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Effect of Fertilizers (Activators) in Enhancing the Microbial Degradation of Endosulfan in Soil

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Abstract: The effect of fertilizers activators on microbial growth and capability in degrading α and β-endosulfan was studied by incubating, two groups of microorganism in the presence and absence of four fertilizers (urea, triple super phosphate, urea + triple super phosphate and cow manure) for 45 days with sample drown every 15 days. Drawn sample were examined for microbial growth, concentration of starting material remained and a mount of sulphate generated. Results indicated that all activators caused significant increase in microbial counts especially the triple super phosphate followed by urea + triple super phosphate, cow manure and urea. Significant reduction in half lives of α and β-endosulfan accompanied with various level of sulphate generation was noticed. Since, the microorganism studied have shown great potential in degrading endosulfan therefore any enhancement in their numbers and activity (caused by activator) will no doubt promote their capability in degrading endosulfan in soil.

Key words: Endosulfan, degrading, microorganism, soil

INTRODUCTION

The chlorinated cyclic sulfite diester endosulfan is a cyclodien ensecticide possessing a relatively board spectrum of activity. Technical-grade is a mixture of two stereo isomers, α and β-endosulfan, in a ratio of 7:3. It is used extensively throughout the world as a contact and stomach insecticide and an acaricide on field crops, vegetables and fruit crops. Because of its abundant usage and potential transport, endosulfan contamination is frequently found in the environment at considerable distances from the point of its original applications (Mansingh and Wilson, 1995; Miles and Moy, 1979). Endosulfan has been detected in the atmosphere, soils, sediments, surface and rain water and food stuffs. It is extremely toxic to fish and aquatic invertebrates (Sunderam *et al*., 1992) and has been implicated in mammalian gonadal toxicity (Sinha *et al*., 1997), genotoxicity (Chaudhur *et al*., 1999) and neurotoxicity (Pual and Balasubramaniam, 1997). These health and environmental concerns have led to an interest in detoxification of endosulfan in the environment.

Detoxification of pesticides through biological means is receiving serious attention as an alternative to existing methods, such as incineration and landfill. A preliminary step in the investigation of enzymatic technologies for endosulfan detoxification is the definitive identification of a biological source of endosufan degrading activity. Microorganisms have increasingly been investigated as a source of xenobiotic-degrading enzymes (Chen and Mulchandani, 1998).

Several studies have reported the isolation of bacteria (Shivaramaiah and Kennedy, 2006), co-culture (Awasthi *et al*., 1997) and mixed culture (Sutherland *et al*., 2000) capable of degrading endosulfan. Mukherjee and Copal (1994) and Tejomyee and Pravin (2006) reported the degradation

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of α-endosulfan by *Aspergillus niger*. Although, *Phanerochataete chrysosporium* (Kullman and Matsumura, 1996) and *Mucor thermohylospora* MTCC 1384 (Shetty *et al*., 2000) have been examined for endosulfan degradation, these fungi were isolated for other degradative activities.

In a bioremediation process, heterotrophic microorganism break down substrates (hazardous compounds) to obtain chemical energy, hence organic pollutants can serve as carbon, energy and nutrient sources for microbial growth and a poor biological energy source when used as a sole carbon (Sutherland *et al*., 2000; Guerin, 1999). Sutherland *et al*. (2000) selected microorganisms for their ability to release the sulfite group from endosulfan and to use this insecticide as a source of sulfur for bacterial growth. Awasthi *et al*. (1997) isolated a bacterial co-culture using endosufan as a sole carbon source.

In this study, microorganisms were isolated through enrichment on endosulfan as a carbon source.

As part of the development of an overall strategy to manage organochlorine pesticide residues, the present study aimed to evaluate the potential effects of fertilizer activators on the microbial degradation of endosulfan under soil conditions.

MATERIALS AND METHODS

This studies were conducted from 2003 to 2006.

Soil Sampling

About 3 kg of soil were taken from top surface soil (10 cm) of Sondos scheme South of Jable Awelia and near Khartoum Kosti high way, Khartoum State (Sudan) using a soil auger. Soil surface was first cleaned from animal or plant debris. The collected samples were left overnight to dry in open air at room temperature. Samples were thoroughly mixed; clods or big particles were broken by hand to get uniform and reasonable size.

Sub-samples (about 1 kg) were placed in paper bag, labeled and taken immediately to University of Khartoum, Faculty of Agriculture, Soil Laboratory for Chemical and Physical Analysis.

