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Microbial Degradation of Endosulfan in Carbon Free Media and Selective Media

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Abstract: The effects of soil microorganisms isolated from highly polluted soil (pesticide stores soil and cotton field), on half-lives of α and β- endosulfan under condition of selective and carbon free media were studied. The results showed significant decrease in half-lives ranging between 58.4 – 81.9% in α-endosulfan compared to 35.5 – 71.6% in β-isomer.

Key words: Endosulfan, Microbial degradation, Sudan, Bio-remediation

INTRODUCTION

Endosulfan is the common name of the insecticidal compounds 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin3-oxide. Endosulfan was first developed in 1956 by Farwerke Hoeschst Ag in Germany. The synthesis of this compound results in production of the two stereo isomers α and β-endosulfan. Endosulfan is often classified as cyclodiene and has the same primary action and target site as cyclodienes. However, it has chemical and physical properties significantly different from other cyclodiene insecticides, that affect both its environment and biological fates [6]. In particular, endosulfan has a relative cyclic sulfate diester group, and as a consequence, its environmental persistence is lower than that of other cyclodienes [18].

In Sudan it was tested for the first time in small scale experiment against cotton pests in season 1966/67 [9]. It is recommended for use against white fly *bemisia tabaci* and *Aphis gossypii* [8].

The period from the early sixties to the late seventies witnessed progressive intensification and expansion in the cropped areas with subsequent increase in pest complexity and damage. This necessitated increase in chemical treatment with negative impact on human health and the environment. Organochlorines were the major group of pesticides, which flourished during this period favored by their high potency against wide range of agricultural and public health pests, cheapness and environmental persistence.

The improper storage of pesticides in Sudan has created many problems. As in many of the developing countries, stores were sub-standard in construction and facilities improperly located (near or within residential areas, water bodies or farming activities) and their staffs were less trained in store management. The poor storage facilities and management practices in Sudan has led huge amounts of the stored pesticides to become obsolete. The total amounts of the stored pesticides in Sudan was estimated at 666 tones, 77.5% in liquid state and 22.5% as solids with about 6459 cubic meters of contaminated storage soil scattered over 43 major and minor sites in the country. The previous and current effort in Sudan was directed towards estimation of quantities and how to get rid of them. Nothing was done towards treatment of affected sites. It is obvious soil is heavy and difficult to transport abroad for decamination. Further, horizontal and vertical movement of contaminants complicated the problem [1]. Therefore, in situe treatments of affected situe appeared move attractive, suitable and could be feasible. Preliminary reports [2] argued the potential use of endogenous soil microorganisms in cleaning highly polluted soil and dump sites.

The biodegradation of persistent compounds is an important mechanism for their dissemination in the environment [3]. In predicting the persistence of synthetic chemicals in soil, sediment and natural water, it is necessary to determine the role of endogenous microorganisms in the over all degradation process.

Microorganisms play an important role in the conversion of cyclodiene insecticides in soil to nontoxic products. In the natural environment microorganisms may provide some protection against toxicity of endosulfan. Pure culture of a range of soil microorganisms have been reported to transform endosulfan to a nontoxic diol metabolite in unsealed liquid cultures [7,12]. Endosulfan can be completely degraded in about two weeks to nontoxic metabolite under anaerobic conditions [10]. Microbial degradation of endosulfan was also reported by Shivaramaiah. and Kennedy [14]. They also, identified endodiol as the

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major degradation product in an undefined mixture of microorganisms obtained from soil suspension. Siddique *et al.* [13] reported that degradation of endosulfan occurred in contaminant with bacterial growth when endosulfan was used as only source of sulfur in the culture, while no growth occurred in the absence of endosulfan.

[12]. investigated the ability of 28 soil fungi, 14 soil bacteria, and 10 soil actinomycetes to degrade insecticide endosulfan. He found that the major metabolites detected were endosulfan sulfate.

