THE POTENTIAL OF SOME INDIGENOUS SOIL MICROORGANISMS IN DEGRADATION OF THE INSECTICIDE ENDOSULFAN ALPHA AND BETA ISOMERS

BY

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Dedication

To the soul of my father,

my great family, mother and brothers

To my uncle Eltyp Elkrial

with love

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Abstract

Nine soil types from sites with various levels and history of contamination by insecticides were chosen as inoculums sources for the present studies.

The presences of four groups of soil microorganisms in these soils were surveyed using selective media. The results indicated that organic nitrogen bacteria is more prevalent and found in all types of soils followed by inorganic nitrogen bacteria and actinomycetes, fungi and bacteria and actinomycetes which lives in poor media.

Soil microorganisms isolated from the nine soil types showed great potential in degrading α and β-endosulfan in selective media after 15 days of incubation. Generally the results indicated that microorganisms isolated from highly contaminated soils had greater potential in degrading the two isomers of endosulfan.

The effects of soil microorganisms isolated from highly polluted soil, on half-lives of α and β- endosulfan under condition of selective and carbon free media were re-studied for longer period. 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.

The effect of fertilizer activator on enhancing the microbial growth and capability in degrading α and β- endosulfan under soil conditions was studied. Results indicated that all activators caused significant increase in microbial counts especially the triple super phosphate. The accompanied reduction in half lives ranged between 20-73% with various levels of endosulfan sulphate generated.

Microbial degradation at elevated concentration (500 mg/l) of endosulfan in carbon free was studied and the results showed that there were no

significant differences in the reduction of half-lives between high (500 mg/l) and low (100 mg/l) concentration.

Tolerant strains of bacteria and fungi from the soil of Rass Elfeel pesticide store (Mangil scheme) were isolated through consecutive exposure to elevated concentration of endosulfan under condition of carbon free media and the results showed that the most tolerant fungi (can tolerate up to1000 mg/l) was *Aspergillus fumigates* while the most tolerant bacteria was *Bacillus sp*. The comparative degradation of endosulfan by tolerant strains and their parents was studied under condition of soil and carbon free media. Results showed that parent strains (present in large number) showed faster decrease in half lives compared to tolerant strains (few numbers). However tolerant strains might have greater potential if they find a chance to propagate in massive numbers.

ملخص الأطروحة

تم اختيار 9 أنواع من الترب تحتوي على نسب مختلفة من متبقيات المبيدات كمصادر للتلقيح. اشتمل البحث على سبع تجارب معملية مختلفة. تم إجراء مسح لأنواع واعداد أربع مجموعات من الكائنات الدقيقة الموجودة في التربة (البكتريا التي تتغذي على النيتروجين العضوي، البكتريا والاكتينوميست التي تتغذي على النيتروجين غير العضوي، البكتريا والاكتينوميست التى تعيش فى الاواسط الفقيرة والفطريات) فى أواسط غذائية اختيارية. أظهرت النتائج وجود هذه الكائنات بنسب متفاوتة و كانت البكتريا التي تتغذي على النيتروجين العضوي اكثر وجوداً في جميع الترب وبنسبة أعلى من بقية الكائنات الأخرى.

تناولت التجربة الثانية دراسة مقدرة الكائنات الدقيقة في هدم مبيد الاندوسلفان بعد تحضينها بهذا المبيد لمدة 15 يوم. أظهرت النتائج أن الكائنات الدقيقة المعزولة من ترب اكثرتلوثاً لها مقدره اعلى فى تحلل المبيد . درس تأثير الكائنات الدقيقة المعزولة من الترب ذات التلوث العالي على فترة بقاء الاندوسلفان في أوساط غذائية اختيارية وفي وسط خالي من اي مصدر للكربون لفترة اطول وذلك بقياس تأثيرها على نصف عمر المبيد، أظهرت النتائج فروقات معنوية تتراوح بين 58 –81,9% في الفا اندوسلفان مقارنة 35.5 - 71.6% في بيتا اندوسلفان.

تمت دراسة تأثير إضافة الأسمدة الزراعية على إعداد ونشاط الكائنات الدقيقة وعلى مقدرتها في تحليل الاندوسلفان في التربة. أظهرت النتائج أن كل الأسمدة أحدثت زيادة ملحوظة في اعداد الكائنات الدقيقة خاصة سماد السوبر فوسفات الثلاثي. أدت هذه الزيادة الي نقص في نصف عمر المبيد بنسبه تتراوح بين 20 – 73% مصحوبا بنسب مختلفة في تكوين كبريتات الاندوسلفان.

تمت دراسة معدل هدم الاندوسلفان في تركيز عالي من المبيد (500 ملم/لتر) في وسط خالي من الكربون .لم تظهر النتائج فروقات معنوية في نقص عمر المبيد عند التركيز العالي (500 ملم/لتر) والتركيز المنخفض (100 ملم/لتر).

تم عزل بكتريا وفطريات من مخزن رأس الفيل (مشروع المناقل) وتم تعريضها لتراكيز متزايدة ومتتالية من الاندوسلفان في وسط خال من الكربون. أظهرت النتائج أن فطر *Aspergillums fumigatus* تحمل حتى تركيز (1000ملم/لتر) وكذلك بكتريا *Bacillus sp.* كذلك تمت مقارنة مقدرة الأنواع الاكثر تحملا مع آباء هذه الأنواع تحت ظروف التربة وفي وسط خال من الكربون على تحال الاندوسلفان. أظهرت النتائج أن الآباء (أكثر تعدادا) لهم مقدرة أسرع في تقليل نصف عمر المبيد مقارنة مع السلالات الآكثر تحملا (أقل تعدادا).

لكن ربما يكون للاخيرة (السلالات الاكثر تحملا) مقدرة اعلي اذا وجدت الفرصة للتكاثر باعداد كبيرة.

CHAPTER ONE

Introduction

Pesticides occupy rather a unique position among many chemicals that man encounters regularly. They are usually added to the environment to kill or injure some forms of live. Most of the chemicals that are used, as pesticides, are not highly selective. But instead are generally, toxic to many forms of life that co-inhabit the environment. Hazards of pesticides usually accompany their mesuses and handling, yet significant ecological implication motivated many researchers, in various contents, to investigate the environmental impact of pesticides use in all ecosystem.

Pesticides in the environment are subject to various degradative processes. These processes include; chemical, physical and biological means. Biological or biodegradation can occur by higher plants or animals or by microorganism. The action of former groups is limited to pesticides directly deposited on them or brought to their vicinity by various ecological factors. However microbial degradation is considered as important route for pesticide conversion in nature.

Microbial degradation refers to the breakdown of pesticides by bacteria, actinomycetes or other microorganisms. Biodegradation can encompass many processes with drastically differing outcomes and consequences. Xenophobic contaminants including pesticides (Lamar, 1990) might be mineralized or converted to completely oxidized products like CO_2, transformed to other compounds that are toxic or nontoxic, accumulated within the organism, or polymerized or otherwise bound to natural material in soils, sediments or water. More than one of these processes may occur for a single pollutant at the same time.

Most microbial degradation of pesticides occurs in the soil which is rich in microorganism compared to other environmental compartments. Soil conditions such as moisture, temperature, aeration, PH and the amount of organic matter affect the rate of microbial degradation because of their direct influence on microbial growth and activity. The frequency of pesticides applications can also influence the microbial degradation. Rapid microbial degradation is more likely when the same pesticide is used repeatedly in a field. Repeated applications can actually stimulate the buildup of microorganism capable of degrading the chemical.

As the population of these organisms increase, degradation rate will be accelerated and subsequence amount of pesticides available to control the pests is reduced. Bioremediation refers to the productive use of biodegradative processes to remove or detoxify pollutants that have found their way into the environment and threats publics' health. Bioremediation is currently used to decontaminate polluted soil, water or sediment with many classes of pollutants.

Before large scale technological application of bioremediation basic research to identify and characterized promising biological processes in required first. Then pilot scale testing follows, before the acceptance by regulators and the public. All processes their effectivenes and safety must be confirm through step-wise well studied programs. Bioremediation is a cheep technology compared to others. Levin and Gealt (1993) estimated the costs of biotreatment of biodegradable contaminants in soil to range between 40$ & 100$ per cubic yard as compared to the costs of 150$ - 250$ per cubic yard for land filling. There are five known types of bioremediation techniques; above ground bioreactors, solid phase treatment, composting, in situe treatments and land forming.

Sudan is considered as one of the large consumers of pesticides in Africa and Arab world. Trials for use of pesticides in Sudan started with the introduction of Brodeaux mixture in 1941 followed by DDT for the control of cotton jassid in Gezira Scheme in 1949. The success of the trial, which started with a single application against single major pest initiated the interests for expansion in the treated area and opened the way for subsequent introduction of other related compounds. Early in the sixties organophosphate became a reliable partner to the organochlorine for control of complex of chewing and sucking insect pests.

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 (Butrous, 1999). 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 (Babiker, 1998). Therefore, in situe treatments of affected situe appeared move attractive, suitable and could be feasible. Preliminary reports (Almahi, 1996, Abdelbagi, *et al*., 2000; 2003) argued the potential use of endogenous soil microorganisms in cleaning highly polluted soil and dump sites.

Ali, (2005) conducted preliminary investigation on the potential role of endogenous microorganism in degradation of endosulfan and γHCH. The preliminary results indicated a promising role of such bioagents. These results initiated our interest to further evaluate such agents under laboratory conditions. The specified objectives of the study are:-

(1) To survey types and counts of naturally occurring microorganisms in nine types of soil with variable levels of contamination.

(2) Isolation of natural microorganism that is capable of degrading endosulfan α & β isomers.

(3) Evaluation of their degradative capability under conditions of;

    (A) Selective media.

    (B) Carbon free media.

(4) Evaluation of potential effects of fertilizer activators on their degradation capability under soil conditions.

(5) Evaluation of the degradative capability of selected mutant microorganism (tolerant to high dose of endosulfan).

GHAPTER TWO

Literature Review

2.1. Organochlorine Insecticides

The organochlorines (oc) are insecticides that contain carbon, hydrogen and chlorine. They are also known by other names: chlorinated hydrocarbon, chlorinated organic, chlorinated insecticides and chlorinated synthetics.

Organochlorines represent a wide group of pesticide chemicals which falls into four subcategories based on chemical nature and mode of action;

(a) Diphenylaliphatics:

The oldest group of organochlorines is the diphenyl aliphatics which included ; DDT, DDD, dicofol, ethylan, chlorobenzilate and methoxychlor .DDT is probably the best known and one of the most notorious chemicals of the 20^{th} century . It is also fascinating and remains to be acknowledged as most useful insecticide developed. More than four billion pounds of DDT were used throughout the world, beginning in 1940 and ending essentially in 1973 when the USA Environmental Protection Agency banned all its uses. The remaining first world countries rapidly followed USA. In 1948 Dr. Paul Muller , Swiss entomologist , was a warded the Nobel prize in medicine for the live saving caused by the discovery of the insecticidal activity of DDT 1939, which effectively control malaria, yellow fever and other insect vector borne diseases.

(b) **Hexachlorocyclohexane**(HCH)

Also known as benzenehexachloride (BHC). The insecticidal properties of HCH were discovered in 1940 by French and British entomologist. In its technical grade, there are five isomers: alpha, beta, gamma, delta and

epsilon. Surprisingly, only gamma isomer has insecticidal properties .Consequently, the gamma isomer was isolated, manufactured and sold as odorless insecticide called lindane. In contrast, the technical grade HCH has a strong odor and flavor, which can even be felt or detected in treated crops and animal products. Because it has very low cost, HCH is still used in many developing countries.

(c) **Cyclodienes:** -

The cyclodienes appeared after world war Π: chlordane 1945, aldrin and dieldrin, heptachlor 1949, endrin 1951, mirex 1954, endosulfan 1956, and chlodecone 1958.There are other cyclodienes of minor importance developed in USA and Germany. Most of the cyclodienes are persistent insecticides and are stable in soil and relatively to the ultraviolet portion of the sun light spectrum. As a result they were used in large quantities as soil insecticides (specially chlordane , heptachlor, aldrin and dialdrin) for the control of termites and soil-borne-insects whose larval stages feed on the roots of plants .The cyclodienes were the most effective, long-lasting and economical termiticides ever developed. Because of their persistence in the environment, resistance developed in several soil insects and in some instances biomagnifications in food chain, most agricultural uses of cyclodienes were banned by EPA between 1975 and 1980 and their uses as termiticides were banned in 1984-1988.

(d) **Polychloroterpene**s: -

Only two polychloroterpenes were developed: toxaphene in 1947 and strobane in 1951. Toxaphene had by far the greatest use as insecticide in agriculture while strobane has relatively insignificant uses. Toxaphene was first used on cotton, in combination with DDT. then in 1955 after several major cotton insects became resistant to DDT, toxaphene was formulated

with methyl parathion, an organophosphorous insecticide. Toxaphene is a mixture of more than 17710 carbon polychlorinated derivatives. These materials persist in soil, though not as long as cyclodienes, and disappear from the surface of plants in 3-4 weeks. This disappearance was attributed more to volatility than to photolysis or plant metabolism. Toxaphene is rather easily metabolized by mammals and birds and is not stored in body fat compared to DDT, HCH and cyclodienes. Despite its low toxicity to insects, mammals and birds, fishes are highly susceptible to toxaphene poisoning, in the same order of magnitude as to cyclodienes. Toxaphene was banned by US- EPA in 1983.

