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Bioremediation of Water Contaminated with Fenitrothion and Butachlor using Microorganisms Isolated from Soil

Sherif H. Abd-Alrahman[1,2], Zidan H. Zidan[3], Mohamed I. Abdel-Megeed[3], Monir M. Almaz[2], Mounir M. Salem-Bekhit[4], Sobhy M. Yakout[1] and Mostafa A.A.[5]

[1]Department of Biochemistry, College of Science, King Saud University, PO Box, 2455, Riyadh, 11451, Saudi Arabia.
[2]Pesticides Residue and Environmental Pollution Dept., Central Agricultural Pesticide Laboratory, Agricultural Research Center, Giza 12618, Egypt.
[3]Department of Plant Protection, College of Agriculture, Ain Shams University, Shobra Elkheima, Egypt.
[4]Department of Pharmaceutics, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh, Saudi Arabia.
[5]Department of Botany and Microbiology, Collage of Science, King Saud University, P. O. 2455, Riyadh, 11451 Kingdom of Saudi Arabia.

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Fenitrothion and butachlor are widely used pesticides in pest control. These pesticides are released into the environment presenting a potential hazard risk. Only limited data are available on the microbial biodegradation of fenitrothion and butachlor. Biodegradation of butachlor by different microorganisms was investigated. Ten bacterial and fungal strains were isolated from an agricultural soil and found to actively utilized butachlor, as a sole source of carbon and energy. Based on their morphological and biochemical categorization, the ten bacterial and fungal isolates were identified as *Micrococcus rosus, Pseudomonas alcaligens, Aspergillus sp., Bacillus licheniformis, Bacillus megatherium, Trichoderma viride, Rhizopus sp., Rhizobium huakuii, Brady rhizobium sp. and E. coli.* Five strains out of these ten of microorganisms *Pseudomonas alcaligens, Bacillus megatherium, Trichoderma viride, Brady rhizobium sp. and E. coli.*, had different degradation rate of fenitrothion and butachlor, while the growth of other five strains were inhibited by two tested pesticides. Our results can conclude that both of the fungus strain *Trichoderma viride* as and bacterial strain *Pseudomonas sp.*, Showed the great degradation rate for both fenitrothion and butachlor that proved they can use for bioremediation of water contaminated with fenitrothion and butachlor the fenitrothion and butachlor residues.

Key words Bioremediation, Fenitrothion, Butachlor, Water contamination, Soil contamination Microorganisms.

The extensive use of pesticides in agriculture has led to their widespread release into environment. Environmental contamination with pesticide residues increasing the accumulation of these pesticides in environment resources and different food chain[1]. Fenitrothion and butachlor are model xenobiotic pollutants in environment for biodegradation studies, because it have been one of the most widely used in agriculture for pest control[2]. Fenitrothion and butachlor have been detected in different environmental sources in several monitoring programs[3-5].

Fenitrothion [O,O-dimethyl-(3-methyl-4-nitro phenyl) phosphorothioate, an organophosphorus pesticide] is mainly used against spruce bud worms and cotton pests. In

* To whom all correspondence should be addressed.
Tel.: +966 561776615;
E-mail: drsherif_hussein@yahoo.com

order to classify the mechanism of its selective toxicity has been investigated in mouse[6], rabbit[7], fish[8] and white rat[9].

Butachlor (N-butoxymethyl-2-chloro- 2,6-diethyl acetanilide) is one of the most widely recommended herbicides. It is a pre-emergence herbicide belonging to chloroacetanilide group used widely in oriental countries for the control of annual grasses for rice cultivation[10,11]. The recent study, reported that the application of butachlor has adverse environmental impact; butachlor was found to flow out with effluents, causing contamination of river and ground water[12], it has toxicity to aquatic organisms[13], and it has genotoxicity to the amphibian animals[14]. Also it could induce apoptosis in mammalian cells[15]. Applying butachlor in soil can cause toxicity to earthworm[16,17], change the microbial populations and enzyme activities[18], and sometimes adversely affects the growth and activities of beneficial microorganisms in soils[19].