Microbial Degradation of Endosulfan

One kilogram of the soil was sterilized by incubation in an oven at 160°C for 3 h and transferred to 1 L conical flask. Six hundred milliliter of distilled water containing a total of 100 mg endosulfan were added. The conical flasks with its content were dried in an oven at 90°C for 36 h to remove the water. The treated soil was removed from the oven and allowed to cool at room temperature. The soil was then crushed into powder by electric mill and divided into 30 sub-samples (50 g each) and each sub-sample was maintained in a separated clean conical flask (100 mL). The flask containing sub-samples were grouped into two sets. The first set was used for studying degradation using organic nitrogen bacterial inoculums (isolated by selective media from soil). This set contained the following treatment:

- Treated soil+1 mL from stock culture of organic nitrogen bacteria
- Treated soil+1 mL from stock culture of organic nitrogen bacteria+1 g urea
- Treated soil+1 mL from stock culture of organic nitrogen bacteria+1 g urea+1 g triple super phosphate
- Treated soil+1 mL from stock culture of organic nitrogen bacteria+1 g triple super phosphate
- Treated soil+1 mL from stock culture of organic nitrogen bacteria+1 g cow manure

The second set was used for studying degradation using inorganic nitrogen bacteria and actinomycetes inoculums; it contains the following treatments:

- Treated soil+1 mL from stock culture of inorganic nitrogen bacteria and actinomycetes
- Treated soil+1 mL from stock culture of inorganic nitrogen bacteria and actinomycetes+1 g urea
- Treated soil+1 mL from stock culture of inorganic nitrogen bacteria and actinomycetes+1 g urea+1 g triple super phosphate
- Treated soil+1 mL from stock culture of inorganic nitrogen bacteria and actinomycetes+1 g triple super phosphate
- Treated soil+1 mL from stock culture of inorganic nitrogen bacteria and actinomycetes+1 g cow manure

All flasks were incubated at 30°C for 60 days and residues of endosulfan were extracted and analyzed using GLC every 15 days.

Extraction of Endosulfan

Ten gram of dried soil from each flask was placed in a jar. Redistilled hexane (80 mL) and acetone (20 mL) were added. The jar was tightly closed and placed in an end over shaker for 2 h. The sample was then left to stand for a while to enable the soil particles to settle down and then filtered in a round button flask through 240 mm filter paper containing 100 mg of anhydrous sodium sulphate to absorb the moisture from the filtrate. The round button flask with its content was placed in a rotary evaporator to reduce the filtrate volume to about 10 mL. The extract was then kept in vials, tightly closed and stored in refrigerator at 5°C for the analysis. GLC analysis was done for α, β isomers and endosulfan sulphate.

GLC Analysis

Agilent 6890N gas liquid chromatograph equipped with Flame Ionization Detector (FID) and fused silica capillary column of 30 m and 0.25 mm i.d., was used for analysis of the extracts. The stationary phase (0.25 mm thickness) used was 5% phenyl methylpolysiloxane. Oven, detector and injector temperature were 250, 270 and 230°C, respectively. Nitrogen (N_2) was used as carrier gas at flow rate of 1 mL min^{-1}. Analysis of sample was done by injection of 0.2 μL. Five concentration of the standards (1, 2, 3, 4 and 5 mg L^{-1}) were injected in the GLC used for constructions of the stander curves. Reanalysis of standard solution were repeated every morning to check for the performance of the machine. Septum was changed when necessary.

Microorganism Counts

Types and counts of microorganisms were done every 15 days to assess relationship between types and number of microorganism and endosulfan degradation in soil.

RESULTS AND DISCUSSION

Organic Nitrogen Bacteria

Table 1 shows the total counts of organic nitrogen bacteria in various treatments. All treatments were highly significantly different from the control. Generally the organic nitrogen bacterial counts decreased in the first 15 days following the treatment, increased after 30 days and decreased again to level slightly higher than the first counts (15 days). Phosphate treatment caused the highest increase in organic nitrogen bacteria counts thought the various time intervals. Other treatments caused variable effects throughout various time intervals (Table 1).