2.1.1 Mode of action

DDT and related compounds, as well as cycloalkanes are axonic poisons which bind to the lipoprotein structure of the Na+ channel, while cyclodienes interact with GABA (γ-amino butyric acid, inhibitory neurotransmitter) receptor and , thus affect permeability of chloride ion, which is responsible of hypo depolarization of the nerve membrane (Mutsomura 1985). The cyclodienes have positive temperature correlation with their toxicity increase with increase in the surrounding temperature. Toxaphene and strobane act on the neurons, causing an imbalance in sodium and potassium ions, similar to that of cyclodene insecticides.

Due to their lipophilic nature organochlorine can easily inter the human body and accumulate in fats which can be metabolized in time of stress (Mathews *et al*., 1974). Further, these insecticides are known to affect the biochemistry of mammalian system in other ways. These include induction of microsomal drug-metabolizing enzymes and influence on steroid metabolism (Matsumura 1985).

2.1.2 Use in Sudan.

Organochlorine insecticides were first introduced in the Sudan in 1945. Trails started by using DDT in Gezira scheme to control cotton pests, followed by Dieldrin, HCH and other organochlorine insecticides. The last four decades from late forties to nineties, an intensive usage of these compounds in the country. Ten million Kg active ingredient of DDT was sprayed in Gezira scheme (Elzorgani, personal communication).

In 1982 the usage of DDT for agricultural purposes was banned. Eight years later the application of dieldrin and other organochlorine insecticides in agriculture sector was restricted to certain pests. Later in 2002 the uses of all OC were banned except endosulfan. Currently, endosulfan is the only OC compound which is still in use on cotton. Endosulfan is extensively used on cotton and it comprise 45% -50% of the annual spray on this crop

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2.1.3 Residues of OC Insecticides

2.1.3.1 Residues in water and sediments

Some of organochlorine (OC) insecticides can stay suspended in water. The level of OC pesticides in water do change from year to year as in USA where level in surface water reached a peak in 1966 and declined sharply in 1967 and 1968 except for BHC, which showed only slight decline. Dieldrin is the most serious pollutant in USA surface water (Matsumura 1985). Long-term contamination in bottom samples of rivers was reported by (Zobic *et al*., 1971).

In Sudan measurable level of γ HCH and heptachlor were detected in runoff and well water samples surrounding pesticides stores in central Sudan

(Babiker 1998).Further Abdelatif (2006) detected measurable levels of some pesticides including OC in Nile water.

2.1.3.2 Residues of OC in food

In Sudan Elzorgani *et al*. (1979) investigated the presence of persistence OC insecticides in the fish fauna of lake Nubia. They found that all the 10 samples examined contained detectable levels of insecticides residues. P.P-DDE was found in all samples while DDT was found only in three muscle samples. The highest level of total residues (184 mg/kg) was found in a muscle sample of *Hydricynus forscallii*. Similar study was conducted on fishes from the Gezira canals, collected specimens of five fish types examined were also, found to contain measurable samples residues (Elzorgani, 1976).

2.1.3.3 Residues in human samples

Humans being at the top of the food chain are the terminal depots for persistent environmental contaminants like OC insecticides. It's well known that OC insecticides and their metabolites can accumulate in human body. Many excellent surveys have been made in various countries on residues of these insecticide and their levels in adipose tissue. In Sudan milk and blood of human population were not properly well covered and reported data of this aspect is very little and fragmented. Direct application in areas of intensive use, drifts during active spray seasons and winds blown dusts were among the anticipated means of residue transport. Levels detected were not high even in the area of intensive use (Elzorgani, 1976)

2.1.3.4 Residues of OC in soil

Elmahi (1996) and Abdelbagi, *et al*., (2000, 2003) reported measurable levels of OC insecticides (Aldrin, dieldrin, heptachlor, DDT, ð-HCH) from the areas of intensive and limited insecticides use in Sudan. Their reports showed higher level in cotton soils than sugarcane, river-rain and rain-fed areas. Babiker (1998) reported the presence of some OC insecticide moving at different rates from Qurashi stores soil.

2.1.4 **Environmental Fate of organochlorine insecticides**

Soil serves as the largest environmental reservoir of the applied pesticides. OC insecticides were known for their long persistence in soil. Their persistence is determined by many factors; The insecticidal properties of the compound itself is the major factor. Other external factor had important role in the fate are deposited surface, leaching and washing by water, evaporation, degradation and activation by soil microorganism, sunlight and transportation through the biological systems to other environments (Matsumura 1985). Additional important factors known to influence the fate of insecticides in soil, are: soil type, moisture, temperature, mode of cultivation, plant cover, mode of application and strength of sorption to soil particles, (Edwards 1964).

Aldrin and dieldrin can be adsorbed by organic matter and this decreases the chance of their disappearance by the action of various factors. Air movement over the insecticides contaminated soil is very important. Spencer *et al.* (1969) reported that the rate of dieldrin disappearance from soil is a function of air movement through soil. Keanrney, *et al.* (1969) reported on the accumulation of organochlorine residue in USA beneath orchard tree and

cotton field and attributed the high concentration to their poor solubility in water .

The fate of OC in the environment brought about by various factors such as physical, biological and chemical factors.

2.1.4.1 **Physical factors**

The major physical factors known to influence the residual fate of OC insecticides in nature are light, surface, moisture and temperature. Sunlight appears to have the most significant contribution in this aspect , particularly the ultraviolet portion of sunlight spectrum. The sunlight reaching the surface of the earth does not have any ultraviolet component below 280 nm. This because the atmosphere effectively eliminates such short wave by absorption in ozone layer. One of the most important factors affecting the rate of sunlight degradation of insecticides is the presence of photosensitizes compounds that facilitate the transfer of the light energy to the receptor chemicals. Another important factor is the medium in which the reacting insecticides are dissolved or suspended. Other physical factors include temperature and aeration. O.C insecticides disappear much faster at high temperature (Spenser, *et al*., 1969).

2.1.4.2 Chemical factors

The most important factors contributing to the degradation of OC pesticides appear to be the chemical nature of insecticides itself. pH play an important role in degradation of OC insecticides and generally OC insecticides persist longer in acidic soil than alkaline soil (Mutsomura, 1985).

2.1.4.3 Biological factors

Metabolism by microorganism, higher plants and animals are the most important biological factors that play significant roles in reducing the level of contamination by pesticides in any given site. Higher plants and animals can reduce the level of contamination through their active uptake and metabolism. However their effects are limited to pesticides deposited directly on them or brought to their vicinity by various transport processes. Their metabolic reactions include oxidation, reduction, dehalogenation, hydrolysis and elimination. Pesticides may be activated into more toxic compound or deactivated into many non- or- less toxic products.

Microbial mediated alteration plays a significant role in the degradation of pesticides and many environmental pollutants in nature (Matsumura, 1985). Generally, soil is rich in microbial fauna and flora while oceanic and atmospheric compartments are devoid of microbial activities. (Alexander, 1965). Microbial activity can by affected by number of factors such as ;

1- Temperature, the cold temperature reduced microbial activity.

2- pH affects types of microorganisms present and the rate of pesticide degradation. Generally, bacteria predominate in soil with pH of 5 or more while acidic soils are dominated by fungi. In acidic soil, with pH 4, in the Pacific North West and north east of USA, DDT does not break rapidly.

3- Aeration of soil and water: aeration is related to the rate of oxygen diffusion which is 10^{-5} less in water than in air. The rate of oxygen diffusion is important in aerobic reactions such as oxidation while oxygen is not a limiting factor for anaerobic reactions such as reduction.

4- Nutrients ; soil is generally regarded as nutrients deficient for microbial growth, which require carbon. The unique characteristic of microorganism is

their ability to utilize a pesticide chemical as carbon source. Also higher plants and animals can do the same by completely mineralizing the pesticide into carbon dioxide but their metabolism is mainly incidental to energy production (Matsumura, 1985). Beside carbon microorganisms need other nutrients such as nitrogen, phosphorus and sulfur. Attempted treatment of soil by microorganisms is called bioremediation, which is currently considered an important mean of cleaning highly polluted soil and dump sites.

5- Soil Depth **:** high microbial population is confined to the top 10-50 cm layer depending on soil type, climate and human activity. Thereafter the microbial activity decrease gradually with depth. Generally were pesticide pass this active layer is not subject to microbial degradation.

6- Toxic substances**:** these include organic material such as phenolic compounds and non-organic material such as ions of Hg, Ag, Cd and Ni.

2.1.5 Endosulfan insecticides

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-benzodioxathiepin 3-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 (Casida, 1993).In particular, endosulfan has a relative cyclic sulfate diester

group, and as a consequence, its environmental persistence is lower than that of other cyclodienes (Van Woerden,1963).

In Sudan it was tested for the first time in small scale experiment against cotton pests in season 1966/67 (El Zorgani, 1979).It is recommended for use against white fly *bemisia tabaci* and *Aphis gossypii* (El Zorgani, 1976)

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2.1.5.1 **Endosulfan toxicity**

Acute oral DT_{50} for rat 70 mg/kg (in aqueous suspension), 10 mg/kg tech (in oil), 76 mg/kg for α isomer, 240 mg/kg for β isomer. Acute percutaneous DT_{50} for rabbits is 350 mg/kg (in oil), for male rats > 400 mg/kg, for female rats 600 mg/kg. The inhalation LC50(4h) for male rat is 0.0345 mg/kg, 0.10126 mg/kg for female rat (FAO\WHO,83,85).

2.1.5.2 **Endosulfan use**

It is non systemic insecticide and acaricide with contact and stomach action. Uses for control of sucking chewing and boring insects and mites on a very wide range of crops.

2.1.5.4 Fate in human body and The Environment

Residues of endosulfan accumulate in the kidneys rather than fats. Elimination from kidneys take place with a DT_{50} of 7d, but there is no sign of accumulation in the kidneys even after long-term feeding. Endosulfan is metabolized rapidly in mammalian organisms to less toxic metabolites and to polar conjugates. The plant metabolites, mainly endosulfan sulfate, were also found in animal and have thus been investigated from a toxicological point of view. Fifty percent of residues are lost in 3-7 days, depending on plant species. Endosulfan (α and β) and their metabolite endosulfan sulfate,

is degraded more slowly. For this reason the sulfate is considered as the most important metabolite. DT_{50} for total endosulfan α, β and sulfate in the field were 5-8 months. No leaching tendency was observed.

Balschmitter *et al*. (1976) had considered all of the possible metabolites of endosulfan which can hypothetically be formed on hydrolysis, oxidation or reduction. They have investigated endosulfan metabolism in mouse and rat using thin layer and gas chromatography. These Chromatographic techniques have identified five of the possible metabolites, including endosulfan sulfate, diol, ether, hydroxyether and lactones.

2.1.6 Problems of pesticides storage in Sudan

Butrous (1990) studied the condition of storage facilities, quality and quantity of obsolete pesticides and the possible methods of disposal of obsolete pesticides in Sudan. The study covered the Blue Nile area (Suki), Elrahad, New Halfa and Gezira scheme. He found that most of the visited sites have no proper pesticides stores, even the existing ones were of poor ventilation and not suitably located. He suggests that the stores required construction and rehabilitation for two years and should be re-constructed in suitable isolated location. Proposed improvements should also include secured fence, Locked gates, pesticide stores and sheds, storekeepers, offices, emergency showers and toilets. He proposed construction of large and small stores and sheds in most of the visited sites. He also suggested that different schemes should coordinate between them in order to organize the use of the small quantities of carry over during the next season therefore they can avoid the accumulation of small quantities through years. He also reported on problems of pesticide empty containers that pose a serious environmental hazard. Containers, and drums with different ages and

conditions were kept on an open ground in non-fenced or poorly fenced areas with no proper disposal programs.

2.1.7 The problems obsolete stocks

The continuous expansion and intensification of the cropped area in Sudan over periods from the 60s of last century have led to the important of huge amounts of pesticides into the country. The poor storage facilities and management practice have led huge amount of these pesticides to become obsolete. According to an investigation in 1998 the total amount of obsolete pesticides in the country was estimated at 666 tons, 77.5% in liquid state and 22.5% as solid with about 6459 cubic meter of contaminated storage soil and about 8046 tons of empty drums scattered over 43 major and minor sites in the country. About 91 types of active ingredients were identified according to its container labeling with additional 45 unknown chemicals. The transformation products of these stocks were also unknown. Substantial amount of these chemicals were 15-25years old and many of them were left on bare ground directly under sun light for long period. Corroded containers drained significant amount of chemicals to underneath soil. There is no proper disposal procedure for empty containers which were thrown carelessly and sometimes used for carrying drinking water.

Many of the pesticides stores were located in close proximity to human residence. Signs of human and animals intoxication were usually encountered during rainy season and windy days (Botrous 1999). The limited investigation carried indicated potential threat to human life and environment.

