Original Article

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RESPONSE OF SOIL MICROORGANISMS TO MALATHION IN SAUDI ARABIA

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Abstract

Microorganisms play an extensive role in the decomposition of many organic matters and are in symbiotic relationship with plants and animals. In this study, concentration containing 57 mg/ml malathion was diluted and used to spray soil sample collected from Riyadh area, Saudi Arabia. Total chromosomal soil microorganisms were isolated from sprayed and non sprayed samples. Absence and presences of PCR band profiles were assumed to represent the absence or presence of bacteria. A maximum 7mg/ml and minimum 0.2mg/4.16 µl of malathion concentration was used in a nutrient agar medium for bacterial growth. The fingerprinting profiles of treated soil samples demonstrated the missing of some fingerprint bands which were present in the profiles of non treated soil samples. It was concluded that malathion had a misbalancing effect on the soil flora bacteria. However, smearing with maximum malathion concentration in nutrient agar supporting bacterial growth was observed. While a minimum concentration in the same nutrient agar was not supporting.

Keywords: Symbiotic, Decomposition, Plant, Redundancy, organic matters

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Introduction

Studies on malathion (organophosphate) insecticide that has been widely used for both domestic and commercial agricultural purposes have indicated that malathion has the potential to produce toxic effects in mammalian systems (1). Randomly amplified polymorphism DNA (RAPD) markers linked to beet necrotic yellow vein virus (BNYVV) resistance genes were used to identify two *Beta vulgaris* accessions (Holly-1-4 and WB42) using bulked segregate analysis. The polymorphism revealed by the RAPD markers in the F_2 generations of WB42 was higher than that of Holly-1-4 (2). More importantly, differences in susceptibility could be related to the frequency of the field treatments. Food safety has acquired great attention by food importer and exporters. Food rejection or acceptance across international borders is based on the compliance with international food regulations (3,4). Identification and characterization of *ace1*-type acetyl cholinesterase likely associated with organophosphate resistance in *Plutella xylostella* has been reported (5). PCR analysis of pinned specimens of Australasian *L. cuprina* collected before the release of organophosphate insecticides reveals no cases of the diazinon-resistance change but several cases of those associated with malathion resistance which explained the preexistence of mutant alleles encoding malathion consuming and thus, resistance (6, 7). PCR-RFLP was also used for estimation of ecological indices of soil microbes in soil samples Carbon isotope signatures generated by amino acid synthesis in plants, fungi, and bacteria suggested

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Fig. 1: Four Kg of soil samples were placed in each of these two black containers. The one at right was sprayed with 57% w/v malathion every day for two months, while the other at left was not.

that 13C fingerprints of amino acids could provide a powerful in situ assay of the biosynthetic sources of amino acids and a potential new tool for understanding nutritional linkages in food webs (8). Organophosphate insecticide (parathion/diazinon) resistance in housefly (*Musca domestica L.*) is associated with the change in carboxylesterase activity.

The recovery of acetyl cholinesterase (AChE) activity of a dominant crop field earthworm (*Drawida willsi*, *Michaelsen*) was investigated under laboratory conditions (8,9).

The effect of soil treatment with brominal (a herbicide) and the insecticide selecron (the equivalent field rates and five-fold) on population counts of bacteria, actinomycetes and celluloytic fungi in soil was tested and soil treatment with the two pesticides on acid phosphatase was promotive at field application rates after some incubation periods, but the enzyme activity was delayed at the higher application doses (10). Alkaline phosphatase activity in treated soil was accelerated with both pesticides even at the higher application rates, suggesting a direct role of alkaline soil pH in increasing resistance of alkaline phosphatase to Pesticides (11).The effect of soil treatment with pesticides on arylsulphatase activity fluctuated between promotion and inhibition, but inhibition was predominant (12,13). Microorganisms play an extensive role in the decomposition of many organic matters and are in symbiotic relationship with plants and animals. During the past few years, Gizan region, Saudi Arabia had an out break of rift valley virus, which curried by mosquitoes and consequently transmitted to human and animal, producing disease and death. Malathion pesticide was chosen to eliminate the vector (mosquitoes) and used as an Arial spray in continuous manner. More importantly, differences in susceptibility could be related to the frequency of the field treatments. This study focuses on the detection of the effect of malathion residues in Saudi soil, and on the flora microorganism. The residual malathion and its product may accumulate in the soil which may hinder the biological activities of certain organisms. However, the frequency of insecticide treatments has been increased in some areas of the kingdom of Saudi Arabia, because of problems with the control of vector (mosquitoes). We know very little about this pesticide in respect of its effect in relation to environmental condition, hazards-pollution, healthy and soil infertility. The present investigation will also recommend farmers to conserve and maintain the ecological balance of the Saudi Arabia environment, maintain the fertility of the soil, warn and advice governmental farm sectors to keep healthy environment and fertile soil away from the ill consequences due to pesticide misuse.

