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Evaluation of Certain Bioactive Agents for Bioremediation of Pesticide-Contaminated Soil

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Abstract: The degradation pattern of carbofuran and ethoprophos in sandy loam soil was studied under laboratory conditions. Residue analysis of the initial samples of the two applied pesticides was relatively high. Graduate dissipation of both pesticides was noticed through the successive intervals. At the end of the experimental period, carbofuran residues detected showed a rapid decrease to 4.66 ppm, meanwhile ethoprophos slightly decreased to 23.83 ppm revealing loss percentages ranged between 94.82 to 73.9%, respectively. Addition of certain bioactive amendments was able to induce the pesticide degradation in the contaminated soil. The microbial capacity of the tested agents, as non specific biomasses, are considerably varied whether exerted their effects early or late after a short time of mixing with the treated soil. Generally, adding of microbian, compost or manure to carbofuran-treated soil fell the half life values ($t_{0.5}$) from 8.86 days to 3.38, 5.66 or 4.83 days, respectively. Similarly, these values declined from 8.04 days to 4.8, 4.57 or 6.3 days after amending ethoprophos-treated soil with the above mentioned agents, respectively. Such materials can be effectively used as accessible tools for control of pesticidal contaminants, particularly in the developing countries.

Key words: Pesticide residues, persistence in soil, enhanced biodegradation, bioactive agents

INTRODUCTION

The fate of pesticides in soil has been the subject of many researchers in the last two decades (Jones *et al*., 1988; Hong and Pehkonen, 1998). There have been numerous investigations which indicated that soil temperature, moisture, PH and microbial viability are the major factors affecting the degradation of pesticides in soil (Forgarty and Tuovinen, 1991; Jones and Norric, 1998). However, microbial degradation plays the key role in the breakdown of soil pesticides, persistence and reducing pollution (Johnston and Camper, 1991; Abdel-Rahman, 1999).

The bioremediation technology of the contaminants is nowadays in progress to dispose the soil pollutants and maintain the environment clean and safer as well (Karpouzas *et al*., 1999; Saad *et al*., 2000). In Egypt, groups of pesticides are recommended and widely used for controlling weeds, nematodes and insects in the different cultivations. The agricultural eco-system in certain regions is contaminated with various types of pesticides (Zidan *et al*., 2002; Nasr and Shokr, 2004).

The present study aims to follow the degradation pattern, under laboratory conditions, of two currently used pesticides (carbofuran and ethoprophos) in soil. Special attention was directed to the potential of three biological amendments, locally available and cheap, to mineralize such compounds.

MATERIALS AND METHODS

A soil sample (4-6 kg) was collected from citrus orchard, not previously treated with pesticides throughout the last three years, at the newly reclaimed regions, Nubaria, Beheira governorate, Egypt. It was taken from the top 20 cm layer; air dried under laboratory conditions and passed through a 2 mm sieve. The physical and chemical properties of the soil are presented in Table 1. The water content of the soil was adjusted to 65% of the maximum water-holding capacity and maintained through the experiment.

The following two pesticides were used

Carbofuran: 2,3-Dihydro-2,2-dimethylbenzofuran-7-yl methyl-carbamate, known as furadan 10% G.

Ethoprophos: O-ethyl-S-S-dipropyl phosphorodithioate, known as Mocap 10% G.

A small amount of each pesticide was distributed in 10 g of fine sand and added to 1000 g soil, resulting in final concentration of 100 μg g^{-1} (100 ppm), based on a.i percentages. The soil treated by carbofuran and/or ethoprophos was kept separately in plastic plots (300 g each) and incubated at 22±2°C and 65±5% R.H. Representative samples (25 g each) from each treatment were taken for determination of pesticide residues at 0.0, one day, 1, 2, 3 and 4 weeks after incubation.