2.2 Microorganism

General

In microbiology of soil it is essential to consider carefully the nature of the environment in which the microorganisms find themselves. The forces that play role in the dynamics of soil populations and the effects of these populations on their environment are governed to great extent by the physical and chemical properties of the soil. Soil is composed of five major components: - mineral matter, water, air, organic matter and living organism. The quantity of these constituents is not the same in all soils but varies with the locality.

Soil contains five major groups of microorganism; the bacteria, actinomycetes, fungi, algae and protozoa (Alexander, 1965). The soil ecosystem includes these microbial groups as well as the inorganic and organic constituents. All of the inhabitants of the particular locality make up the community. The collection of cells or filaments of the individual species that are represented in the community are considered as distinct population. Bacteria are especially prominent because of their many population in any given soil and because the fact that they are the most abundant group, usually more numerous than the other four combined.

2.2.1 Bacterial

The number of bacterial cells in the soil is always great, but the individuals are small, rarely more than several micrometers in length. Because of the minute size of the bacteria and the large cells or extensive filaments of the other four groups, the bacteria probably account for appreciably less than half of the total microbiological cell mass. In adequately aerated soil the

bacteria and fungi dominate whereas bacteria alone account for almost all the biological and chemical changes in environments containing little or no oxygen. Although many transformations similar to those of the bacteria are carried out by the other groups ,the bacteria stand out because of their capacity for rapid growth and vigorous decomposition of variety of natural substrates. Number of bacteria is high, normally ranging from a few hundred million to three billion in each gram of soil (James and Sutherland (1939).

In the soil bacteria exist as mats chumps, filaments, called colonies around the soil particles wherever food and other conditions are favorable. The standard methods of examining soils for viable counts often give variable numbers and the errors in sampling and in sample preparation are frequently far greater than the variations inherent in the counting procedure itself. This limitation can be minimized by the use of many composites prepared from numerous boring made in the field. It is far better to use many sub sample than numerous replicate plates per dilution since the variation among duplicate soil samples is far greater than the variation between replicate plates or replicate dilution (James and Sutherland 1939).

The bacteria isolated from soil can be placed in two broad division; the indigenous or autochthonous species that are true residents and the invaders or allochthonous organism . Indigenous population may have resistant stages and endure for long periods without being active metabolically ,but at sometime these natives proliferate and participate in the biochemical functions of the community. Allochthonous species, by contrast, do not participate in significant way in community activities (Alexander, 1965)

Environmental conditions affect the density and composition of the bacterial flora. The non biological factors can frequently alter greatly the

community and its biochemical potential . The primary environmental variables influencing soil bacteria include moisture, aeration, temperature, organic matter, acidity and inorganic nutrient supply. Many lesser variables such as cultivation, season and depth have been described and are of undoubted significance but their influence arises from combinations of the primary determinants

2.2.2 Actinomycetes

The true bacteria are distinctly different from the filamentous fungi and many morphological characteristic separate the two broad types. There is, however, transitional group between bacteria and fungi, with overlapping boundaries it more primitive and its more develop neighbors. These are the actinomycetes. They have no taxonomic validity since these organism were classified as bacteria in a strict since, all being members of the order actinomycetales, but not all genera of the actinomycetales are considered to be actinomycetes in common parlance. The actinomycetes are microorganisms that produce slender, branched filaments that develop into a mycelium in all soil genera except for the genus *Actinomyces*.

The relation of actinomycetes to fungi is clear in three properties; (a) Mycelium of higher actinomycetes has the extensive branching characteristic of the fungi; (b) Like fungi many actinomycetes form an aerial mycelium as well as conidia; and (c) Growth of actinomycetes in liquid culture rarely results in the turbidity associated with unicellular bacteria, instead it occurs as distinct clumps or pellets. On the other hand, the morphology and size of hyphae, conidia and of the individual fragments of species whose mycelium undergoes segmentation are similar to structures found among the bacteria(Alexander, 1965).

In addition some actinomycetes genera produce no aerial mycelium, and they closely resemble Mycobacterium and the coryneform bacteria in general morphology staining reactions and physiology.

Recognition of colonies of the most abundant actinomycetes on agar media is relatively simple providing the incubation period is sufficiently long. Whereas the usual bacterial colony consist of a large population of individuals derived from single cell by binary fission, while that of the actinomycetes prior to sporulation consist of one organism, a mycelium derived from a single propagative unit. The colonies of some genera developing on the agar surface may have a firm consistency and adhere tenaciously to the solidified substratum; In certain types of these genera, the surface appears powdery and often becomes pigmented when the aerial spores are produced. In the organisms having a simple mycelium, the colony has a more mealy consistency and often crumbles when touched (Alexander, 1965).

Actinomycetes are numerous and widely distributed not only in soil but in variety of other habitats including composts, river mud sand lake bottoms. They are present in surface soil and also in the lower horizons to considerable depths. In abundance, they are second only to the bacteria, and the viable counts of the two are sometimes almost equal. Particularly in environments of high pH, a large proportion of the total community consists of the actinomycetes.

A variety of microscopic or planting methods have been used in ecological investigation, but only the latter techniques are truly quantitative for these microorganisms. Despite the frequent use of plating, it is remarkable that the count do not seem to be greatly affected by the composition of the medium, an indication that the organism can utilize a variety of organic nutrients.

Counts may be made upon the plates that are used for bacteria enumeration but special media are often preferable. In either instance, the period of incubation must be some what longer than that for the bacteria because of the slow growth characteristic of actinomycetes. A useful selective media for enumeration are those containing chitin (Kuznetsov, and Yangulova, 1970).This polysaccharide used at high percentage of the more common actinomycetes and by a relatively lower percentage in bacteria and fungi. Media supplemented with antibacterial compounds are also sometimes employed, (Ottow,1972). In their normal habitats the actinomycetes may occur as conidia or as the vegetative hyphae, and both form can give rise to colonies on agar media. Thus a determination of colony numbers on agar media will not differentiate between propagative units derived from a single conidium, unbroken cluster of conidia, or hypha fragments, and the onset of sporulation in soil will result in high counts despite the lack of appreciable change in total protoplasmic mass.

The size of the community depends on the soil type, particularly on certain physical characteristics, organic matter content and pH of the environment. Planting estimates give values ranging from 105 – 108 per gram in temperate zones, but lower figures have been found in regions of Antarctica, acid peat, tundra and in water logged soils and counts in excess of 100 million have been encountered occasionally. By and large, actinomycetes make up from 10 to 50 percentage of total community determinates by planting in both virgin and cultivated land.

In qualitative and quantitative terms, the actinomycetes flora are governed by the surrounding habitat. The stage of the live cycle that predominates, the size of the community, its biochemical transformations and the genera and species found are determined by the forces acting within the ecosystem. Any

one biological system is in the last analysis are reflection of the other biological systems functioning in association or in opposition and of the physical and chemical characteristics of the ecosystem. For the actinomycetes, the primary ecological influences include the organic matter status, pH, moisture and the temperature. Season of year and depth in the profile are also of no little consequence, but the role of these two variables seems to be largely an outcome of interactions among the primary determinants.

Actinomycetes are affected directly by the presence of a viable carbon and their number is especially great in land rich in organic matter. This is true whether examination is by plate counting or by one of several microscopic techniques. In general, site high in carbonaceous materials and humus have larger number than habitats poor in organic matter.

Most of actinomycetes are aerobic, except those of the genus actinomyces which is anaerobic or micro aerophilic. The majority of actinomycetes are mesophilic but several thermophilic species occurred in strerptomycetes which exhibit a strong resistance to heat and dryness (Jagnow, 1957). Many of actinomycetes are distinguished by a high acid tolerance (Stapp, 1952; Szabo *et al.,* 1975) except few species e.g. strerptomycetes, which grows best in alkaline media and primarily occur on substrates with slightly alkaline reduction. Some species of *strerptomyces* show a marked sensitivity to acid. Shaking of the soil cause breaking of hyphae and consequently increase in the number of colonies (Skinner.1957). Actinomycetes decrease with the depth of soil, moreover the number of different species in deeper layers is much reduced. Szabo *et al*., (1975) found that sterile types dominate deeper layer (β- horizon). While sporulating types more frequently occur in (α- horizon). They explain this by the better aeration, dryness and

low temperature.

2.2.3 Fungi

In most well- aerated, cultivated soils fungi account for a large part of the total microbial protoplasm. Although enumeration procedures used with other microbial groups tend to suggest that fungi are not a major soil inhabitants, they do in fact make up significant part of the biomass because of the large diameter and extensive network of their filaments, especially in the organic layer of the woodland and forest soils do the fungi dominate the microbial protoplasm contained within the decomposing litter, but acid environments in general have the fungi as major agents of decay.

Several techniques have been developed for the study of the fungal flora, each with its own advantages. No single procedure adequately describes the entire generic composition of the flora nor does any one method depict accurately the mass or biochemical capacities of the hyphae. The approach most frequently used for enumeration is the plate count in which dilutions of soil specimen in sterile water are plated on a suitable agar medium. Because bacteria and actinomycetes are usually more numerous than fungi conventional laboratory media can not be used as the development of fungal colonies on the Petri dishes will be suppressed. Early microbiologists overcame the problem of suppression on solid media by acidifying the agar to pH 4.0, a reaction at which few bacteria and actinomycetes but most fungi develop. On the other hand acidification is not necessary provided that appropriate bacteriostatic agents are included in the counting medium. Penicillin, novobiocin, rose Bengal and streptomycin have thus been used to inhibit bacteria and actinomycetes. Population estimates of fungi based on plate counting are open to serious criticism. Since colonies appearing on the

agar may be derived from a spore or a fragment of vegetative mycelium, the active or dormant nature of the viable unit in the original sample is unknown. Furthermore then readily sporulating genera appear in large number on the agar plate because each individual spore may give rise to a colony. It is not surprising that fungi sporulating profusely, for example, Pencillium and sperogillus spp; are isolated frequently. The mere act of shaking often introduces an error into the population estimate, for the agitation tends to rupture the mycelium and sporulating body in to an indeterminate number of fragments each of which may produce a single colony. For these and other reasons the result of plate counts must be interpreted with considerable care, bearing in mind the numerous short coming of the technique. Fungi may be investigated in a number of ways not involving soil dilutions. Each additional technique serves to help in the characterization of the composition of the flora. Procedure for direct microscopic observation of the upper soil crust in site has been used occasionally, but special apparatus required and the spare data obtained have limited the widespread adoption of these method. For the purposes of enumeration, conventional plate counts have been most widely used since, although the results are far from unequivocal, this procedure permits a degree of quantification. Such estimate of microbial density reveal the presence of populations typically ranging from 20,000 to many as 1,000,000 fungal propagules per gram. The propagule being considered as any spore, hyphae, or hypha fragment that is capable of giving rise to a colony. At best plate count values of fungi rarely amount to more than a few percent of bacteria count (Alexander, 1965).

The abundance and physiological activity of the fungul flora of different habitats vary considerably and the community and its biochemical activities

undergo appreciable fluctuation with time at any single site. Both the generic composition and the size of the flora vary with the type of soil and with its physical and chemical characteristics. Whether a given microorganism will be able to survive, adapt itself and become established in specific habitat will be determined by the surrounding environment. The major external influences imposed on the fungal community include the organic matter status, hydrogen ion concentration, organic and inorganic fertilizers, the moisture regime, aeration, temperature, position in the profile, season of year and the composition of the vegetation (Alexander, 1965).

2.2.4 Relationship between pesticides and microorganism

One of the most active fields of research in soil microbiology is concerned with the relationship between pesticides and microorganisms. In the last few years innumerable studies have established the effect of many of these compounds on indigenous populations and the ways in which the micro flora alters a multitude of chemicals to which they are exposed. This extensive research has been prompted by the importance of pesticides for food production and the potential or actual environmental hazards associated with wide spread use of toxic compounds.

2.2.5 Effects of pesticides on microorganism

The rate of application of certain pesticides to soil, such as some fungicides, my be high so that the micro flora is exposed to level that could seriously affect individual populations. Most herbicides, by contrast are applied at low rate so that one might expect little or no significant toxicity. On the other hand the potency varies with chemicals; hence, the impact of low concentration of one toxicant may sometimes be greater than a second

toxicant present at a higher level in the soil. Furthermore, the duration of effectiveness of a pesticide its persistence is governed by the chemical structure and environmental conditions so that the longevity of the inhibitor must also be considered. Therefore the influence of chemicals on the community or its constituent population is determined by the particular pesticide, the concentration present and the persistence (Alexander, 1981).

Evaluation of possible harm can be conducted in several ways. The substance may be included in various levels in nutrient solutions inoculated with the pure cultures or in agar media inoculated with soil dilutions; the data so obtained is useful, but interpretation of the results in ecological terms is frequently difficult because interactions between soil constituents and toxicants would not be evident from test laboratory media. Soil colloids adsorb or hydrolyze certain organic toxicants, both processes frequently leads to a loss of potency. Drawing conclusions solely from cultural tests is also is risky inasmuch as the duration of toxicity differs in soil and culture media. One population may alter the introduced molecule making it less or possibly more harmful to a sensitive population than would be anticipated from tests involving just a single species. Because of these reasons evaluations are commonly conducted directly with soil and the rate of CO2 evolutions, O2 consumption , nitrogen mineralization, nitrification or another biochemical process is determined. The soil may be treated with appropriate substrates before the experiment is conducted to enhance the transformation of interest chemical. Alternatively, population response may be evaluated using total count of major microbial groups or enumerations of individual genera or species in order to assess the potential harm.