Table 1: Average count of organic nitrogen bacteria per gram of fertilizer treated soils

	Time (days)		
Fertilizer	15	30	45
Control	1.3×10^4	2.2×10^4	1.0×10^4
Urea	0.3×10^4	1.6×10^5	9.8×10^4
Urea + phosphate	0.4×10^4	2.2×10^5	1.4×10^5
Phosphate	1.0×10^4	3.4×10^5	2.3×10^5
Organic fertilizer	0.4×10^4	1.9×10^5	8.8×10^4
Grand means	3.4×10^4	9.2×10^5	5.7×10^5
SE±	164.9**	544.8**	95.7**
CV	0.8	0.1	0.03

** Values significantly different; Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; Control: The soil was not treated with fertilizers; SE: Standard error; CV: Coefficient of variation

Table 2: Half lives, percentage reduction in half lives and percentage of degradation after 45 days of α-endosulfan incubated with organic nitrogen bacteria in fertilizer treated soil

Treatments	Slope	R^2	$\tau_{½}$ (days)	Reduction in $\tau_{½}$%	Degradation after 45 days (%)
Fertilizer-free soil	2.13	0.9200	22.6	37.1	91.1
Urea treated soil	2.28	0.9021	17.0	52.7	100.0
Urea + phosphate treated soil	2.03	0.7283	12.9	63.9	100.0
Phosphate treated soil	2.18	0.6742	11.2	69.0	100.0
Organic fertilizer treated soil	2.07	0.8630	15.5	56.9	100.0
Sterilized fertilizer-free soil	1.63	0.9099	36.0	0.0	66.2

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; R^2: Coefficient of determination; $\tau_{½}$: Half lives

Table 3: Half lives, percentage reduction in half lives and percentage of degradation after 45 days of β-endosulfan incubated with organic nitrogen bacteria in fertilizer treated soil

Treatments	Slope	R^2	$\tau_{½}$ (days)	Reduction in $\tau_{½}$%	Degradation after 45 days (%)
Fertilizer-free soil	1.51	0.9519	29.7	26.4	67.2
Urea treated soil	2.01	0.7331	11.5	71.6	100.0
Urea + phosphate treated soil	2.14	0.8572	14.5	63.9	100.0
Phosphate treated soil	2.15	0.8622	14.7	63.6	100.0
Organic fertilizer treated soil	2.19	0.9258	19.5	52.8	100.0
Sterilized fertilizer-free soil	1.38	0.9363	40.4	0.0	57.5

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; R^2: Coefficient of determination; $\tau_{½}$: Half lives

Table 2 and 3 show the half live of endosulfan α and β incubated for a total of 45 days in soil treated with different fertilize. The reduction in half lives ranged between 37.1- 69.5% in α-endosulfan compared to 26.4 -71.6% in β-isomer. The best enhancement in the bacterial capabilities in degrading endosulfan was obtained in soil treated with phosphate 69% reduction in half lives (Table 2). Which the best promotions of bacterial capability in degrading β-endosulfan was noticed in soil treated with urea (Table 2).

The generation of sulphate in the control treatment (bacteria inoculated activator free soil) starts at the 15th day and steadily increased until the 30 days and became non-detectable after decreased at slow rate after 45 days, the level of sulphate reached 10 mMl L^{-1} after 30 days and became non- date cable after 52 days (Fig. 1). Generally the organic nitrogen bacterial treatments (alone or combined with activator), induces significant change in the pattern of sulphate generation curves (Fig. 1-5).

Inorganic Nitrogen Bacteria and Actinomycetes

Table 4 and 5 show the total counts of inorganic nitrogen bacteria and actinomycets in various treatments. All treatments significantly enhanced the microbial counts throughout various time intervals. Phosphate and phosphate+urea treatments caused the highest increase in the counts of inorganic nitrogen bacteria and actinomycetes throughout the various time intervals.

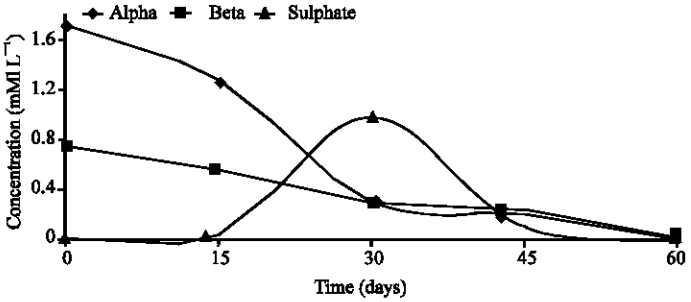

Fig. 1: Effect of organic nitrogen bacteria in degradation of endosulfan (α and β) and generation of sulphate in treated free soil