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Table 1: Mechanical and physical characteristics of the tested soil

	Particle size distribution						
Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	C_aC_3 (%)	Texture	PH	Organic matter (%)
66.0	4.18	7.5	18.5	3.6	Sandy loam	8.2	0.4

In parallel, another similar experiment was done to evaluate three selected bioactive agents in enhancing the degradation of the tested pesticides in soil. These were:

Microbian: A mixture of four microbial species in equal portions (*Bacillus megatherium*, *Azotobacter* sp., *Azospirillium* sp. and *Pseudomonus* sp.). It produced by General Organization of Agric. Fund, Ministry of Agric. Egypt and used commercially as biofertilizer.

Compost elwadi: Wastes of food industries that exposed to technological bacteria fermentation and maturation processes using certain beneficial bacteria to be standardized organic fertilizer. It produced by Delta Bio-Tec, Egypt.

Organic manure: Cattle dung was spread over a sheet under the natural conditions, for five days, before use. About 300 g of the original soil were treated by carbofuran and/or ethoprophos at the concentration mentioned before (100 ppm). The contaminated soils were mixed thoroughly with microbian, compost and/or cattle manure at the rate of 2, 4 and/or 4 g/100 g soil, respectively. They incubated under the above mentioned conditions. Samples for determination of pesticide residues were taken at the same intervals. Results were based on the average of three replicates.

Determinations of pesticide residues: The procedures for extraction, clean up and determination was as follows:

Extraction and clean up: For both compounds, the method adopted by Krause *et al.* (1986) was used for extraction, through partitioning by chloroform. Twenty five grams soil was shaken mechanically with 50 mL of acetone- water (3/1 v/v) for 1 h in 500 mL glass stopper bottle. The extract was carefully decanted and filtered through a clean pad of cotton. Fifty milliliter from filtrate was concentrated by using a rotary evaporator on a water bath at 30°C to remove acetone and then extracted twice by 50 mL chloroform. The combined chloroform was dried through anhydrous sodium sulfate and the evaporated near dryness at 40°C.

The clean up procedure for carbofuran extract was done according to the method of Al-Samariee *et al.* (1988). The extract was mixed with two grams of activated charcoal, and shaken for 2 min. The mixture was filtered through filter paper and the supernatant rinsed with additional 50 mL chloroform and concentrated just to dryness using a rotary evaporator at 30°C. The residues were ready for HPLC determination. No clean up was needed in the case of ethoprophos extract.

Residue determination: Quantitative analysis of carbofuran was performed by HPLC, Agilent 1100 Series with work station. The U.V. Diod- array detector set at 220 nm and the analytical column Nucleosil- C18, 5 μm (4×250 mm) was used. The mobile phase was acetonitrile-water at flow rate 1 mL min^{-1}. The retention time of carbofuran under these conditions was 3.7 min.

Ethoprophos was detected and determined using GLC, HP 6890 serial equipped with Flame Photometric Detector (FPD) operated in the phosphorus mode (529 nm filter) under the following conditions: capillary column PAS 1701 30 m×0.32 mm×0.25 μm. Detector temperature: 250°C, Injection temperature: 245°C, Oven temperature program: Initial into temperature: 160°C, Initial time 2 min. Riserate: 10°C min^{-1}. Final temperature: 240°C. Hold time 8 min. Nitrogen carrier gas: 3 mL min^{-1}. Hydrogen flow: 75 mL min^{-1}. Air flow: 100 mL min^{-1}. Under these conditions, the retention time for ethoprophos was 4.15 min.

The experiments were performed with analytical grade chemicals and the purity of pesticides was more than 95%. The obtained data were corrected according to the rates of recovery which were 89.6 and 92.2% for carbofuran and ethoprophos, respectively. The half-life ($t_{0.5}$) values were calculated using Moye's *et al.* (1987) equation.

RESULTS AND DISCUSSION

Persistence of the tested compounds: The stability and the rate of degradation of the applied dose are somewhat varied according to the nature and structure of the tested compounds.