The possible suppression of microbial groups has been tested with many herbicides, insecticides and fungicides and the abundance of a variety of

dissimilar population has been evaluated in comparisons with treated and untreated soil. Most populations are either not reduced in numbers or are not greatly affected by those pesticides that are normally present at low concentrations, as the case of herbicides and many insecticide. (Tyunyayeva *et al*. 1974)

Notable exceptions are algae, many of which are markedly inhibited by herbicides (Cullimore, 1975). This is not surprising as much of these chemicals are chosen for their effectiveness against certain other chlorophyll- containing organisms, namely weed species. Those insecticides that alter abundance particular heterotrophic populations are usually the ones present in high concentration, although response vary with the type of soil. The spectrum of dominant species may be drastically modified as common genera are suppressed and new groups come to the front. This setup is at times short-lined but the changed community may, alternatively, endure for long periods. Compound that are found in concentration high enough to injurious level do not affect all population to a similar degree; one species may decline appreciably, a second may suffer only modest harm, while a third is entirely resistant (Venkataraman and Rajyalakshmi.1971). Differences in sensitivity also are evident in organisms having more than one form so that hyphae of fungi or vegetative cells of bacteria are affected either more or less than are fungal conidia and sclerotia or bacteria endospores.

2.2.6 Effect of Pesticides persistence

How long pesticides persistence in soil is of great practical importance because it reflects the time that the pest will be subject to control. At the same time the persistent insecticides has special position in environmental

pollution because the remain long enough in soil: (a) to be assimilated by plants and accumulate in edible portions, (b) to adhere to edible portions of root crops, (c) to be transmitted with eroding soil particles to nearby water ways, or (d) to be accumulated in earth worms and then show up in high levels in birds feeding on worms.

Synthetic organic compounds may disappear from soil by a variety way. Some are volatile and evaporated from the land to the overlying air. Few are transported vertically with moving water and the ground water may then receive significant quantities. Reasonable numbers are subject to chemical reactions often hydrolytic to yield non toxic products. Such non microbial conversions, although leading to changes in toxicity of the original molecule, do not result in a complete degradation or mineralization. In many instance pesticides disappearance is attributable to microbial activity, a biological contribution verified by comparing changes in concentration with time in samples of natural soil and in samples that have been sterilized or treated with inhibitors to retard microbial or metabolism growth.

Many genera of hetrotrophs use pesticides molecules as substances either co-metabolizing the molecules or using them as nutrients. Many species of *Agrobacterium*, *Arthrobacter, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Klebsiella, Pseudomonas,* and *Xanthomonas* among bacteria*; Alternaria, Aspergillus, Cladosporium, Fusarium, Glomerella, Mucor, Pincillium, Rhizoctonia,* and *Trichoderma* among fungi*;* and *Micromonospora, Nocrodia*, and *Streptomyces* among actinomycetes modify one or more of synthetic chemicals. The diversity of substance is matched by a diversity of species. Nevertheless, its rarely possible to predict which species or even which genus is responsible for a particular transformation in nature (Alexander, 1981).

Many observations that microorganisms are important or essential for ridding natural environments of pesticides point to two practical matters. First for those substances that are modified or degraded by members of microflora, it seems plausible to expect that environmental factors governing hetrotrophic population would have a comparable influence on chemical destruction. Although the populations bringing about the transformation of individual organic compounds are generally unknown, the available information suggests the environmental factors influencing the community as a whole have comparable effects on the degradation of those pesticides known to be subject to microbial metabolism. Thus the rate of degradation is often enhanced by increasing of temperature or by rising the moisture level of dray soil, and rates of decomposition are frequently greater in soil rich in organic matter rather than poor soil. This is presumably because of the more vigorous community. Similarly, for molecules that are acted on by indigenous populations, a previous application generally leads to a greater activity on retreatment, probably because of the abundance of cells containing the needed enzymes (Suess, 1970).

Second, the soil inhabitants play a key role in detoxication and in mineralization of a multitude of organic molecules, the prolonged durability of the compounds designated as persistence is itself convincing evidence that the community has little or no action on the long-live chemical. The molecules that thus endure have been termed recalcitrant, that is, they are stubborn and fail to be metabolized or mineralizing at significant rates. The period of persistence is frequently given as the time required for half the chemical to be lost, but it is often expressed as the time for detectable level of the substance to disappear entirely. However, no one expression of persistence has been accepted by all investigations. Furthermore, the live of

a chemical in nature is greatly influenced by the particular soil, local condition of temperature and rainfall, and agricultural practice. Typical data are not directly applicable to all soils and all climatic regions. The quantity of information on longevity of such toxicants is enormous, yet the reasons for varying duration of effectiveness are still largely uncertain. This is well illustrated by parathion. In some soils nearly all the parathion is gone in 30 days (Iwata *et al*. 1973) yet the chemical is at times be found in other areas 16 year following the last application (Stewart *et al*., 1971).

2.2.7 Microbial metabolism of pesticides

The microbial metabolism of pesticides can be divided into;

(a) The chemical supported growth, serving as a source of carbon, energy and occasionally nitrogen. In this instance the population density of the active species rises in soil treated with pesticide and the cells multiply at the expense of the chemical. Concomitant with rise in abundance is an increasing rate of disappearance of the compound. Organism of this sort can be isolated readily from the natural habitat by means of enrichment cultures containing the pesticides as a source of carbon, nitrogen, or sulfur. The consequent utilizing of the chemical as nutrient leads to a breakdown of the toxic substance into some kind of intermediates typical of intracellular processes and these are used to sustain growth.

(b) The chemical although metabolized does not serve as source of nutrients. The transformation in these instances is by co-metabolism as metabolism by microorganism of a compound that the cell is unable to use as a source of energy or un essential nutrient heterotrophy of the sort therefore can not be isolated by enrichment culture in which the medium contains pesticide as nutrient (Alexander, 1965).

Metabolism of toxicants, heterotrophy may bring about one of a verity of reactions or classes of reactions. The kind of transformation depends on the particular species. These reaction falls into several broad categories: (a) Detoxication the conversion of a molecule inhibitory in the concentration used, to a nontoxic product. (b) Degradation, the transformation of a complex substrate into simple product, degradation is often (but not universally) considered to be synonymous with mineralization, in this instance the products are CO_2, H_2O and sometime NH3 or chloride if the molecule contains nitrogen or chlorine. (c) Conjugation, complex formation, or addition reactions, in which an organism makes the substrate more complex or combined the pesticide with cell metabolites. Conjugation or the formation of addition products may be accomplished by the organism catalyzing a reaction that lead to addition of an amino acid, organic acid or methyl or ether groups to the substrate. (d) Activation, the conversion of a nontoxic substrate to more toxic molecule than the actual pesticide. (e) Defusing, the conversion of a nontoxic molecule, which would be pesticidal were it subject to enzymatic activation, to a nontoxic product that no longer is subject to activation (McRae and Elexander.1963). (f) Changing the spectrum of toxicity. Some pesticides are toxic to one group of organism, the pests they are designed to control but they are metabolized to yield products inhibitory to entirely dissimilar organisms (Ishida, 1964).

Because of the many transformations that the microflora can bring about and the possibility that the product may be harmful to plants, animals or humans, a vast amount of research on the metabolism of numerous herbicides, insecticides and fungicides has been conducted in the past few years. These studies are not easy to perform because most of the chemical

exist initially in the soil in concentrations of few parts per million or less so that intermediate would be found at still lower levels. Characterization of these trace quantities of intermediates has been facilitated, however by the availability of instruments capable of detecting even such minute amounts.

2.2.7 Microbial degradation of endosulfan

The biodegradation of persistent compounds is an important mechanism for their dissemination in the environment (Alexander, 1981; Marcae, 1990; Wallnofer& Engelhardt, 1990). 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 (Elzorgani & Omer, 1974 and Marten, 1976). Endosulfan can be completely degraded in about two weeks to nontoxic metabolite under anaerobic conditions (Guerin & Kennedy, 1999). Microbial degradation of endosulfan was also reported by Perschied *et.al*. (1973). They ,also, identified endodiol as the major degradation product in an undefined mixture of microorganisms obtained from soil suspension. Tariq *et al*. (2000) 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.

(Martens,1976). 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.

2.2.8 The use of microorganisms in cleaning polluted Soils (Bioremediation)

From the view point of toxicologists, the disappearance of pesticides residues from their original form of a given location doses not mean the end of problem, rather than indicating that problem starts there. The question is whether such disappearance means actual degradation of hazardous chemical or whether it is actually a signal that the insecticide has been translocated or metabolized in some ecosystem converted various pesticides into less or more toxic chemicals e.g. DDE, Dieldrin, Heptachlor epoxide, Aldrin, and Heptachlor in environment. Any environmental changes which result in the creation of biologically active, but not necessary toxic substances is of concern. Therefore alteration products having long residual live are what actually cause problems.

The process of treating contaminated soils by microorganisms is called bioremediation. Such treatment seems attractive, easy, simple to apply and not expensive. However it was practically faced with major obstacles, specially in soil contaminated with synthetic organic compounds. In order to develop an effective bioremediation strategy for soil contaminated with organic pollutants, the emphasis should provide a comprehensive overview of the complexity of the soil system as it relates to bioremediation (Rao *et al*., 1993).

The soil environment is dynamic and includes gas, liquid and solid phases. It is imperative in any soil bioremediation process to have a good understanding of these phases and how they interact. This includes not only the chemical characteristics of soil colloids, but also the physical arrangement of components. During the development of bioremediation strategies it is imperative to consider also the soil complexity and variability. Instead the potential impacts of soil on both the contaminant and microorganism should be integrated into the strategy from its inception. The researcher should be familiar with what type of soil minerals present and how such minerals might bind to the organic contaminant or participate in the affinity of the compound to soil organic matter and the effect of binding might have on contaminant bioavailability. In situations in which soils are inoculated with specific microorganism it is also necessary to consider what impact the soil will have on microbial survival and activity.

CHAPTER THREE

Material and Methods

3.1 Reagents and chemicals

Analytical grade endosulfan (99.5 pure) was obtained from the Agricultural Research Corporation, (Wed Medani). This grade is a mixture of two di-astereoisomers; α-endosulfan and β-endosulfan (7:3 respectively). Acetone (99.8 pure), Hexane (99.8 pure), Ethanol (99.8 pure) and other solvents were obtained from Fischer, company.

3.2 Soil samples

3.2.1 Areas of samples

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

3.2.2 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.

3.2.3 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 reasonable uniform size.

3.3 Preparation of media

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 $FeSO_4$, 1 g $CaCO_3$ and 25 g agar 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, 0.3 g NaCl and 25 g agar 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, 5 g NaCl and 15 g agar to one liter meat extract.

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 2	Cotton field from Kapelgedad in Gezira scheme
Gezira 3	Kapelgedad Residential area
Gezira 4	Gorashi Pesticides Store
Managil 1	Inside of Raselfeel pesticides store in Managil extension
Managil 2	Cotton field in Raselfeel Mangil extension
Elrahad 1	Inside of Elfaow pesticides store in Elrahad scheme
Elrahad 2	Cotton field in Elrahad scheme
Elrahad 3	Elfaow Residential areas

(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 $NaNO_3$, 20 g glucose and 30 g agar 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.

3.4 Survey of naturally occurring microorganisms

The following experiments were conducted to identify types and counts of naturally occurring microorganisms in soil samples.

A number of sterilized Petri dishes were prepared and grouped in nine sets. Fifteen ml were carefully pipetted form each stock media (SAA, NA, MPA, and CHA) and placed in the center of each Petri dish.

From each soil sample , the nine soil types, one g was placed in sterilized test tube containing ten ml distilled water; the contents were well shaken to give the first dilution (10^{-1}). The other five dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) of each soil sample were prepared by serial dilution from the respective preceding concentration (i.e. by adding one ml of the preceding concentration to nine distilled water).

From each of the six serial dilution of each soil sample one ml was transferred to the agar surfaces in the center of each plate (described earlier). The inoculums was spreaded over the agar surfaces using flamed glass spreader and inoculated at 30º C for two days for MPA, four days for CHA and ten days for NA and SAA. The viable bacterial colonies were counted (per plate) using a colony counter.

For NA media the following formula was used for counting under the photomicroscope (Tepper, *et al*, 1994).

$$N = \frac{\sum a.144.Bn}{C}$$

Where:

N = Number of bacteria or actinomycetes per g soil

∑a/n= the average of ten times count on the plate (of bacteria or Actinomycetes) under the microscope photo glass

144 = Constant

n = Dilution number

C = Dry weight of soil

B = Microscope glass pore

The experimental unit were arranged in a completely randomized design with three replications. Counts of various types of microorganism in soil samples were recorded.

3.5 Microbial degradation of α and β-endosulfan in selective media

This trial was conducted to study the capability of the isolated soil microorganisms in degrading α and β-endosulfan in selective media.