Endosulfan was selected for the present study because of its extensive use in Sudan where it comprises 20-40% of the annual spray in irrigated cotton and it has many health and environmental problems. Endosulfan is highly to moderately toxic to mammals (LD 50- 76 mg/k for α and 240 mg/k for β isomers). Non-target organisms specially fishes, birds and beneficial arthropods such as natural enemies. Previous studies in Sudan [1] have indicated that its toxicity to the aphid predator. Endosulfan is greatly misused in Sudan and responsible for over 90% of the documented poison incidences reported by the National chemical laboratory [2]. Endosulfan also was reported to constitute a major fraction of obsolete pesticides stocks in Sudan.

The specified objectives of the study are:-

Isolation of natural microorganism that is capable of degrading endosulfan α & β isomers and Evaluation of their degradative capability under conditions of selective media and carbon free media.

MATERIAL AND METHODS

Soil Samples:

Areas of Samples: Top soil surface (0-10 cm) samples were collected from five sites and used in this study for isolation of endosulfan degrading microorganisms. The soil was collected from different locations described in Table (I).

Sample Collection Methods: A soil auger of 10cm length and 5cm diameter was used to randomly collect the soil samples to depth of 10cm. In each site five augers samples were taken from different location and mixed thoroughly to make composite sample. The collected samples were placed in paper bags, labeled and immediately transported to the microbiology laboratory ElNeleen University, Khartoum.

Preparation of Samples: The samples were left over night to dry in open air at room temperature. Each sample was then mixed thoroughly. The clods and big particles were broken by hand to reach a.

Preparation of Media:

Preparation of Carbon Free Media: One liter of liquid media was prepared according to the method described by Tepper, et.al. [17], to one liter conical flasks. One g K2HPO4, 0.5 g MgSO4 .7 H2O, 0.5 g NaCl, 0.001 g FeSO4.7 H2O , 0.01 g MnSO4. 4 H2O, 0.05 g CaCO3 were added. The volume was completed to one liter distilled water. The flasks containing these media were autoclaved for 20 minutes at 121˚ C, allowed to cool at room temperature and kept in the refrigerator as stock media at 5˚ C.

Microbial Degradation of Endosulfan in Carbon Free Media: A total of 36 conical flasks (100 ml) and culture media (carbon free media) were autoclaved separately for 20 min. at 121˚ C. Two hundred and fifty micro liters of acetone containing 100 mg endosulfan were added to each flask in laminar flow hood. The acetone was allowed to evaporate using gentle air flame. Fifty ml of culture media was added to each flask. All flasks were grouped in four sets as follows:

(a) One ml of organic nitrogen bacteria isolated from Managl1, Rahad1and Rahad2 soil (isolated by selective media)
(b) One ml of inorganic nitrogen bacteria and actinomycetes isolated from Gezira4, Rahad1and Rahad2 soil (isolated by selective media)
(c) One ml of bacteria and actinomycetes which live in poor media isolated from Gezira1, Managl1and Gezira4 soil (isolated by selective media)
(d) One ml of fungi isolated from Gezira4, Rahad1and Rahad2 soil (isolated by selective media)

All flasks were incubated at 30˚ C for 45 days. Flasks were arranged in a completely randomized design with three replicates.

Preparation of Media Selective: Four types of selective media were prepared in four conical flasks (1500 ml) following the method of Tepper, *et al.*, (1994) these include:

(a) Starch agar (SAA): This medium was used for inorganic nitrogen bacteria and actinomycetes. The medium was prepared by adding 10 g starch, 2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 1 g $MgSO_4$, 1 g NaCl, 1 g $FeSO_4$ and 1g $CaCO_3$ to one liter distilled water .

(b) Nitrate agar (NA): This medium was used for bacteria and actinomycetes which live in poor media such as, *Mycobacterium*, *Arthrobacterium*, *Micromonospora*, and *Nocardia.* The media was prepared by adding 0.2 g $NaNO_2$, 1 g $NaNO_3$, 0.2 g $FeSO_4$, 1 g Na2CO_3, 0.5 g K_2HPO_4 and 0.3 g NaCl to one liter distilled water.