The initial deposit of carbofuran was 90.04 ppm. Only 5.95% of this amount can be degraded within the first day indicated that its degradation proceeded slowly and continued to increase gradually thereafter (Table 2).The compound lost 61.04 % of its active ingredient at the 2nd week. Degradation of carbofuran from a previously untreated field in the UK was characterized by a short lag period followed by rapid degradation

Table 2: Degradation pattern of carbofuran residues in soil after incubation with microbian, compost and/or manure under laboratory conditions

	Carbofuran		Carbo. + microbian		Carbo.+ compost		Carbo. + manure	
Periods	Amount (ppm)	Loss (%)	Amount (ppm)	Loss %	Amount (ppm)	Loss (%)	Amount (ppm)	Loss (%)
Zero time*	90.04	0.00	89.28	0.00	86.18	0.00	86.46	0.00
One day	84.68	5.95	60.64	32.08	62.44	27.54	72.62	16.01
1st week	56.88	36.83	25.56	71.37	43.58	49.83	37.06	57.14
2nd week	35.08	61.04	23.4	73.8	39.0	54.75	22.78	73.65
3rd week	13.34	85.18	3.2	96.42	35.4	58.92	1.82	97.89
4th week	4.66	94.82	ND	>99.9	6.3	6.3	92.68	ND
$t_{0.5}$	8.86		3.38		5.66		4.83	

* = 1 h after treatments, $t_{0.5}$ = the half life period, ND = None Detected

Table 3: Degradation pattern of ethoprophos residues in soil after incubation with microbian, compost and/or manure under laboratory condition

	Ethoprophos		Ethopr.+ microbian		Ethopr + compost		Ethopr. + manure	
Periods	Amount (ppm)	Loss (%)	Amount (ppm)	Loss (%)	Amount (ppm)	Loss (%)	Amount (ppm)	Loss (%)
Zero time*	91.23	00.00	89.33	00.00	84.0	00.00	88.35	00.00
One day	75.43	17.32	63.78	28.6	55.25	34.21	72.6	17.83
1st week	50.43	44.7	29.9	66.54	36.55	56.5	40.68	53.96
2nd week	38.68	57.61	21.45	76.0	24.6	70.7	38.68	56.22
3rd week	33.98	62.75	20.3	77.28	17.43	79.27	27.1	69.33
4th week	23.83	73.9	18.3	79.51	16.25	80.65	13.33	84.91
$t_{0.5}$	8.04		4.8		4.57		6.3	

* = 1 h after treatments, $t_{0.5}$ = the half life period, ND = None Detected

Karpouzas *et al.* (1999). At the end of the experimental period, the residue level did not exceed 4.66 ppm and about 5% of carbofuran amount that incorporated were still adsorbed onto the soil particles.

Hafez and Thiemann (2002) pointed out that carbofuran was slowly dissipated in acidic and neutral soils (pH≥7), but appeared to be somewhat faster in alkaline soils (pH≥9).

As for ethoprophos, about 17.32% of its initial amount was diminished one day after application. Graduate dissipation of the applied concentration was noticed throughout the successive intervals (Table 3). At the 4th week, the last sample contained 23.83 ppm; indicating that 26.6% of the initial dose was still remained unchanged in the contaminated soil. The insecticide ethoprophos seems to be slightly more stable and persistent than that of carbofuran in the soil. However, the obtained $t_{0.5}$ values were not significantly different and reached 8.86 and 8.04 days for carbofuran and ethoprophos, respectively. In a field experiment, Nasr and Shokr (2004) mentioned that the initial deposits of carbofuran, cadusofos and ethoprophos were decreased by 93.19, 70.1 and 76.97% loss, respectively, within the first five days after application in the sandy clay loam soil. They added that carbofuran was degraded more rapidly than the other two compounds.

Effect of the bioactive agents: Addition of microbian to carbofuran-treated soil considerably induced the insecticide degradation as indicated by the high rate of loss (32.08 %), one day after treatment, in comparison to the other two amendments (Table 2).

Microbian as ready bacterial preparation was able to express its action and displayed its activity within as short time. Such biodegradation became more obvious in the successive samples where complete disappearance was recorded at the 4th week after incubation. Saber and Gomaa (1993) pointed out that microbian as a multi-strain biofertilizer lead to a significant reduction in the rate of mineral fertilization as well as reduction of high doses of pesticides that control rhizosphere diseases. El-Kbbany (2002) also mentioned that microbian has the potential to break down a great variety of pesticides including dieldrin which was hard to mineralize by other biofertilizers.