Microbial inoculums were prepared by shaking 10 g of soil sample (10 g from each of the nine soil types) in 100 ml media (all media) at 25° C·

Shaking period was over night for MPB and CHB and seven days for NB and SAB media. The solid particles were allowed to settle down during one hour waiting period and aliquots of the supernatants were used to inoculate the media.

A total of 108 test tubes and nutrient culture media (NB, MPB, SAB and CHB) were autoclaved separately for 20 minute at 121 $^{\circ}C$. Fifty microliters of acetone containing 0.5 mg endosulfan was aseptically added to each sterilized test tube in laminar flow hood. Acetone was allowed to evaporate by gentle heating on flame. Nine ml of media were added to each sterilized test tube (27 tubes for each media). Then one ml of supernatant solution from the source flasks was added to inoculate the spike tubes. Uninoculated spiked test tubes (12 tubes, three for each media) were included as control to compensate for any chemical degradation. The culture was incubated at 30^{o} C for 15 days (Round one enrichment culture). There after 0.01 ml of the culture was transferred on to 10 ml fresh media containing 50 mg/L endosulfan and incubated for further 15 days (Round two enrichment cultures). A completely randomized design with three replicates was used in this experiment.

3.5.1 Stock culture

Round one and round two cultures were centrifuged at 800 rpm for 10 min. to obtain media free from microorganisms the supernatant was removed to extract the remaining endosulfan residues. The solid fraction (containing microorganism cells) was re-suspended in 100 ml of sterile media (four types of media) and kept in the refrigerator to use in further studies.

3.5.2 Extraction of endosulfan from the culture

The supernatant in section 3.5.1 was removed by carefully decanting and placed in 100 ml separating funnel. Ten ml of hexane and acetone (3:1 respectively), were added followed by one ml methanol, the contents were vigorously shaken for five min. and allowed to stand for one minute until separation of layers. The hexane layer was collected in a clean test tube and the aqueous layer was re-extracted by similar method. Hexane fractions from the two extracts were recombined in closed test tubes. The solvent was dried up by passing the extracts through anhydrous sodium sulphate on filter paper and collected in clean closed test tube. The solvent was then stripped off by heating in a water bath at 68˚ C till dried and residues were re-solved in 10 ml Hexane and stored in the refrigerator at 5˚ C for analysis by GLC

3.5.3 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˚ C, 270˚C and 230˚ C respectively. Nitrogen (N_2) was used as carrier gas at flow rate of one ml\minute. Analysis of sample was done by injection of 0.2 micro liter. Five concentration of the standard (10^{-1}, 2 x 10^{-1}, 3 x 10^{-1}, 4 x 10^{-1}, 5 x 10^{-1} mg/l) were injected in the constructions of the calibration curve. Reanalysis of standard solution were repeated every morning to check for the performance of the machine. Septum was changed when necessary. The minimum detection for α and β-endosulfan were 1.8 and 1.6 respectively.

3.6 Degradation of α 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 from stock culture. Three soil types were selected as sours of bacteria based on the results of previous experiments (3.5).

(b) One ml of inorganic nitrogen bacteria and actinomycetes from stock culture. Three soil types were selected as source of bacteria and actinomycetes based on the results of previous experiments (3.5).

(c) One ml of bacteria and actinomycetes which lives in poor media from stock culture. Three soil types were selected as source of bacteria and actinomycetes based on the results of previous experiments (3.5).

(d) One ml of fungi from stock culture. Three soil types were selected as source of fungi based on the results of previous experiments (3.5).

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

3.6.1 Extraction and analysis

About 10 ml were taken from each flask every 15 days extracted and analyzed as described in section 3.5.2 and 3.5.3 respectively.

3.7 Microbial degradation of endosulfan in carbon free media

The purpose of this experiment was to assess the ability of isolated cultures in utilizing endosulfan as carbon source.

One liter of liquid media was prepared according to the method described by Tepper, *et.al*. (1994), to one liter conical flasks. One g K_2HPO_4, 0.5 g $MgSO_4$ .7 H_2O , 0.5 g NaCl , 0.001 g $FeSO_4$.7 H_2O , 0.01 g $MnSO_4$. 4 H_2O , 0.05 g $CaCO_3$ were added. The volume was completed to one liter distilled water.

A total of 36 conical flasks (100 ml) and culture 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 from stock culture. Three soil types were selected as sours of bacteria based on the results of previous experiments (3.5).

(b) One ml of inorganic nitrogen bacteria and actinomycetes from stock culture. Three soil types were selected as source of bacteria and actinomycetes based on the results of previous experiments (3.5).

(c) One ml of bacteria and actinomycetes which live in poor media from stock culture. Three soil types were selected as source of bacteria and actinomycetes based on the results of previous experiments (3.5).

(d) One ml of fungi from stock culture. Three soil types were selected as sours of fungi based on the results of previous experiments (3.5).

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

3.7.1 Extraction and analysis

About 10 ml were taken from each flask every 15 days and extracted and analyzed as described in section 3.5.2 and 3.5.3 respectively.

3.8 Impact of fertilizer and amendments activators in enhancing the microbial degradation of endosulfan in endosulfan treated soil

The purpose of this experiment was to investigate the effect of microbial activators in enhancing the microbial capability in degrading endosulfan residues in polluted soil.

3.8.1 Soil sampling

About three Kg of soil were taken from top surface soil (10 cm) of Sondos scheme south of Jable Awelia near Khartoum Kosti high way, Khartoum state 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 a uniform reasonable size.

Sub-samples (about one kg) were placed in paper bag, labeled and taken immediately to University of Khartoum faculty of agriculture, soil laboratory for chemical and physical analysis. The soil chemical analysis was made according to Page *et al.* (1982), while the physical analysis was made according to Black *et al.* (1965).

3.8.2 Microbial degradation of endosulfan

One kg of soil was sterilized by incubation in an oven at 160˚ C for three hours and transferred to one liter conical flask. Six hundred ml 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 hours 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 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 bacterial inoculums; it contained the following treatment.

1) Treated soil + one ml from stock culture of bacteria
2) Treated soil + one ml from stock culture of bacteria + one gram urea
3) Treated soil + one ml from stock culture of bacteria + one gram urea + one gram triple super phosphate
4) Treated soil + one ml from stock culture of bacteria + one gram triple super phosphate
5) Treated soil + one ml from stock culture of bacteria + one gram cow manure

The second set was used for studying degradation using fungal inoculums; it contains the following.

1) Treated soil + one ml from stock culture of fungi
2) Treated soil + one ml from stock culture of fungi + one gram urea
3) Treated soil + one ml from stock culture of fungi + one gram urea + one gram triple super phosphate

4) Treated soil + one ml from stock culture of fungi + one gram triple super phosphate
5) Treated soil + one ml from stock culture of fungi + one gram 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.

3.8.3 Extraction of endosulfan

In this experiment the methods described by Bollen *et al*., (1958) were followed to extract endosulfan. Ten grams 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 two hours. 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 ten 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 and endosulfan sulphate according to method described in section 3.5.3

3.8.4 Microorganism counts

Beside the above studies the types and counts of microorganisms were done according to methods as previously described in section 3.4 every 15 days to assess relationship between types and number of microorganism and endosulfan degradation in soil.

3.9 Microbial degradation of high endosulfan concentrations in carbon free media

The purpose of this experiment was to investigate the microbial degradation of high concentration of endosulfan in carbon free media. The microorganisms used in this experiment were isolated from stock culture following the method described in section 3.5.2. Media was prepared as described in section 3.7.1.

Conical flask containing 500 ml media was autoclaved for 20 minutes at 121˚ C, and allowed to cool at room temperature. Five ml of acetone containing 250 mg endosulfan were added to the flask in a Laminar flow hood. The acetone was dried by gentle heating over flame for five minutes. Ten ml from the treated media were taken by sterilized pipette to sterilized test tubes (10 ml in each) and the test tubes were then inoculated with the following groups of microorganism as follows.

1) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml fungi.
2) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml organic nitrogen bacteria.
3) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml *Nocardia*.
4) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml *Arthrobacterium.*

5) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml *Micromonospora*.
6) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml *Mycobacterium*.
7) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml *Bactoderma.*
8) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml Bacteria and actinomycetes which live in poor media.
9) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml mixed microorganisms (i.e. including all of the previous microorganisms).
10) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml Distilled water as control.

Test tubes were arranged in a completely randomized design with three replicates. All test tubes were incubated for a total of 30 days and contents of endosulfan (α and β) and endosulfan sulphate analyzed every ten days. About two milliliters were taken from each test tube every ten days for a month period. Samples were extracted according to the method as described in section 3.5.2. And analyzed by GLC for α, β and endosulfan sulphate according to the method as described in Section 3.5.3.

3.10 comparative Degradation of endosulfan by mutant microorganism and their Parents

3.10.1 Mutant strains procedure

From stock cultures described in Section 3.5.2 one ml was taken and placed in sets of sterilized test tubes containing CHB media for fungi and MPB media for bacteria. The media were prior treated with 200 mg/l endosulfan. The test tubes were incubated for seven days at 30˚ C. The growth of microorganisms in these test tubes was observed by locking for turbidity of the media. Then counts of microorganisms in each test tube were estimated following the method described in Section 3.4. One ml was taken from each of the seven days incubated test tube and transferred to sterilized set of another test tubes each containing ten milliliter of CHB media (for fungi) or MPB (for bacteria) treated with higher concentration of endosulfan (400 mg/l). Test tubes were incubated for another seven days, growth and count of microorganisms were observed and recorded as indicated above. Microorganisms were subject to further consecutive elevated concentrations of endosulfan (600, 800 or 1000 mg/l) and effects on growth and counts were determined. Units were arranged in a completely randomized design with three replicates.

3.10.2 Identification of microorganisms

The microorganism tolerant to high concentrations of the endosulfan were identified as follows.

(a) Bacteria

Culture of sample on nutrient agar media

One ml was taken by sterilized pipette from tests tubes containing the most tolerant microorganisms (test tube containing 1000 mg/l endosulfan) and placed in a Petri dish containing sterilized nutrient agar. The inoculated plates were then incubated at 37˚ C for 24 hours. This procedure was replicated four times. The plates were checked for shape, colour and other general characteristics of the colonies growth.

Gram stain

The colonies obtained in the four plates were then subjected to Gram stain test as described in Brough (1999). One drop of distilled water was added to sterilized slides, and then small portion of colony was taken by the loop on a drop of water, and then was spreaded over the slide. The drop was allowed to dry by exposure to air at room temperature. Then the smear was fixed by heating on a flame and stained by three types of stains (Crystal violet stain for 60 seconds, lugols iodine for 60 seconds, and decolorized by alcohol for 10 seconds). After each stain the smear was rapidly washed by water. Lastly the smear was dried by exposure to air and examined under oil by microscope 100 x magnifications. The slides were examined for Gram positive rod with central and terminal to sub terminal spores and results were recorded.

Inoculation in Mannitol salt agar

Small portion of colonies grown in nutrient agar plates were taken by sterilized loop and inoculated in Mannitol salt agar. They were incubated at 37˚ C over night. Shape and colour of colonies were recorded.

(b) Fungi:

Culture in PDA

One ml was taken from test tubes containing the most tolerant fungi (test tube containing 1000 mg/l endosulfan), and placed in a Petri dish containing sterilized PDA media. The inoculated plates were incubated at 25˚ C for seven days. This procedure was repeated four times. The plates were checked daily for hyphal shape and colour.

Lacto phenol cotton blue stain (LPCB)

One drop of LPCB was placed in a sterilized slide, then small amount from the growing culture were taken using a loop and placed in the LPCB drop. The Slide was then covered and examined under microscope at 10 x and 40 x magnifications for hyphal characteristics.

3.10.3 The comparative degradation experiment

The purpose of this experiment was to study the relative capability of tolerant stains (compared to their parents) degrading endosulfan in the liquid media and soil.

(a) Degradation under liquid media

A pre cleaned and sterilized conical flask (500 ml) was prepared. Three hundreds ml liquid media described in Section 3.7.1 was placed in the flask. The flask with its contents was autoclaved at 121˚ C for 20 minutes. Then allowed to cool at room temperature. Five milliliter acetone containing 150 mg endosulfan were added to sterilized media, and then acetone was

evaporated by gentile flame. The treated media was Sub-divided into 30 sets each composed of 10 ml in sterilized test tubes. Test tubes in triplacate were then treated with endosulfan (500 mg/l) and inoculated with one ml of either bacteria or fungi as follows;

(A) Bacteria

1) Organic nitrogen bacteria (parents).
2) Organic nitrogen bacteria exposed to 200 mg/l endosulfan
3) Organic nitrogen bacteria exposed to 600 mg/l endosulfan
4) Organic nitrogen bacteria exposed 1000 mg/l endosulfan (tolerant strains).