Materials and methods

Sterilized distilled water, 10 mer RAPD primers, master mixture, Malathion 57% w/v, Nutrient agar broth, soil, DNA template, PCR machine from (Company MWGAG Biotech, Model Primus 96 plus), plastic dish, incubator, autoclave, smearing loops, burner Bunsen, eppendorf centrifuge model 5415C, balance ADAM AFP-210 , 20µl pipette, 200µl pipette, 0.2ml PCR tubes, 1.5ml micro centrifuge tubes, Extraction buffer (pH 8.0) consisting: 50mM NaCl, 50mM Tris-HCl, pH 7.6, 50mM EDTA, 5% SDS autoclaved to sterilize), Phenol (pH 8.0), 24:1 chloroform: isoamyl alcohol, and 100% isopropanol (filtered to sterilized), RAPD analysis primer 1, 10 mer sequence 5·GGTGCGGGAA-3, 65% G+C content. Sample soils were collected from depth of 10cm near by a garden belonging to the King Saud University. In two small black containers of a holding capacity four kg each, were filled with four Kg of sample soils and stored at home temperature. One of the sample soils was sprayed with 57%

w/v malathion every day exactly; a period reaching of two months and the other was not. Having weighed 0.5 gram of the sprayed and non sprayed sample soil, 1ml of sterile distilled water was added to each of the samples. Mixed by shaking and overtaxing, followed by centrifugation at 800g/minute and supernatant were saved. Total chromosomal DNA was extracted from the supernatants of each sample, Sprayed and non sprayed. The extraction buffer consisted of: 50mM NaCl, 50mM Tris-Hcl, pH 7.6, 50mM EDTA, 5 % SDS, Phenol (pH 8.0), 24:1 chloroform: isoamyl alcohol, and 100% isopropanol (filtered to sterilized). But in our isolation method phenol and isoamyl purification step was not necessary so it was omitted. The PCR reaction consisted 12.5µl of master mixture (containing all the necessary reagents of PCR reaction except primer and DNA template), 2.5µl of primer and 1.5µg of DNA template isolated from each sample, sprayed and non sprayed. The reaction volume was completed to 25µl with sterile distilled water and the thermal cycler machine was programmed as: 95c for 2minnutes one cycle, (94c for 0.30 sec, 48c for 0.30sec, 72c for 0.30 sec, 35 cycles) and 72c for 10min. Broth agar nutrient medium was sterilized by autoclaving and cooled then poured in Petri dishes. The Petri dishes were dried and inoculated with 57% w/v malathion in different concentrations mg/ml: 0.57mg, 0.1425mg, 0.07125mg 0.04mg and were dried at 37ċ in an incubator. The smear consisting of the microbes freshly isolated from the non sprayed soil samples were smeared on the agar broth sucked with malathion in the Petri dishes and incubated at 37c for 28 hours

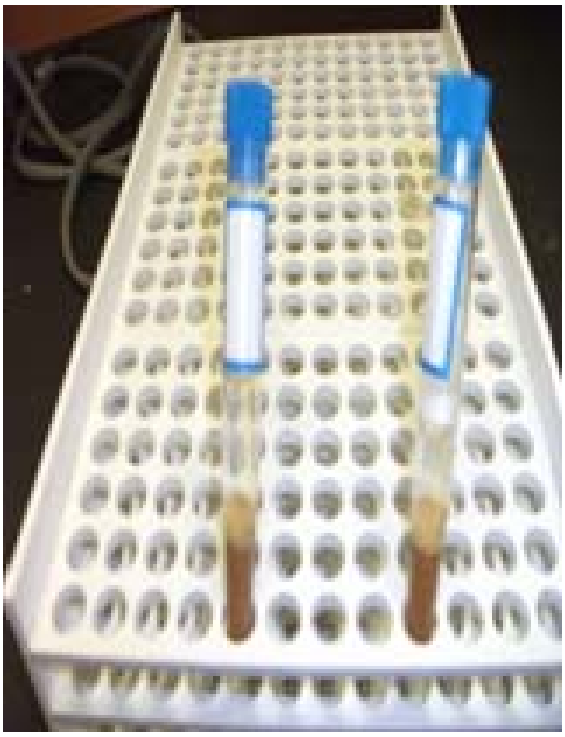

Fig.2: The supernatant containing the bacteria extracted from 0.5 gram of the sprayed and non sprayed sample soil, after added 1ml of sterile distilled water and mixed by shaking and vortexing, followed by centrifugation at 800g/minute.