Mixing compost with the contaminated soil accelerated carbofuran degradation, particularly at two points; the first occurred within the first week (from 27.54 to 49.83% loss) and the second was more pronounced and ranked between 3rd -4th week after the treatment.

Little change in carbofuran decomposition was detected, one day after adding the cattle manure. A remarkable raise of its activity, however, was noticed soon as indicated by the high reduction (57.14 %) of carbofuran at the first week and on forth. Microbial activity of animal wastes often improve with the time elapsed during natural fermentation. Abdel-Rahman (1996) found that mixing atrazine polluted- soil with sheep manure for 3 weeks at 65% which (water holding capacity), reduced atrazine concentration to about 21% of its original concentration and sometimes exceed the activity of some fungi or bacteria species.

Table 3 clarify that amending the polluted soil with any of these biodynamic materials produced significant reductions in ethoprophos residues in the soil. One day

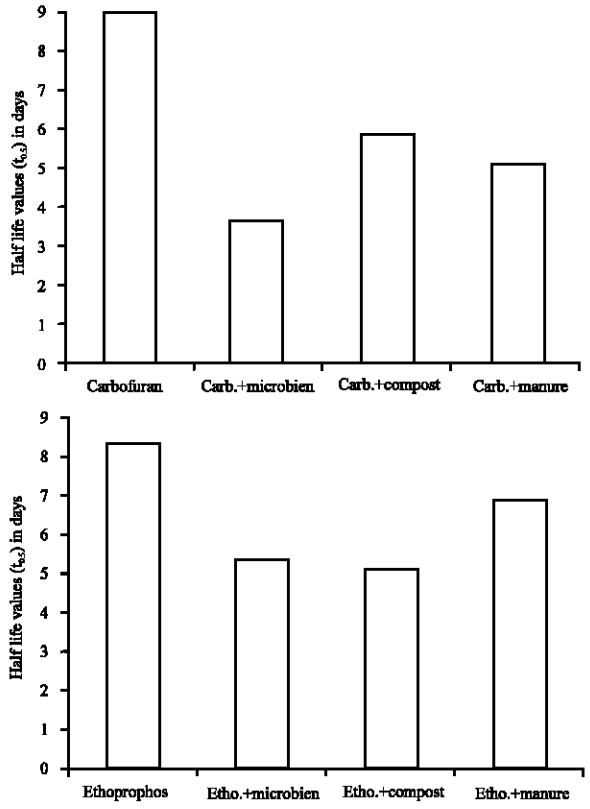

Fig. 1: Dissipation rate of carbofuran and/or ethoprophos residues in soil after incubation with microbian, compost and manure under laboratory conditions

after incubation microbian, compost or the manure, the residue levels decreased by 28.6, 34.21 or 17.83%, respectively. The activity of these additives represents 1.7, 2.0 and 1.0 folds of the normal dissipation of ethoprophos alone (17.32%), respectively. Again, the manure became active after a lag interval, then its activity steady increased where 84.91% of ethoprophos residues were dissipated at the end of the experimental period. Doyle *et al.* (1978) found that degradation of pesticides metabolized by dealkylation reaction was inhibited by sewage sludge, but enhanced by dairy manure. In general, the calculated half lives ($t_{0.5}$) declined from 8.04 days to 4.8, 4.57 and 6.3 days after amending ethoprophos treated soil with microbian, compost or the manure, respectively.

The bioremediation capacity of the tested agents is considerably varied whether exerted their effects early or late after a short time of mixing with the treated soil.

The microbial communities usually include wide range of species or enzymes required for chemical biodegradation. Even though the soil received various pesticides, an active microbial population which present could either adapted to these pesticides or capable of degradation them via existing enzymes and may serve as a source of nutrients (Johnston and Camper, 1991).

It may be inferred from this laboratory study that the rate of ethoprophos dissipation sandy loam soil was somewhat differed and has more persistency than that of carbofuran (Fig. 1). Addition of the tested bioagents to the polluted soil enhanced the degradation of both compounds and therefore they can be effectively used as accessible tools for control of pesticidal contaminants.

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