Microbial degradation is an important process affecting the fate and behavior of pesticides. The bioremediation techniques have become a popular alternative to chemical or physical remediation because of their relatively low cost and minimal impact on the environment[20]. Techniques using microorganisms to degrade contaminates in polluted sites have been commercially available since the 1970s[21]. Nowadays, the use of microorganisms to biodegrade this kind of waste is almost imperceptible, as the most popular way of managing with them is thermal utilization[22]. Bacteria and fungi show the biggest capability of degrading pesticides. Bacteria, actinomycetales (special group of bacteria), and fungi show the biggest capability of degrading pesticides.

The aim of this study was determining the ability of different microorganisms isolated from soil to transform fenitrothion and butachlor to investigate biodegradation rate of to provide basic information for developing regulations regarding environmental contamination by fenitrothion and butachlor and their potential public health effects.

MATERIALS AND METHODS

The 127 soil samples were collected from the surface 10 cm layer at the agricultural ground of Kalubia, Egypt. Twenty-Five grams were collected for each soil sample in plastic bags and transported at once in cold storage containers to laboratory for further investigation. The mass soil was a little air-dried, thoroughly mixed, and then sieved through 0.20 mm mesh sieve for incubation experiment and chemical analysis, respectively. The soil homogenate was inoculated in 50-ml nutrient broth (Sigma, USA) and Sabouraud dextrose broth (Difco, USA). All purified microorganisms were tested for their abilities to grow in the presence of fenitrothion and butachlor individually in (nutrient agar medium): Beef extract 3.0gm, Peptone 5.0gm, Agar-agar 20.0 gm. and Distilled water to make 1 litter (H_2O) 1.0L at pH 7.2 [23]. The resulting colonies were repeatedly subculture in medium containing 10 ppm fenitrothion and butachlor to confirm their pesticides-catabolising ability. Inoculated plates were incubated at (30°C ±2) for 7days. The growth of microorganisms used for standing the toxicity of pesticide was determined and recorded as growth or inhibition. Identification and characterization of the isolates *Micrococcus rosus, Pseudomonas alcaligens*, *Aspergillus* sp., *Bacillus licheniformis*, *Bacillus megatherium*, *Trichoderma viride*, *Rhizopus* sp., *Rhizobium huakuii, Brady rhizobium* sp. and *E. coli*. were carried out on the basis of the colony morphology, biochemical characteristics and Polymerase Chain Reaction used as a supporting tool in the identification of the bacterial isolates[24].

Microorganisms, which were able to utilize fenitrothion and butachlor as a sole carbon and/or nitrogen source, were tested for their abilities to degrade these pesticides. Tested microorganism was incubated into liquid aqueous (basal medium): KH_2PO_4 1.0gm, K_2HPO_4 1.0gm, NH_4NO_3 1.0gm, $MgSO_4.7H_2O$ 0.2 gm, $CaCl_2$ 0.02gm, $Fe(SO_4)_3$ 0.01gm and Distilled water to make 1 liter (H_2O)1.0 L at pH 7.0 [25], with pesticide used for 21 day at (30°C ±2). Samples were taken from treatments and control at intervals of 0, 1, 3, 7, 14, 21 and 30 days for determination. Each culture was filtered through whatman No.1 filter paper. Pesticide residues were extracted from filtrate using the method adopted by[26].

Analyses of fenitrothion and butachlor residues were performed using certified analytical standard of butachlor and fenitrothion. The Stock solution of fenitrothion and butachlor was prepared

by dissolving 50 mg of the analyte (accurate weight) in 50 mL n-hexane to obtain concentration 1 mg mL^{-1}. A working standard solutions of 0.05, 0.1, 0.25, 0.5 and 1.0 µg mL^{-1} were prepared by appropriately diluting the stock solution with n-hexane. Stock solution was stored at -20 ± 2 °C, and working standard solutions were stored in ≤ 4 °C when not in use. Calibration curves were generated by plotting peak area versus concentration. Standard calibration curves were presented excellent linearity with regression coefficient r > 0.995 with good separation and repeatability for the two tested pesticides. The calibration curve and recovery validation study were all repeated three times (n = 3).