Results

Samples were sprayed with malathion (Fig. 1). Bacteria were extracted from sample soil (Fig. 2). A very pure DNA was gained from both samples; sprayed and non sprayed (Fig. 3).The finger printing profiles of sprayed sample indicated the absence of band profile which was present in the non sprayed samples, thus indicating the absences of some bacteria (Fig. 4) and (Fig. 5). We have found a minimum concentration of malathion 0.04mg/ml which inhabited the growth of some bacteria while a maximum concentration, 0.57mg/ml supported. After smearing one ml of 57mg / ml malathion on 4 nutrient agar petri dishes 2 with out bacteria and 2 with bacterial inoculums, we observed an absence of bacterial growth on the 2 petri dishes above, proving that malathion was not contaminated with microorganism, while the others 2 bottom fully support the growth of bacteria (Fig. 6).

Discussion

It is necessary to investigate the level of soil microbial activities and its interaction with the pesticides and discover the period of stability and steady activity of the soil microorganism. This research highlights the existing problem of releasing massive quantities of extremely toxic chemicals in the environment. The residual malathion and its product accumulation may interfere with the biological activities of certain organisms. However, we know very little about this pesticide in respect of its effect in relation to environmental condition,

hazards- pollution, healthy and soil infertility. Thus, to achieve the goal of understanding, we comprehensively carried this investigation. Malathion is an organophosphate insecticide that has been widely used for both domestic and commercial agricultural purposes. However, it has the potential to produce toxic effects in mammalian systems. In a study of *Pseudomonas aeruginosa* AA112 which was isolated from soil using enrichment technique could utilize malathion as a sole carbon source and a source of energy. *Pseudomonas aeruginosa* AA112 was able to grow in MSMPY medium containing 42.75 mg/ml malathion. However, the optimum concentration of malathion which supported the maximum bacterial growth was found to be 22. 8 mg/ml. Malathion was used as an initial source of energy and carbon when it was found without additional carbon sources (in MSM medium) while it was utilized as second source of energy and carbon in a nutrient. The findings of this study are in agreement with the findings of other research which discovered that malathion had been used as source of energy and carbon (10).

Fig.3: Lines from 1 to 5 contain DNA extracted from treated and non treated soil samples. Total chromosomal DNA extracted from the supernatants of each sample, sprayed and non sprayed, using an extraction buffer consisting of: 50mM NaCl, 50mM Tris-Hcl, pH 7.6, 50mM EDTA, 55% SDS. With out Phenol pH 8.0, 24:1 chloroform: isoamyl alcohol.

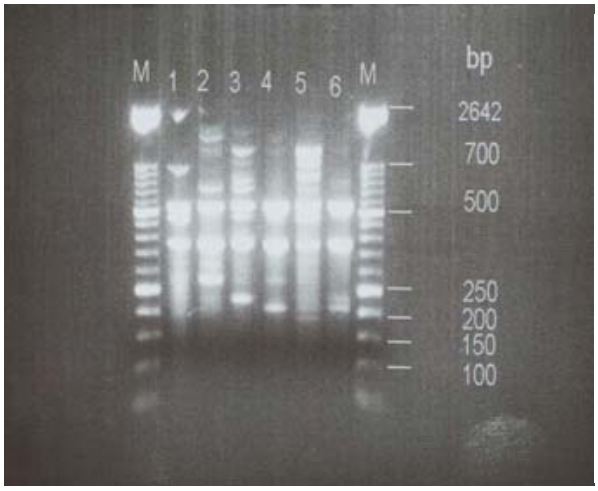

Fig.4: Lines from 1 to 6 contain DNA fingerprints of non treated soil samples. Line M left and line M right contain 2642bp DNA marker. The result of PCR reaction using DNA template from non sprayed sample soil, consisted 12.5µl of master mixture, containing all the necessary reagents of PCR reaction except primer and DNA template, 2.5µl of primer and 1.5µg of DNA template isolated from each sample, sprayed and non sprayed. The reaction volume was completed to 25µl with sterile distilled water and the thermal cycler machine was programmed as: 95c for 2min one cycle, 94c for 0.30 sec, 48c for 0.30sec, 72c for 0.30 sec, 35 cycles and 72c for 10min.