(c) Meat Peptone Agar (MPA): This medium was used for organic nitrogen bacteria. The medium was prepared by adding 7.5 g of peptone and 5 g NaCl to one liter meat extract.

(d) Chabecks media (CHA): This media was used for fungi. The media was prepared by adding 0.5 g KCl, 0.5 g $MgSO_4$, 1 g K_2HPO_4, 0.01 g $FeSO_4$, 2 g $NaNO_3$ and 20 g glucose to one liter distilled water then 4 ml of lactic acid were added. The flasks containing these media were autoclaved for 20 minutes at 121° C, allowed to cool at room temperature and kept in the refrigerator as stock media at 5° C.

Degradation of A and β-endosulfan by Selected Microorganism in Selective Media: This experiment was done to investigate the potential role of selected microorganisms in degrading endosulfan.

A total of 36 conical flasks (100 ml) and culture media (NB, MPB, SAB and CHB) were autoclaved separately for 20 min. at 121 C°. Two hundred and fifty microliters of acetone containing 100 mg endosulfan were added to each flask in laminar flow hood. Acetone allowed to evaporate using gentle air flame. Fifty ml of culture media was added to each flask (nine flasks for each media). All flasks were grouped in four sets and inoculated as follows:

(a) One ml of organic nitrogen bacteria isolated from Managl1, Rahad1and Rahad2 soil (isolated by selective media)
(b) One ml of inorganic nitrogen bacteria and actinomycetes isolated from Gezira4, Rahad1and Rahad2 soil (isolated by selective media)
(c) One ml of bacteria and actinomycetes which live in poor media isolated from Gezira1, Managl1and Gezira4 soil (isolated by selective media)
(d) One ml of fungi isolated from Gezira4, Rahad1and Rahad2 soil (isolated by selective media)

All flasks were incubated at 30º C for 45 days. Flasks were arranged in a completely randomized design with three replicates.

Extraction and Analysis: About 10 ml were taken from each flask every 15 days extracted and analyzed used GLC

RESULTS AND DISCUSSION

Microbial Degradation of Endosulfan in Carbon Free Media: Tables 10, 11 showed the half-lives of endosulfan α and β incubated with selected isolates of microorganisms for a period of 45 days in carbon free media. Based on the percentage reduction in half lives it is clear that the α endosulfan degrade faster compared to β - endosulfan (60.1 to 79.2% in α VS 49.4 to 72.7% inβ).

The results generally indicated that all microorganisms used in this experiment caused relatively similar effects on α endosulfan isomers (Table 2). while different groups of microorganism showed variable effects on β, actinomycetes and bacteria which live in poor media are more efficient in degrading β- endosulfan than others (Table 3).

The best strain of organic nitrogen bacteria capable of degradation of α-endosulfan was that isolated from El Faw cotton field soil (Rrahad 2).

Degradation of α and β-endosulfan by Selected Microorganisms in Selective Media: Tables 4 and 5 show the half lives of endosulfan α and β incubated with selected isolates of microorganisms in selective media. Based on the percentage reduction in half lives, it is clear that the α isomer degrade faster compared to β isomer (47.5 -81.9 % in α VS 35.5 - 71.6 % in β isomer).

The results generally indicated that actinomycetes and bacteria are more efficient in degrading both α and β- endosulfan compared to the fungi, actinomycetes and bacteria which lives in poor media which cause more degradation in α-endosulfan than in β- isomer. Fungi causes relatively similar effects on both endosulfan isomers (58.4-47.5% in α VS 68.03-47.6% in β.

The best strains of actinomycetes capable of degradation α endosulfan was that isolated from Kapelgidal pesticides store soil (Gezira I). The best isolated of organic nitrogen bacteria was the isolate from Elfaw cotton field (Rahad 2).

Microorganisms Isolates from highly polluted sites (pesticide stores, cotton field) were selected for further studies. The selected Isolates were incubated with endosulfan under conditions of both selective or carbon – free media for longer period of time. The results showed a Significant decrease in half lives. The role of soil microorganism in shorting the half lives of endosulfan was reported by many authors [11,10,16,13,4].