Agilent 6890 (USA) gas chromatography coupled with electron capture detector (GC-ECD) was used for determination of butachlor residues, for fenitrothion residues GC-with nitrogen phosphorus detector (GC-NPD) was used. Separation using capillary column HP-5 (30 m × 0.25 mm × 0.25 µm). Nitrogen was used as the carrier gas at a flow rate 2ml/min. The following temperature program was employed: initial temperature of 180 ºC held for 1 min; increased at 25 ºC min^{-1} to 220, held for 2 minutes; yet another increase at 3 ºC min^{-1} to reach 245 ºC. The injector temperature was 220 ºC. The injection volume was 1 µl for all standard and samples. Data analysis was performed using Chemistation software.

Data were statistically evaluated by one-way analysis of variance (ANOVA). Determination the differences among means were carried out by using the least significant differences (LSD) test. All statistical analyses were done using the Statistical Package for social sciences (SPSS 16.0) program.

RESULTS AND DISCUSSION

This study aimed to determine which of the studied microorganisms *Micrococcus rosus, Pseudomonas alcaligens*, *Aspergillus* sp., *Bacillus licheniformis*, *Bacillus megatherium*, *Trichoderma viride*, *Rhizopus* sp., *Rhizobium huakuii, Brady rhizobium* sp. and *E. coli*., is fenitrothion and/or butachlor resistant, and then the materials consisting of such microorganisms were analyzed on microorganism's biodegrading fenitrothion and butachlor content. To identify bacteria and fungi strains are responsible for biodegradation.

Results in Table 1 showed that the inhibition of different microorganisms' growth by fenitrothion and butachlor, this results reflected that the different resistant levels in the tested microorganisms to tested pesticides. Five strains of microorganisms *Micrococcus rosus, Aspergillus sp., Bacillus licheniformis*, *Rhizopus* sp. and *Rhizobium huakuii*., had partial or total growth inhibition while the other five strains *Pseudomonas alcaligens*, *Bacillus megatherium*, *Trichoderma viride, Brady rhizobium* sp. and *E. coli*., showed a great growth without any inhibition. Biological degradation takes place when microorganisms consume or break down pesticides[27-29]. This study was reported that different biodegradation rates were obtained for both fenitrothion and butachlor with *Pseudomonas alcaligens*, *Bacillus megatherium*, *Trichoderma viride, Brady rhizobium* sp. and *E. coli*. When they used as only source of nitrogen and carbon.

Fenitrothion showed different reduction percent 48.27, 68.52, 83.17, 87.39, 100 and 100 % after 30 days for *Bacillus megatherium*, *Trichoderma viride*, and *Brady Rhizobium* sp., *E. coli* and *Pseudomonas* sp., respectively. *Brady rhizobium* sp. and *Pseudomonas* sp. Showed significant highest degradation rate of fenitrothion ($p \leq 0.05$), that might be they can use fenitrothion as a source for both of carbon and nitrogen Figure

Table 1. Pesticide-tolerance of microorganisms

Microorganism	Pesticides were treated at conc. (5 ppm)	
	Fenitrothion	Butachlor
Micrococcus rosus	+	-
Pseudomonas sp.	-	-
Aspergillus sp.	+	-
Bacillus sp.	+	+
Bacillus megatherium	-	-
Trichoderma viride		--
Rhizopus sp.	+	-
Rhizobium sp.	+	+
Brady rhizobium sp.	-	-
E. coli	-	-

(+): growth was inhibited (-): no inhibithion

1. Butachlor showed different reduction percent 43.8, 54.04, 100, 78.49, 84.41 and 100 % after 30 days for *Bacillus megatherium*, *Trichoderma viride*, *and Brady Rhizobium* sp., *E. coli. and Pseudomonas* sp., respectively. *Trichoderma viride* and *Pseudomonas* sp. Showed significant highest degradation rate of fenitrothion ($p \leq 0.05$), that might be they can use fenitrothion as a source for both of carbon and nitrogen Fig. 2.

