved in the hydrolysis of organophosphates has been reported (Ali, 2011). A data reported by (Aboul-Soud *et al.*, in 2011) suggested that the administration of vitamin E (α-tocopherol) and selenium (Se) against Malathion may partially protect against malathion induced hepatic oxidative stress. Such pragmatic

acts may impose a highly divers response on the dynamic biological system leading to the collapse of soil function. Number of respond wells between the groups of carbon sources in the control and the treated in fifteen March, as noted in our investigation the highest response wells was Carbohydrate, Carboxylic acid and Amino acid while Miscellaneous, Polymers, Amin and Phosphate compound were the lowest. In16 March, it was observed that the highest value was carbohydrate while phosphate compound was the lowest. In 17 March, carbohydrate was (21:27) whereas phosphate compound was (2:3). Also in 18 March carbohydrate was 25 for control and 24 for treated and amino acid (19:16) and carboxylic acid (17:17) while the other group were low. The following days 21, 22, 23 March saw similar results where the carbohydrate was given the highest numbers compared to the rest of groups. In 24 March, carbohydrate, amino acid and carboxylic acid recorded high numbers while the control in phosphate did not give any response. 25 March, the groups with high numbers were carbohydrate, carboxylic acid and amino acid whereas polymers , miscellaneous, amin and phosphate compound were the lowest numbers. In 25 April also saw no respond to the control in phosphate compound while the treated for the same group was 3. day 26 April, carbohydrate was 26:16 , amino acid was 16:11, carboxylic acid 15:12, polymers 3:2, miscellaneous 7:6, amin 4:1 and phosphate compound 3:3. In 27 April we noticed a negative result for phosphate compound, Amin and Phosphate compound were the lowest. Soil microflora have been suggested as a potential candidate for the detoxification of pesticides (Community level physiological profiles (CLPPs) based on sole carbon source oxidation have been used as a fast and reproducible tool to study soil microbial functional diversity because the utilization of available carbon is the key factor governing microbial growth in soil. Although pesticides have been extensively used for controlling insects and disease pathogens of plants, little is known regarding the impacts of applying these pesticides on the microbial community and the dynamic biological system, and still is difficult to determine the composition of microbial communities in soil.

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