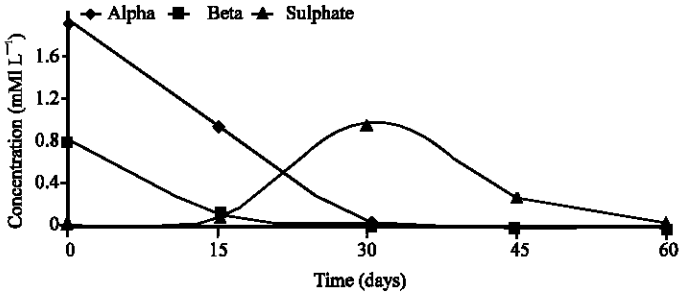

Fig. 2: Effect of organic nitrogen bacteria in degradation of endosulfan (α and β) and generation of sulphate in urea treated soil

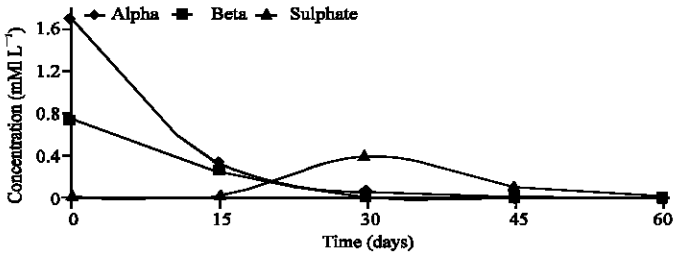

Fig. 3: Effect of organic nitrogen bacteria in degradation of endosulfan (α and β) and generation of sulphate in urea and phosphate treated soil

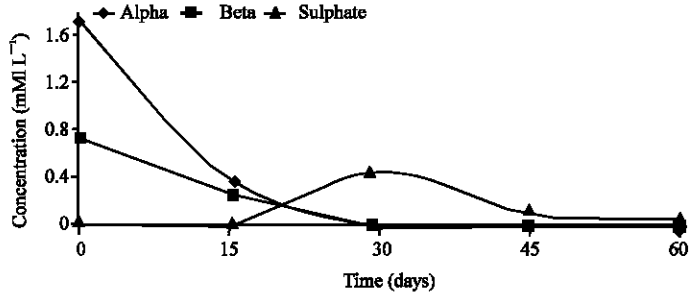

Fig. 4: Effect of organic nitrogen bacteria in degradation of endosulfan (α and β) and generation of sulphate in phosphate treated soil

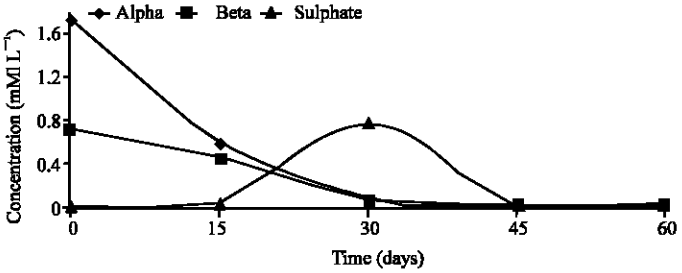

Fig. 5: Effect of organic nitrogen bacteria in degradation of endosulfan (α and β) and generation of sulphate in cow manure treated soil

Table 4: Average count of inorganic nitrogen bacteria per gram of fertilizer treated soils

	Time (days)		
Fertilizer	15	30	45
Control	3.8×10^4	6.6×10^4	2.3×10^4
Urea	9.6×10^4	1.2×10^5	2.8×10^4
Urea + phosphate	6.3×10^4	1.3×10^5	9.1×10^4
Phosphate	1.0×10^5	1.1×10^5	3.5×10^4
Organic fertilizer	6.1×10^4	1.1×10^5	2.5×10^4
Grand means	36.2×10^4	5.3×10^5	2.0×10^5
SE±	19.7	15.7	17.9
CV	0.01	0.01	0.02

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; Control: The soil was not treated with fertilizers; SE: Standard error; CV: Coefficient of variation

Table 5: Average count of actinomycetes per gram of fertilizer treated soils

	Time (days)		
Fertilizer	15	30	45
Control	1.2×10^4	1.3×10^4	0.5×10^4
Urea	4.6×10^4	1.4×10^5	4.5×10^4
Urea + phosphate	3.3×10^4	1.7×10^5	9.3×10^4
Phosphate	3.9×10^4	2.0×10^5	9.2×10^4
Organic fertilizer	3.3×10^4	1.3×10^5	0.8×10^4
Grand means	1.6×10^5	6.5×10^5	2.0×10^5
SE±	76.1	65.87	46.2
CV	0.08	0.02	0.04

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; Control: The soil was not treated with fertilizers; SE: Standard error; CV: Coefficient of variation

Table 6 and 7 show the half lives of endosulfan α and β incubated with inorganic nitrogen bacteria and actinomycetes for a total of 45 days in soil treated with difference fertilizer. The reduction in half lives ranged between 46.8-62.2% in β-endosulfan compared to 36.5-72.4% in-endosulfan. The best promotion in the capability of inorganic nitrogen bacteria and actinomycetes was obtained in soil treated with phosphate+urea; 62.2% reduction in half live of α (Table 6) and 72.4 in β-endosulfan (Table 7).