(B) Fungi

1) Fungi (parents).
2) Fungi exposed to 200 mg/l endosulfan
3) Fungi exposed to 600 mg/l endosulfan
4) Fungi exposed to 1000 mg/l endosulfan

(a) Degradation under soil conditions

A pre cleaned and sterilized conical flask (1000 ml) containing 500 g soil (from soil sample mentioned in Section 3.7.1) was prepared. The flask with its contents was sterilized in an oven at 160˚ C for three hours. The flask was allowed to cool at room temperature. Two hundreds ml distilled water containing 150 mg endosulfan were added to sterilized soil. The treated soil was sub-divided into 30 sets each composed of 10 g in sterilized flasks (50 ml). Flasks in triplicate were then treated with endosulfan (500 mg/kg) and inoculated with one ml of either bacteria or fungi as follows;

(a) Bacteria

1) Organic nitrogen bacteria (parents).
2) Organic nitrogen bacteria exposed to 200 mg/l endosulfan
3) Organic nitrogen bacteria exposed to 600 mg/l endosulfan
4) Organic nitrogen bacteria exposed to 1000 mg/l endosulfan

(b) Fungi

1) Fungi (parents).
2) Fungi exposed to 200 mg/l endosulfan
3) Fungi exposed to 600 mg/l endosulfan
4) Fungi exposed to 1000 mg/l endosulfan

All test tubes and flasks were arranged in a completely randomized design with three replicates and incubated at 30 C° for a total of 60 days. The level of starting material and endosulfan sulphate generated was checked at 15 days interval

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3.10.4 Extraction and analysis

About one ml was taken from each test tube in Section 3.10.3 every 15 days for 60 days and extracted according to the method described in section 3.5.2.

One gram of soil was taken from each flask in Section 3.10.3 every 15 days for 60 days and extracted according to the method as described in section 3.8.3.

GLC analysis was done according to method described in section 3.

CHAPTER FOUR

Results

4-1 survey of naturally occurring soil microorganisms

The average counts of soil microorganism (organic nitrogen bacteria inorganic. nitrogen bacteria, actinomycetes, bacteria and actionmycetes which lives in poor media and Fungi) naturally occurring in nine types of soil of variable level of contamination with pesticides was carried out. Results of survey were displayed in Table 1.2 and 3. The average counts of organic nitrogen bacteria ranged between (1.2×10^3 - 9.1×10^4) where as the average counts of fungi ranged between (0.2×10^2 - 2.8×10^3) the highest counts of bacteria where found in Raselfeel cotton field soil (Mangil 2). However the lowest counts of bacteria and fungi were found in Kabalgidad pesticides store (Gezira 1) and Quarashi pesticide store (Gizera 4) respectively, (Table 1). The average counts of inorganic nitrogen bacteria ranged between (0.2×10^2 - 7.9×10^3) and the average counts of inorganic nitrogen actinomycetes ranged between 0.1×10^2 - 0.6×10^3 the highest counts of inorganic nitrogen bacteria and actinomycetes were found in Gezira cotton field soil (Gezira 2). On the other hand the lower counts of inorganic nitrogen bacteria and actiormycetes were found in soil of Rahad (Rahad 1) pesticides store (Table 2). It is worth to mention that the highest percentage of actinomycetes was associated with soil highly polluted with pesticides (pesticides store).

The average counts were ranges between 1.0×10^3 - 8.5×10^3 the highest counts of bacteria and actinomycets which lives poor media were found in

Table1. Average count of organic nitrogen bacteria and fungi in different soils

Soil types	Count/g Soil	
	Organic Nitrogen bacteria	Fungi
Kapelgedad Pesticides Store	1.2×10^{3}	0.1×10^{3}
Kapelgedad Cotton field	6.6×10^{5}	1.8×10^{3}
Kapelgedad Residential Areas	3.1×10^{4}	0.9×10^{3}
Gorashi pesticides store	7.0×10^{4}	$0.\ 2\times10^{2}$
Raselfeel Pesticides Store	3.7×10^{4}	0.1×10^{3}
Raselfeel Cotton field	9.1×10^{5}	2.8×10^{3}
El Faw Pesticides Store	2.1×10^{4}	0.1×10^{3}
El Faw Cotton field	4.2×10^{5}	0.9×10^{3}
El Faw Residential Areas	9.3×10^{4}	0.4×10^{3}
SE ±	211^{**}	182.2^{**}
C.V.	16.3	4.49

SE = Standard Error

C.V. =Coefficient of Variant

Table 2. Average count of inorganic nitrogen bacteria and actinomycetes in different soils

Soil types	Count/g soil	
	Inorganic Nitrogen bacteria	Inorganic Nitrogen Actinomycetes
Kapelgedad Pesticides Store	0.4×10^{2}	0.1×10^{3}
Kapelgedad Cotton field	7.9×10^{3}	0.6×10^{3}
Kapelgedad Residential Areas	0.4×10^{2}	0.1×10^{3}
Gorashi pesticides store	0.3×10^{3}	0.1×10^{3}
Raselfeel Pesticides Store	0.2×10^{2}	0.1×10^{2}
Raselfeel Cotton field	3.8×10^{3}	0.1×10^{3}
El Faw Pesticides Store	0.3×10^{2}	0.1×10^{2}
El Faw Cotton field	1.2×10^{3}	0.1×10^{3}
El Faw Residential Areas	0.4×10^{3}	0.1×10^{2}
SE ±	114.7^{**}	23.7^{**}
C.V.	*1.4*	*3.9*

SE = Standard Error

C.V. =Coefficient of Variation

Table3. Average count of bacteria and Actinomycetes living in poor media in different soil types.

Soil types	Actinomycetes/g soil			Bacteria /g soil		total
	Nocardia	***Bactoderma***	***Micromonospoa***	***Mycobacterium***	***Arthrobacterium***	
Kapelgedad Pesticides Store	0	0.2×10^3	0.1×10^3	1.5×10^3	0.5×10^3	2.3×10^3
Kapelgedad Cotton field	1.4×10^3	2.5×10^3	0	0.2×10^2	1.3×10^3	5.2×10^3
Kapelgedad Residential Areas	0.4×10^2	0.4×10^3	0.5×10^3	0.4×10^3	0.8×10^3	2.1×10^3
Gorashi pesticides store	0.1×10^2	0.1×10^3	0.7×10^3	0.9×10^3	0.8×10^3	2.6×10^3
Raselfeel Pesticides Store	0.2×10^2	0.1×10^3	0.4×10^2	1.3×10^3	0.1×10^3	1.6×10^3
Raselfeel Cotton field	0.6×10^3	0.8×10^3	0.1×10^3	0.4×10^3	6.5×10^3	8.5×10^3
ElFaw Pesticides Store	0.1×10^3	0.3×10^3	0.2×10^3	1.3×10^3	0.2×10^3	2.1×10^3
El Faw Cotton field	0.5×10^3	0.2×10^3	0.0	0.4×10^2	0.3×10^3	1.0×10^3
El Faw Residential Areas	0.2×10^3	0.1×10^3	0.4×10^3	0.1×10^3	0.3×10^3	1.1×10^3
SE ±	23.9^{**}	60.5^{**}	32.5^{**}	36.0^{**}	40.5^{**}	31.3^{**}
C.V.	*1.4*	*2.3*	*2.8*	*1.0*	*0.7*	1.6

SE = Standard Error
C.V. =Coefficient of Variation

Raselfeel cotton field soil (Managil 2). In this type of soil, Managil 2, the average counts of various types of bacterial and actinomycetes were, *Mycobacterium (*$0.4x10^3$), *Micrromonospora (*0.1×10^3) *,Arthrobacterium,* (6.5×10^3), *Nocardia (*0.6×10^3) and *Bactoderma (* 0.8×10^3) *Nocardia* was not observed in the Gezira pesticide store soil (Table 3).

4-2 Scanning of the capability of isolated microorganisms in degradation of α and β- endosulfan in selective media

Tables 4-7 summarize the results of microbial degradation of endosulfan under condition of selective media in Round I (In initial incubation for 15 days) the rate of α-endosulfan degradation ranged between 10.29 - 43.1 % while that of β-endosulfan ranged between 10.3 - 38.7 % (Tables 4 and 5). However in Round 2 (incubation with microorganism isolated from round 1 for another 15 days), the rate of α endosulfan degradation increase and range between 19.0 - 82.1 % while the percentage of β-endosulfan loss ranged between 18.5 - 82.5 % (Tables 6 and 7).

It is worth to mention that the percentages of loss in α-endosulfan exceeded that of β-endosulfan (Tables 4-7). It also noticeable that inoculums from highly polluted soils (storage soil and cotton field) caused significant degradation of both α and β-endosulfan compared to inoculums from less polluted soil (residential area).

Table 4. Amount and percentage of α- endosulfan (mg/l) remaining after incubation in selective media for 15 days with microorganisms isolated from nine soil types, Round 1. (Figures in parenthesis are %)

Soil types	NB	MPB	SAB	CHB
Kapelgedad Pesticides Store	30.4 (87)	26.1 (75)	30.6 (88)	24.2 (70)
Kapelgedad Cotton field	27.3 (78)	29.9 (86)	26.8 (77)	23.3 (67)
Kapelgedad Residential Areas	30.9 (89)	30.7 (88)	26.8 (77)	27.2 (78)
Gorashi pesticides store	27.6 (79)	28.2 (81)	24.5 (70)	24.7 (71)
Raselfeel Pesticides Store	26.9 (77)	27.5 (79)	26.6 (77)	24.4 (70)
Raselfeel Cotton field	31.4 (90)	30.2 (87)	26.7 (77)	25.9 (74)
El Faw Pesticides Store	29.5 (85)	24.2 (70)	25.5 (73)	26.4 (76)
El Faw Cotton field	29.6 (85)	28.4 (82)	21.5 (62)	19.9 (57)
El Faw Residential Areas	29.4 (84)	26.7 (77)	27.7 (80)	25.2 (72)
Control	34.1 (98)	33.1 (95)	32.1 (92)	32.6 (94)
SE ±	1.7	1.2*	1.3**	1.0**
C.V.	0.97	0.72	0.86	0.69

Initial concentration of α- endosulfan was 35 mg/l

NB = Nitrate Broth Media

MPB = Meat Peptone Broth Media

SAB = Starch amino Broth Media

CHB = Chabecks Broth Media

SE = Standard Error

C.V. = Coefficient of Variation

Table 5. Amount and percentage of ß-endosulfan (mg/l) remaining after incubation in selective media for 15 days with microorganisms isolated from nine soil types, Round 1 (Figures in parenthesis are %)

Soil type	NB	MPB	SAB	CHB
Kapelgedad Pesticides Store	12.1 (81)	11.2 (75)	10.6 (71)	10.4 (70)
Kapelgedad Cotton field	11.5 (77)	12.6 (84)	11.9 (80)	9.7 (65)
Kapelgedad Residential Areas	13.5 (90)	11.1 (74)	10.9 (73)	11.3 (76)
Gorashi pesticides store	12.7 (85)	11.8 (79)	10.6 (71)	10.3 (69)
Raselfeel Pesticides Store	12.4 (83)	12.0 (80)	11.3 (76)	10.3 (69)
Raselfeel Cotton field	13.5 (90)	10.1 (68)	10.8 (72)	11.1 (74)
El Faw Pesticides Store	12.6 (84)	12.4 (83)	10.6 (71)	10.8 (72)
El Faw Cotton field	12.1 (81)	11.4 (76)	9.2 (62)	10.4 (70)
El Faw Residential Areas	12.5 (84)	12.2 (82)	11.7 (78)	10.2 (68)
Control	14.5 (97)	14.6 (98)	13.3 (89)	13.3 (89)
SE ±	0.50*	0.64**	0.41**	0.45**
C.V.	0.70	0.67	0.64	0.73

Initial concentration of ß-endosulfan was 15 mg/l

NB = Nitrate Broth Media

MPB = Meat Peptone Broth Media

SAB = Starch amino Broth Media

CHB = Chabecks Broth Media

SE = Standard Error

C.V. = Coefficient of Variation

Table 6. Amount and percentage of α- endosulfan (mg/l) remaining after incubation in selective media for 15 days with microorganism isolated from nine soil types, Round 2 (Figures in parenthesis are %).

Soil types	NB	MPB	SAB	CHB
Kapelgedad Pesticides Store	22.4 (64)	26.3 (76)	25.1 (72)	19.3 (56)
Kapelgedad Cotton field	25.4 (73)	26.2 (75)	25.4 (73)	20.6 (59)
Kapelgedad Residential Areas	26.6 (76)	26.6 (76)	23.4 (67)	23.4 (67)
Gorashi pesticides store	23.3 (67)	19.6 (56)	23.9 (69)	6.3 (18)
Raselfeel Pesticides Store	24.1 69)	20.6 (59)	26.9 (77)	21.6 (62)
Raselfeel Cotton field	28.2 (81)	28.3 (81)	26.5 (76)	18.3 (53)
El Faw Pesticides Store	27.8 (80)	17.8 (51)	24.5 (70)	16.4 (47)
El Faw Cotton field	28.1 (81)	26.3 (76)	18.2 (52)	17.8 (51)
El Faw Residential Areas	27.6 (79)	24.3(70)	25.5 (73)	17.5 (50)
Control	34.2 (98)	34.3 (98)	34.4 (99)	33.8 (97)
SE ±	0.98**	0.73**	0.89**	1.00**
C.V.	0.63	0.51	0.61	0.89

Initial concentration of α-endosulfan was 35 mg/l

NB = Nitrate Broth Media

MPB = Meat Peptone Broth Media

SAB = Starch amino Broth Media

CHB = Chabecks Broth Media

SE = Standard Error

C.V. = Coefficient of Variation

Table 7. Amount and percentage of ß-endosulfan (mg/l) remaining after incubation in selective media for 15 days with microorganism isolated from nine soil types, Round 2.
(Figures in parenthesis are %).