Reduction in half lives was greater in α-endosulfan compared to β-endosulfan this results is in conformity with the results of Sutherland, *et al.*, [15] who mentioned that bacteria degraded α-endosulfan more than β isomer. Generally inoculums from soil of longer history of exposure to pesticide showed greater capability in degrading endosulfan compared to relatively recent or less exposed soils. This agree with Alexander [3] who reported that longer usage of pesticides in soil increases the microbial tolerance which is expected to have greater capability in degrading pesticides contaminants.

Different results were obtained when endosulfan was used as a source of carbon (in media free from other carbon sources). Results from this trail indicated

Table 1: Soil samples for enrichment and microbial enumeration studies.

Sample code	Sample description
Gezira 1	Inside of Kapelgedad pesticides store in Gezira scheme
Gezira 4	Gorashi Pesticides Store
Managil 1	Inside of Raselfeel pesticides store in Managil extension
Elrahad 1	Inside of Elfaow pesticides store in Elrahad scheme
Elrahad 2	Cotton field in Elrahad scheme

Table 2: Half lives, percentage reduction in half lives and percentage degradation after 45 days incubation α- endosulfan with selected soil microorganisms in carbon free media.

Microorganism	Soil code	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Actinomycetes and Bacteria which lives in poor media	Gezira1	1.87	0.8722	15.0	61.3	97.2
	Managil1	1.94	0.828	13.0	66.6	99.3
	Gezira 4	1.96	0.7951	11.9	69.2	99.7
Inorganic Nitrogen Bacteria and Actinomycetes	Gezira 4	1.78	0.8502	15.5	60.1	95.3
	Rahad 1	1.81	0.6104	08.1	79.2	98.9
	Rahad 2	1.82	0.7286	11.5	70.6	99.2
Organic Nitrogen Bacteria	Managil1	1.90	0.8031	12.6	67.7	99.2
	Rahad 1	1.87	0.7198	10.5	72.9	99.7
	Rahad 2	1.87	0.6768	09.5	75.5	99.0
Fungi	Gezira 4	1.85	0.8402	14.3	67.3	96.7
	Rahad 1	1.76	0.7384	12.7	67.4	91.7
	Rahad 2	1.96	0.7888	11.8	69.6	99.5
CONTROL	Control	0.96	0.9534	38.9	00.0	54.4

R^2 = Determination coefficient
$\tau_{½}$ = Half lives
Gezira1 = Kapelgedad Pesticides Store
Gezira 4 = Gorashi pesticides store
Managil1 = Raselfeel Pesticides Store
Rahad 1 = El Faw Pesticides Store
Rahad 2 = El Faw Cotton field

Table 3: Half lives, percentage reduction in half lives and percentage degradation after 45 days incubating β- endosulfan with selected soil microorganisms in carbon free media.

Microorganism	Soil code	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Actinomycetes and Bacteria which lives in poor media	Gezira1	1.94	0.7738	12.6	72.7	100
	Managil1	1.6	0.6486	13.3	71.0	86.7
	Gezira 4	1.91	0.8384	15.3	66.8	93.2
Inorganic Nitrogen Bacteria and Actinomycetes	Gezira 4	1.93	0.946	18.5	59.8	93.7
	Rahad 1	1.85	0.8912	17.7	61.7	90.0
	Rahad 2	1.77	0.7833	15.1	67.2	80.0
Organic Nitrogen Bacteria	Managil1	1.61	0.9311	21.1	54.1	81.5
	Rahad 1	1.62	0.8005	18.2	60.6	87.1
	Rahad 2	1.61	0.9609	23.3	49.4	81.5
Fungi	Gezira 4	2.05	0.9285	17.2	62.6	96.7
	Rahad 1	1.82	0.8116	15.1	67.2	94.7
	Rahad 2	1.77	0.7833	15.1	67.2	91.4
CONTROL	Control	1.03	0.9275	46.0	00.0	54.6

R^2 = Determination coefficient
$\tau_{½}$ = Half lives
Gezira1 = Kapelgedad Pesticides Store
Gezira 4 = Gorashi pesticides store
Managil1 = Raselfeel Pesticides Store
Rahad 1 = El Faw Pesticides Store
Rahad 2 = El Faw Cotton field

Table 4: Half lives, percentage reduction in half lives and percentage degradation after 45 days incubation α- endosulfan with selected soil microorganisms in selective media.