The generation of sulphate in the control treatment (bacteria and actinomycetes, activator free soil) starts at the 15th day, steadily increased until the 30 days and thereafter decreased at slow rate and became non-detectable after 40 days. The level of sulphate reached 0.6 mMl L^{-1} after 30 days (Fig. 11). However, the organic nitrogen bacteria and actinomycetes treatments induces significant change in the pattern of sulphate generation (Fig. 6-10), it starts from the first day and reach its peak (0.9 mMl L^{-1}) at the 30 days, thereafter decreased at a faster rate and became non-detectable after 45 days.

Table 6: Half lives, percentage reduction in half lives and percentage of degradation after 45 days of α-endosulfan incubated with inorganic nitrogen bacteria and actinomycetes in fertilizer treated soil

Treatments	Slope	R^2	$\tau_{½}$ (days)	Reduction in $\tau_{½}$%	Degradation after 45 days (%)
Fertilizer-free soil	1.97	0.8951	19.3	46.8	91.1
Urea treated soil	2.23	0.8593	15.4	57.2	100.0
Urea + phosphate treated soil	2.27	0.8181	13.7	62.2	100.0
Phosphate treated soil	2.18	0.8244	14.3	60.2	100.0
Organic fertilizer treated soil	2.03	0.6899	14.1	60.1	100.0
Sterilized fertilizer-free soil	1.63	0.9099	36.0	0.0	66.2

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; R^2: Coefficient of determination; $\tau_{½}$: Half lives

Table 7: Half lives, percentage reduction in half lives and percentage of degradation after 45 days of β-endosulfan incubated with inorganic nitrogen bacteria and actinomycetes in fertilizer treated soil

Treatments	Slope	R^2	$\tau_{½}$ (days)	Reduction in $\tau_{½}$%	Degradation after 45 days (%)
Fertilizer-free soil	1.29	0.8061	25.6	36.5	67.2
Urea treated soil	2.19	0.8469	14.2	64.9	100.0
Urea + phosphate treated soil	2.99	0.7192	11.2	72.4	100.0
Phosphate treated soil	2.12	0.8303	13.7	66.2	100.0
Organic fertilizer treated soil	2.47	0.8366	22.1	45.3	100.0
Sterilized fertilizer-free soil	1.38	0.9363	40.4	0.0	66.2

Fertilizers in various treatments were applied at a rate of 1 g per 50 g soil; R^2: Coefficient of determination; $\tau_{½}$: Half lives

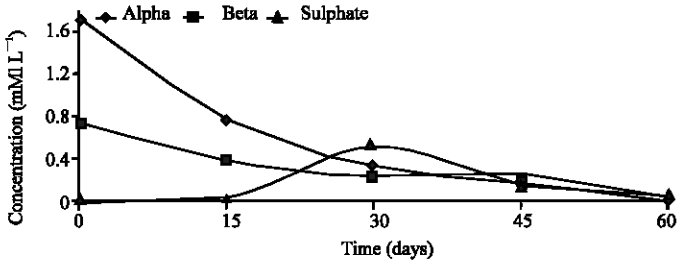

Fig. 6: Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α and β) and generation of sulphate in treated free soil

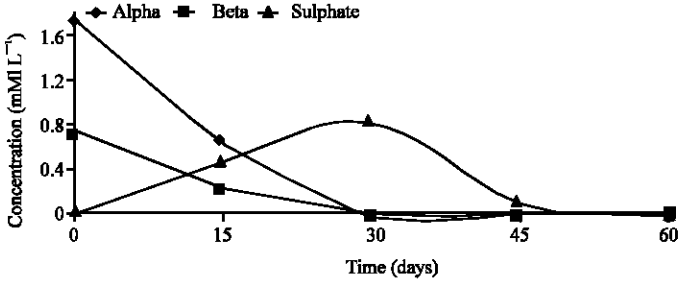

Fig. 7: Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α and β) and generation of sulphate in urea treated soil