Soil types	NB	MPB	SAB	CHB
Kapelgedad Pesticides Store	9.7(65)	10.0(67)	10.4 (70)	8.2(55)
Kapelgedad Cotton field	9.9(66)	11.2(75)	10.8 (72)	8.8(59)
KapelgedadResidential Areas	11.2(75)	10.6(71)	9.4 (63)	10.0 (67)
Gorashi pesticides store	9.3(62)	7.8(52)	10.1(68)	2.7(18)
Raselfeel Pesticides Store	10.4(70)	8.4(56)	11.1(74)	8.6(58)
Raselfeel Cotton field	11.4(76)	11.9 (80)	10.6 (71)	7.4 (50)
El Faw Pesticides Store	11.3 (74)	10.9 (73)	9.5 (64)	6.8(46)
El Faw Cotton field	11.0 (74)	11.4 (76)	8.5 (57)	7.1 (48)
El Faw Residential Areas	12.2 (82)	10.1 (68)	10.0 (67)	7.0 (47)
Control	14.2 (95)	13.4 (90)	13.9 (93)	13.6 (91)
SE ±	0.79*	0.46*	0.42**	0.66**
C.V.	1.2	0.76	0.69	1.4

Initial concentration of ß-endosulfan was 15 mg/l

NB = Nitrate Broth Media

MPB = Meat Peptone Broth Media

SAB = Starch amino Broth Media

CHB = Chabecks Broth Media

SE = Standard Error

C.V. =Coefficient of Variation

4.3 degradation of α and β-endosulfan by selected microorganisms in selective media

Tables 8 and 9 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).

4.4 Microbial degradation of endosulfan in carbon free media

Tables 10, 11 and Fig. (1-24) show 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% in β).

The results generally indicated that all microorganisms used in this experiment caused relatively similar effects on α endosulfan isomers (Table 10). while different groups of microorganism showed variable effects

Table 8. 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 9. 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
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

Table 10. 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 11. 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

Fig. 1. The percentage of α-endosulfan remaining at various time intervals after inoculated with bacteria and actinomycetes which live in poor media isolated from Kabelgidad pesticide store soil.

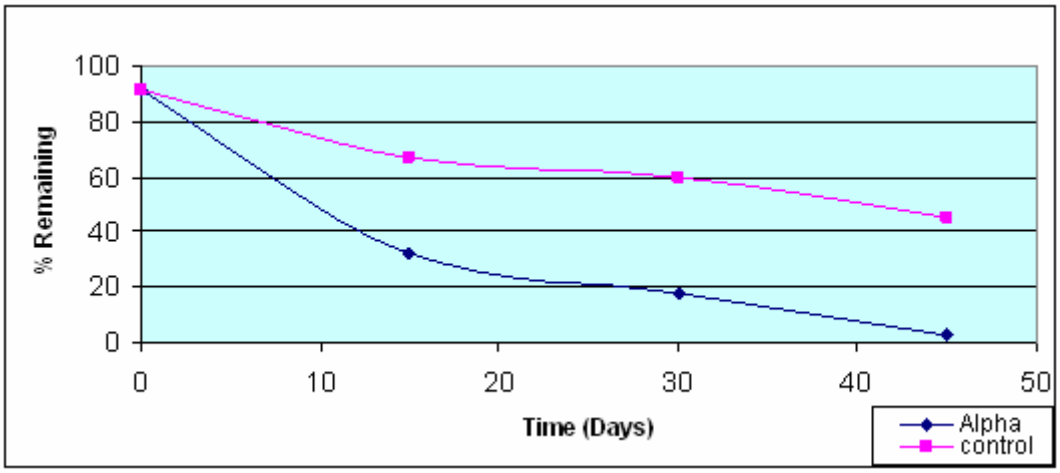


Fig. 2. The percentage of α-endosulfan remaining at various time intervals after inoculated with bacteria and Actinomycetes which live in poor media isolated from Gorashi pesticide store soil.

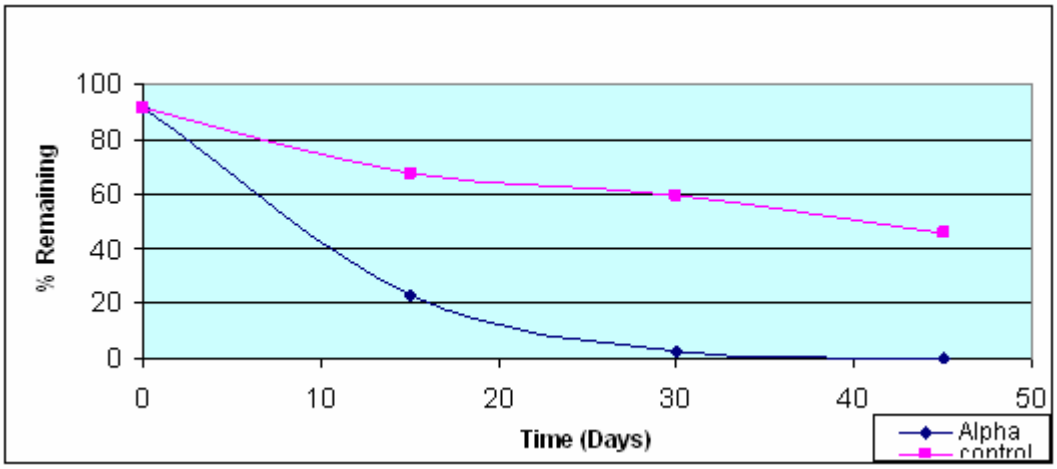


Fig. 3. The percentage of α-endosulfan remaining at various time intervals after inoculated with bacteria and Actinomycetes which live in poor media isolated from Raselfeel pesticide store soil.

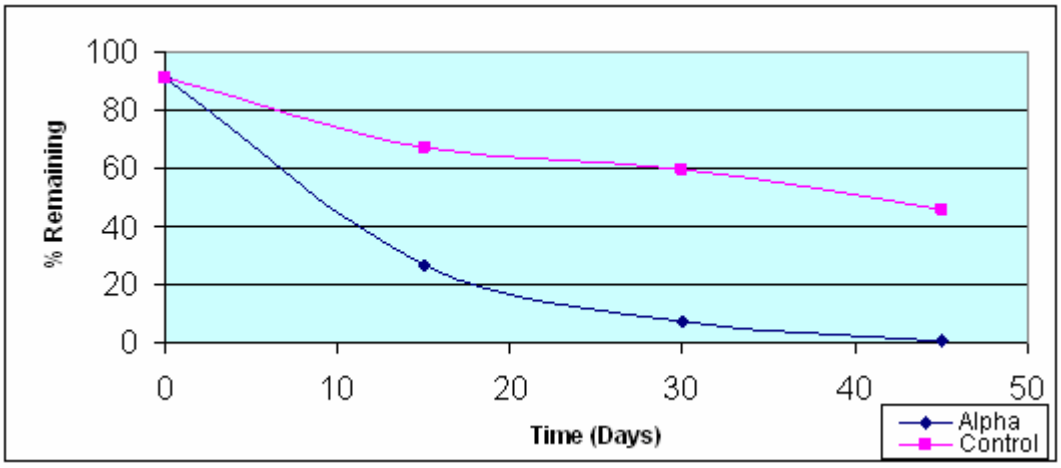


Fig. 4. The percentage of α-endosulfan remaining at various time intervals after inoculated withfungi isolated from Gorashi pesticide store soil.

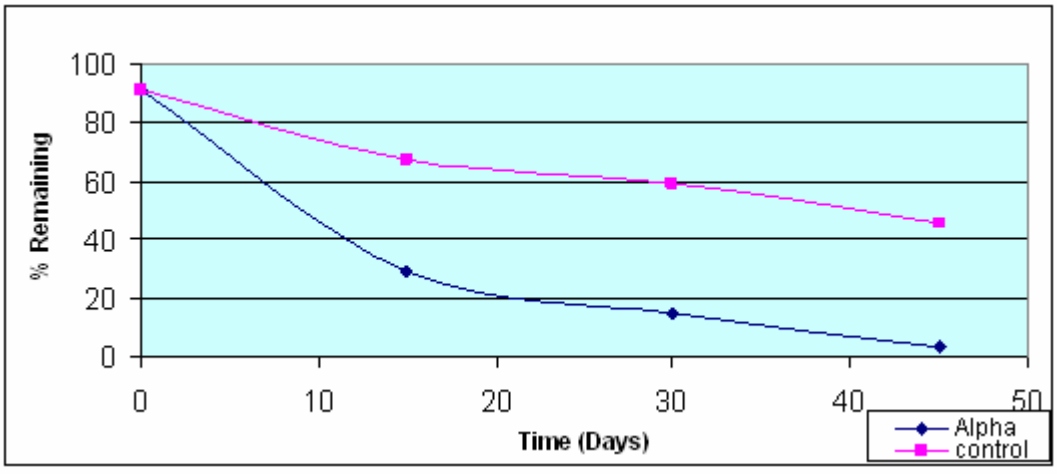


Fig. 5. The percentage of α-endosulfan remaining at various time intervals after inoculated with Fungi isolated from Faw pesticide store soil.

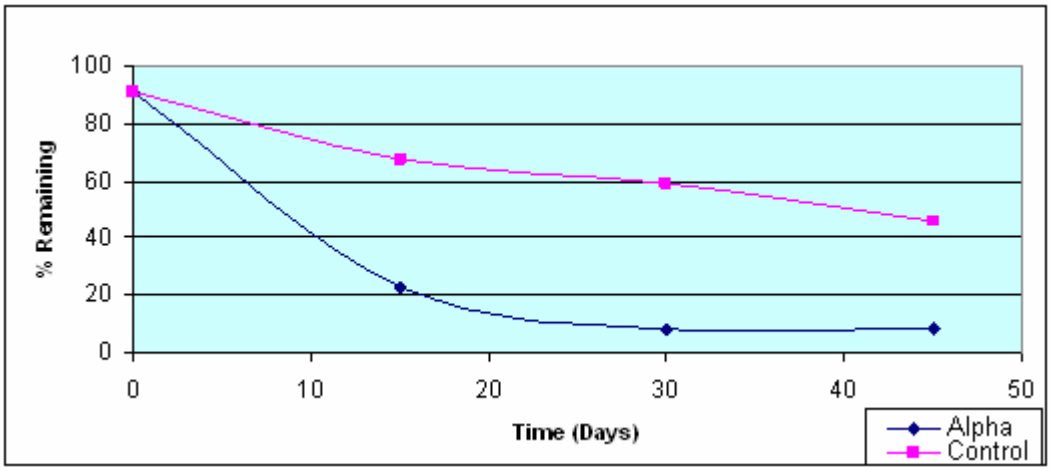


Fig. 6. The percentage of α-endosulfan remaining at various time intervals after inoculated with fungi isolated from Raselfeel cotton field soil.

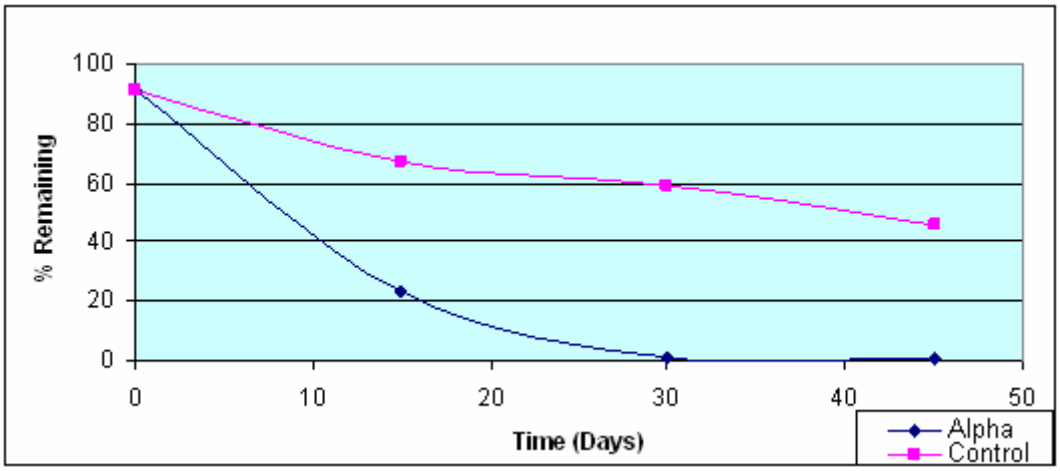


Fig. 7. The percentage of α-endosulfan remaining at various time intervals after inoculated with Inorganic Bacteria and actinomycetes isolated from Gorashi pesticide store soil.