The mineralization rate of the pesticides depends on environmental factors, which was proved by Kodam[30]. Changing the substrate reaction and incubation temperature, he determined optimal parameters of simazine biodegradation for Penicillium steckii and Moraxellaovis. In case of fungi, best were: pH 7 – 8, 30°C, and traces of glucose and yeast extract in substrate (over 50% subsidence of simazine in 5 days); in case of bacteria: pH 5 and 35°C. Nearly 100% of simazine was degraded after 40 days of incubation of mixed bacteria culture (therein Arthrobacter sp.) in bioreactor. It was found that simazine herbicides were used by microorganisms both as a carbon and nitrogen source[31]. Al l of these experiments together with our tests results as well as many others shows the possibility of using different microorganism's populations to the process of contaminated systems biodegradation. Results of pesticides residue analysis proved that the strain Trichoderma viride as a fungus of the Trichoderma genus and bacterial strain Pseudomonas sp. having fundamental impact on

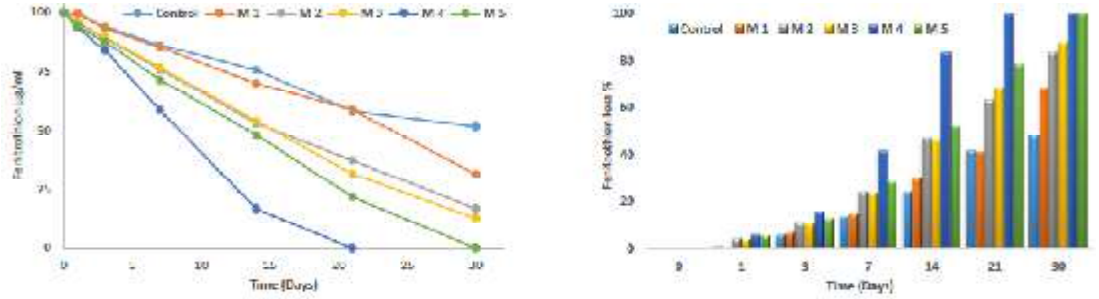

Fig. 1. Degradation rate and reduction percentage of fenitrothion by different microorganism's strains

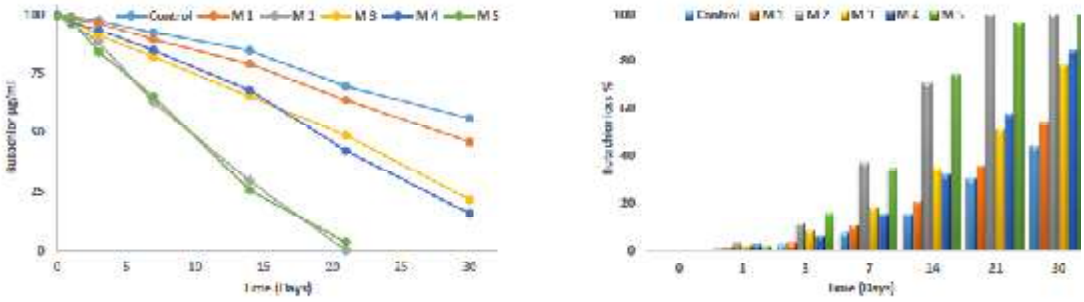

Fig. 2. Degradation rate and reduction percentage of butachlor by different microorganism's strains

biodegradation of fenitrothion and butachlor were added individually to culture media, which were might be used for environmental bioremediation.

CONCLUSIONS

Ten different microorganisms' strains isolated from soil were tested to degrade fenitrothion and butachlor, results showed growth inhibition of five microorganisms and five of these tested microorganisms had different degradation rate of fenitrothion and butachlor. According the data *Trichoderma viride, E. coli*. *Pseudomonas* sp., have the highest degradation rate for fenitrothion and butachlor ($p \leq 0.05$). These results presented that all of this microorganisms can used

to degrade the fenitrothion and butachlor residues. These results provide basic information for developing regulations regarding the safe disposal of and bioremediation of fenitrothion and butachlor using eco-friendly method. We suggest that further studies on using these microorganisms in the degradation of different pesticides, to protect the environment and public health.

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