Microorganism	Soil code	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Actinomycetes and Bacteria which lives in poor media	Gezira1	2.10	0.8285	13.5	81.3	100
	Managil1	2.06	0.992	20.9	71.1	100
	Gezira 4	2.22	0.8898	16.0	77.8	100
	Control	0.64	0.9618	72.3	00.0	31.9
Inorganic Nitrogen Bacteria and Actinomycetes	Gezira 4	1.87	0.6000	08.3	81.9	100
	Rahad 1	2.33	0.8872	18.5	59.9	100
	Rahad 2	2.22	0.8985	16.4	64.3	100
	Control	1.03	0.9551	46.1	00.0	52.6
Organic Nitrogen Bacteria	Managil1	2.19	0.8494	14.8	74.9	100
	Rahad 1	2.02	0.9095	17.8	69.3	100
	Rahad 2	2.11	0.793	13.4	77.5	100
	Control	0.89	0.8887	59.3	00.0	42.6
Fungi	Gezira 4	2.02	0.9761	19.3	47.5	100
	Rahad 1	1.92	0.8271	15.3	58.4	100
	Rahad 2	2.10	0.8895	15.4	58.1	100
	Control	1055	0.7847	36.5	00.0	78.8

R^2 = Determination coefficient
$\tau_{½}$ = Half lives
Gezira1 = Kapelgedad Pesticides Store
Gezira 4 = Gorashi pesticides store
Rahad 1 = El Faw Pesticides Store
Rahad 2 = El Faw Cotton field

Table 5: Half lives, percentage reduction in half lives and percentage degradation after 45 days incubation β- endosulfan with selected soil microorganisms in selective media.

Microorganism	Soil code	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Actinomycetes and Bacteria which lives in poor media	Gezira1	2.22	0.8996	16.7	67.0	100
	Managil1	1.94	0.999	22.7	55.0	93.2
	Gezira 4	2.04	0.986	19.1	62.3	100
	Control	0.92	0.903	50.5	00.0	43.6
Inorganic Nitrogen Bacteria and Actinomycetes	Gezira 4	2.09	0.983	20.3	43.8	100
	Rahad 1	2.29	0.871	18.3	49.3	100
	Rahad 2	1.95	0.953	23.3	35.5	100
	Control	1.21	0.931	36.1	00.0	59.3
Organic Nitrogen Bacteria	Managil1	2.30	0.955	21.2	62.3	100
	Rahad 1	2.33	0.928	24.2	57.7	100
	Rahad 2	2.06	0.886	15.9	71.6	100
	Control	0.85	0.925	56.3	00.0	38.1

Table5: Continue

Fungi	Gezira 4	1.73	0.862	27.2	47.6	93.3
	Rahad 1	1.52	0.728	15.1	68.0	83.6
	Rahad 2	2.19	0.936	20.1	57.5	100
	Control	0.95	0.922	47.3	00.0	50.2

R^2 = Determination coefficient
$\tau_{½}$ = Half lives
Gezira1 = Kapelgedad Pesticides Store
Gezira 4 = Gorashi pesticides store
Managil1 = Raselfeel Pesticides Store
Rahad 1 = El Faw Pesticides Store
Rahad 2 = El Faw Cotton field

a relatively slower rate of degradation compared to selective media. α-endosulfan is again more subject to faster degradation rate. This could be explained by the report of Awasthi [5] who described endosulfan as a poor biological energy source.

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