In our case, the result of high concentration of malathion that supported the growth of bacteria, there may be some bacteria that were using the high concentration as source of energy and carbon and thus, were surviving. While the results of low concentration inhibited, because the bacterial population in this concentration were not in position to consume malathion as carbon and energy source and thus, were inhibited. Malathion degradation pathway and the genes that attributed to the degradation of malathion are located on the chromosome and at least three proteins of high molecular weight that are involved in malathion utilization have been discovered. Further investigation of the bacteria that grew in high concentration in our case is needed. As this type of bacteria would be useful to metabolize malathion residues in the environment and thus, inactive it and for bioremediation of an environmental pollution.

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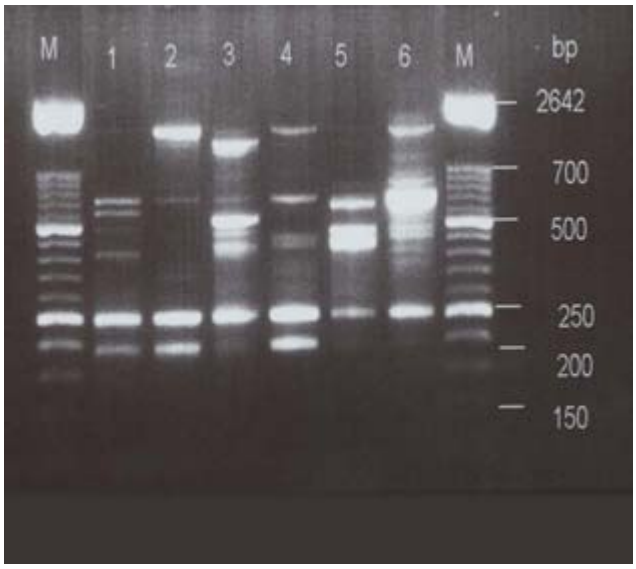

Fig.5: Lines from 1 to 6 contain DNA fingerprints of treated soil samples. Line M left and line M right contain 2642bp DNA marker. The result of PCR reaction using DNA template from sprayed sample soil, consisted 12.5µl of master mixture, containing all the necessary reagents of PCR reaction except primer and DNA template, 2.5µl of primer and 1.5µg of DNA template isolated from each sample, sprayed and non sprayed. The reaction volume was completed to 25µl with sterile distilled water and the thermal cycler machine was programmed as: 95c for 2min one cycle, 94c for 0.30 sec, 48c for 0.30sec, 72c for 0.30 sec, 35 cycles and 72c for 10min.

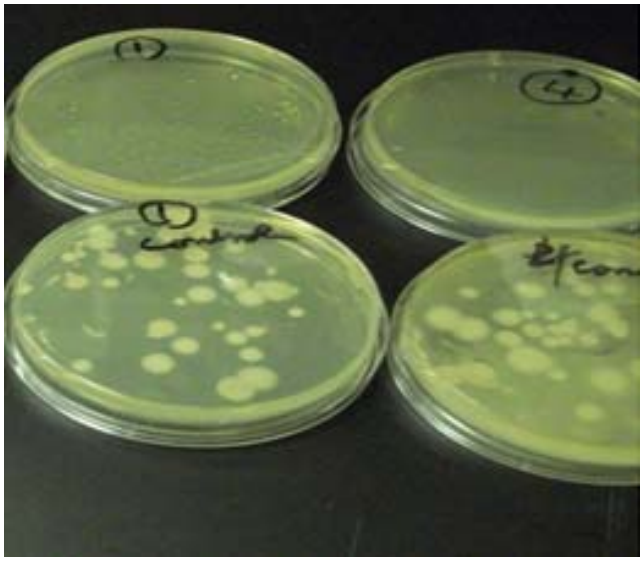

Fig.6: Petri dishes 1 and 4 above contain only malathion for the test of malathion possibility contamination. Petri dishes 1 and 4 bottom contain malathion and bacteria extracted from soil samples. Bacterial growth was observed on this bottom Petri dishes containing 0.57mg/ml malathion and bacteria from soil samples after incubation at 37ċ for 28 hours.

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