The effect of fertilizers activators on microbial growth and capability in degrading α and β-endosulfan was studied by incubating two groups (inorganic nitrogen bacteria and actinomycetes and organic nitrogen bacteria) of microorganism in the presence and absence of four fertilizers (urea, triple super phosphate, urea+triple super phosphate and cow manure) for 60 days with sample drown

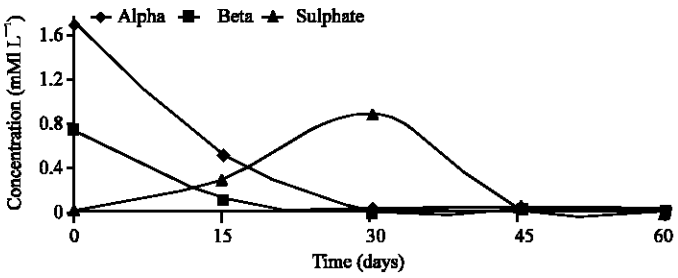

Fig. 8: Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α and β) and generation of sulphate in urea and phosphate treated soil

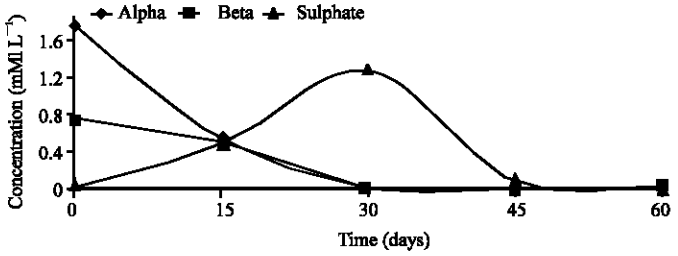

Fig. 9: Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α and β) and generation of sulphate in phosphate treated soil

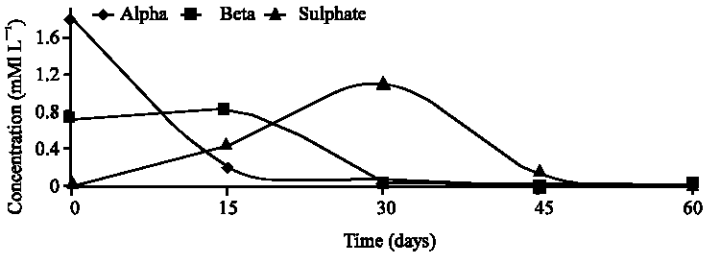

Fig. 10: Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α and β) and generation of sulphate in cow manure treated soil

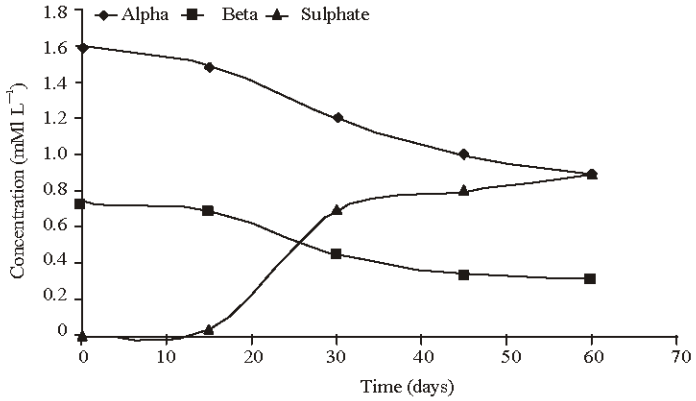

Fig. 11: Degradation of endosulfan (α and β) and generation of sulphate in sterilized fertilizer-free soil

every 15 days. Drawn sample were examined for microbial growth, concentration of starting material remained and a mount of sulphate generated. Results indicated that all activators caused significant increase in microbial counts especially the triple super phosphate followed by urea + triple super phosphate, cow manure and urea. The enhancement of counts and activity of microorganism in soil as a result of addition of fertilizer was previously reported by Alexander (1977) and Al-Hassan *et al.* (2004). Significant reduction in half lives of α and β-endosulfan accompanied with various level of sulphate generation was noticed. Since, the microorganism studied have shown great potential in degrading endosulfan therefore any enhancement in their numbers and activity (caused by activator) will no doubt promote their capability in degrading endosulfan in soil as reported by Siddique *et al.* (2003) and Al-Hassan *et al.* (2004) who studied the effect of amending soil with four different sources of organic matter on the degradation rate of α and β-endosulfan isomers.

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