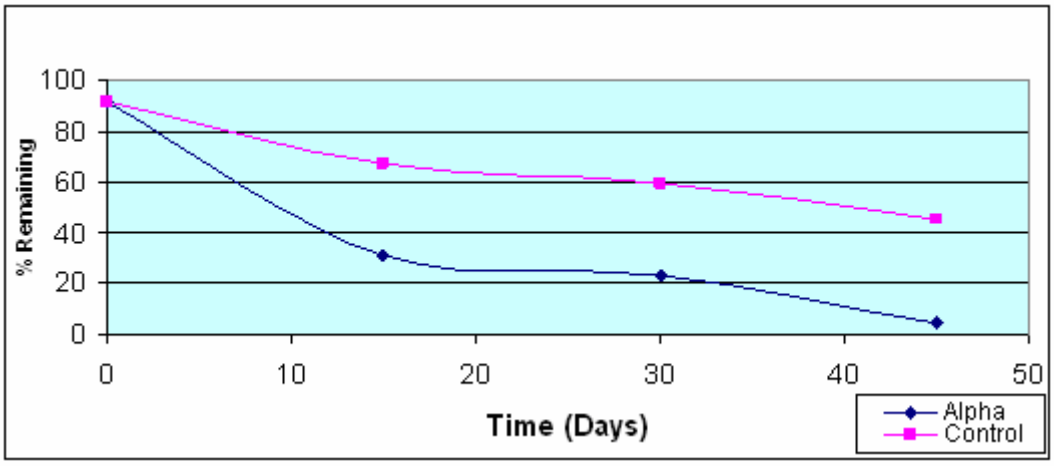


Fig. 8. The percentage of α-endosulfan remaining at various time intervals after inoculated with Inorganic Bacteria and actinomycetes isolated from Faw pesticide store soil.

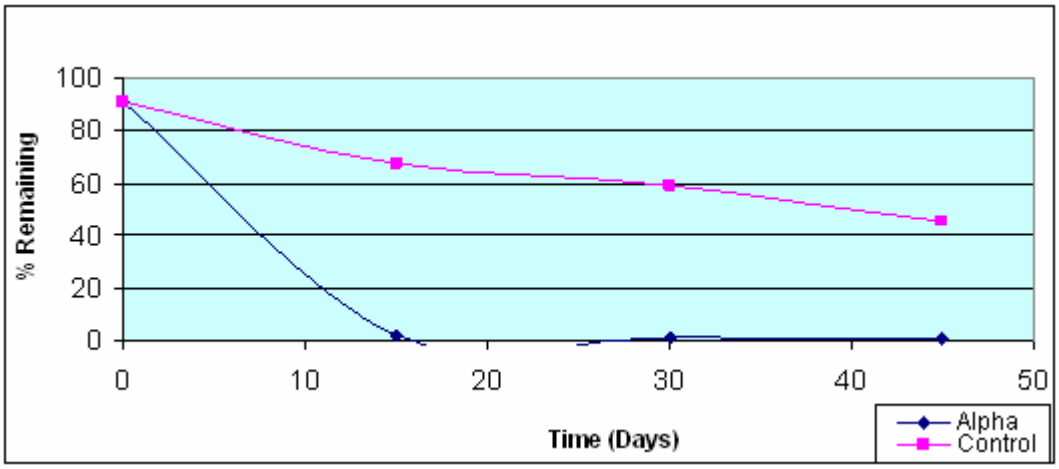


Fig. 9. The percentage of α-endosulfan remaining at various time intervals after inoculated with Inorganic Bacteria and actinomycetes isolated from Elfaw cotton field soil.

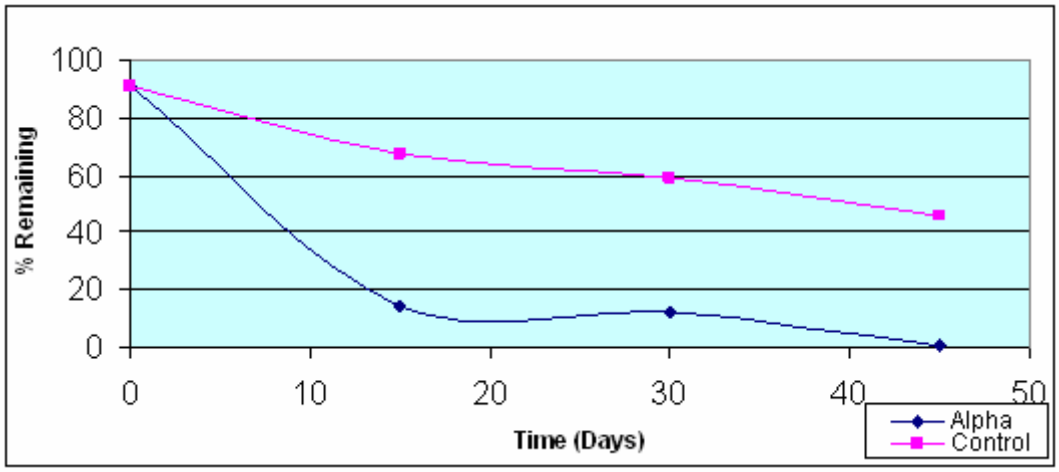


Fig. 10. The percentage of α-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Raselfeel pesticide store soil.

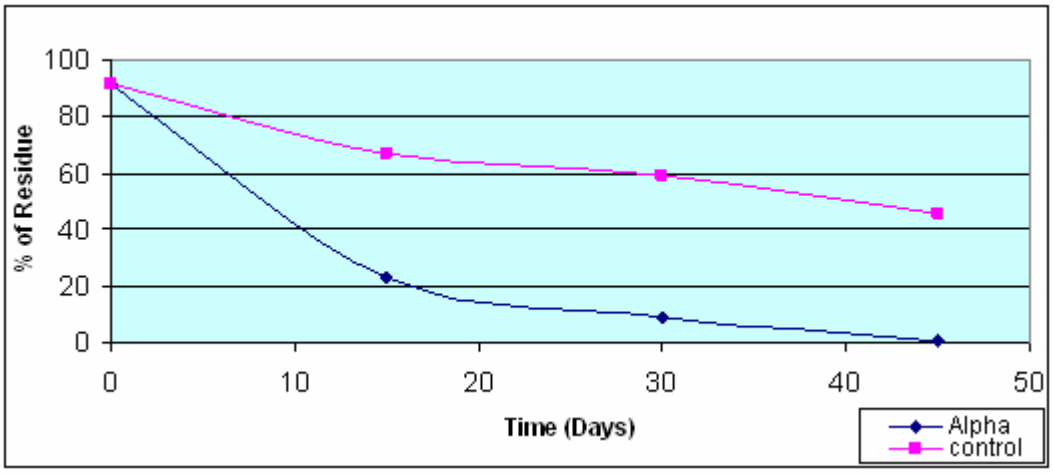


Fig. 11. The percentage of α-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Faw pesticide store soil.

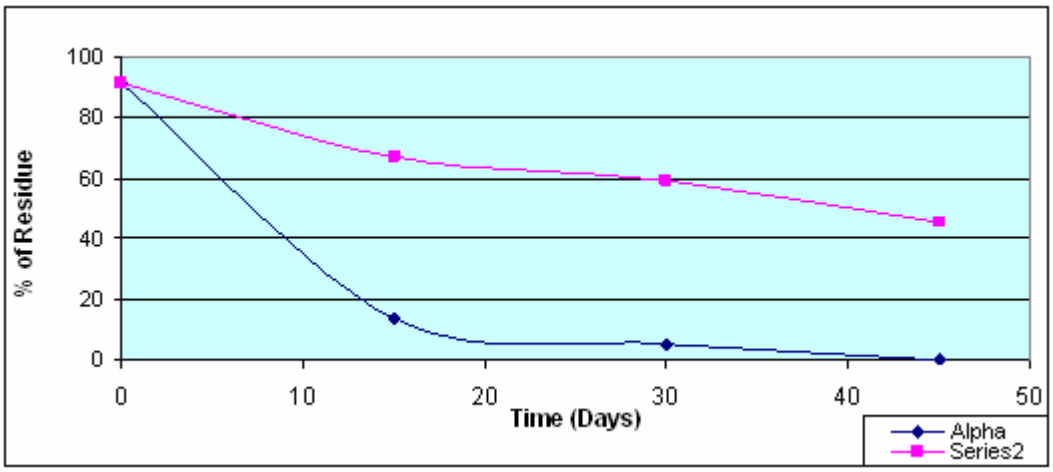


Fig. 12. The percentage of α-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Rahad cotton field soil.

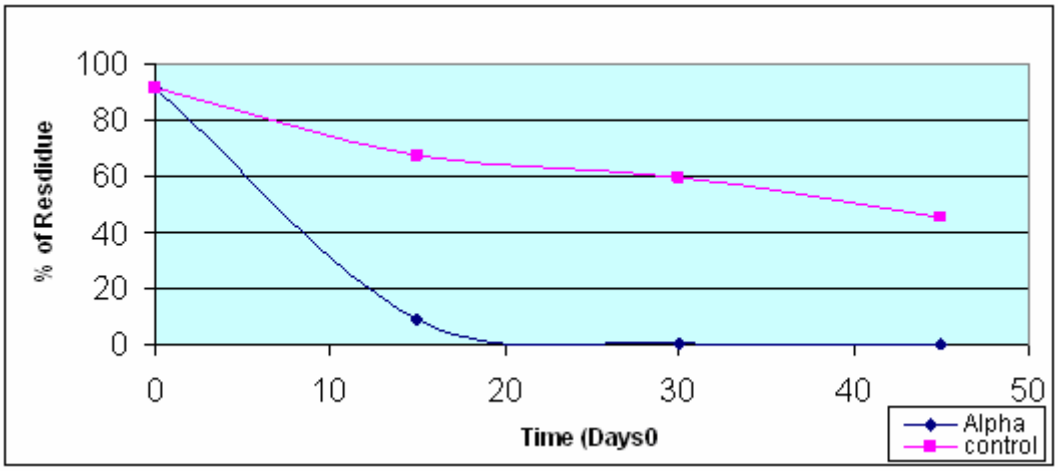


Fig. 13. The percentage of β-endosulfan remaining at various time intervals after inoculated with bacteria and Actinomycetes which live in poor media isolated from kabelgidad pesticide store soil.

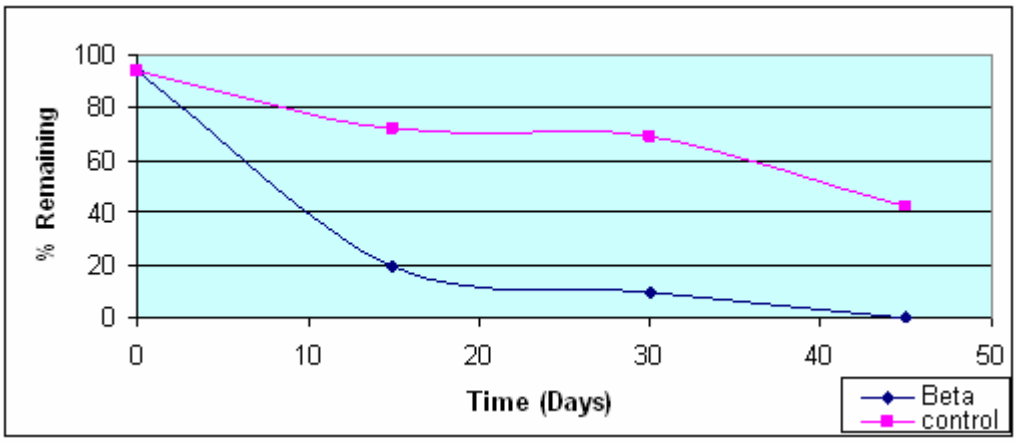


Fig. 14. The percentage of β-endosulfan remaining at various time intervals after inoculated with bacteria and actinomycetes which live in poor media isolated from Gorashi pesticide store soil.

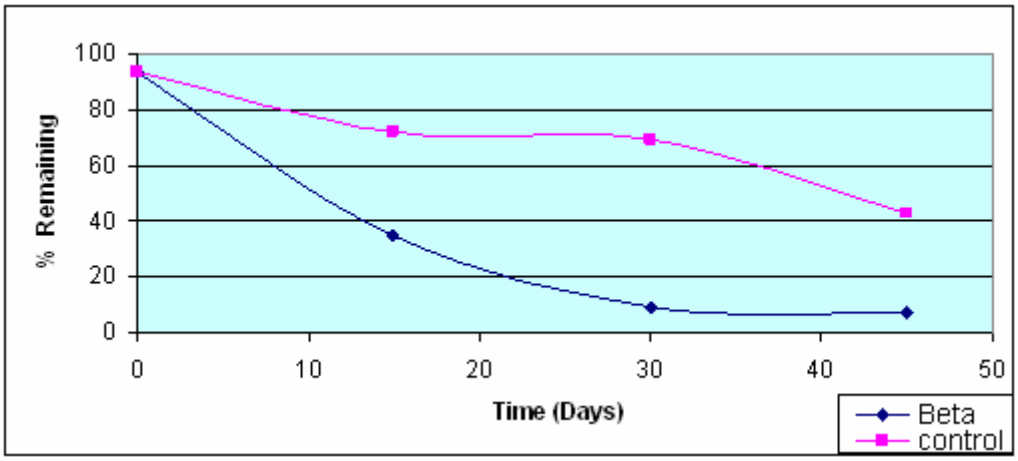


Fig. 15. The percentage of β-endosulfan remaining at various time intervals after inoculated with bacteria and actinomycetes which live in poor media isolated from Raselfeel pesticide store soil.

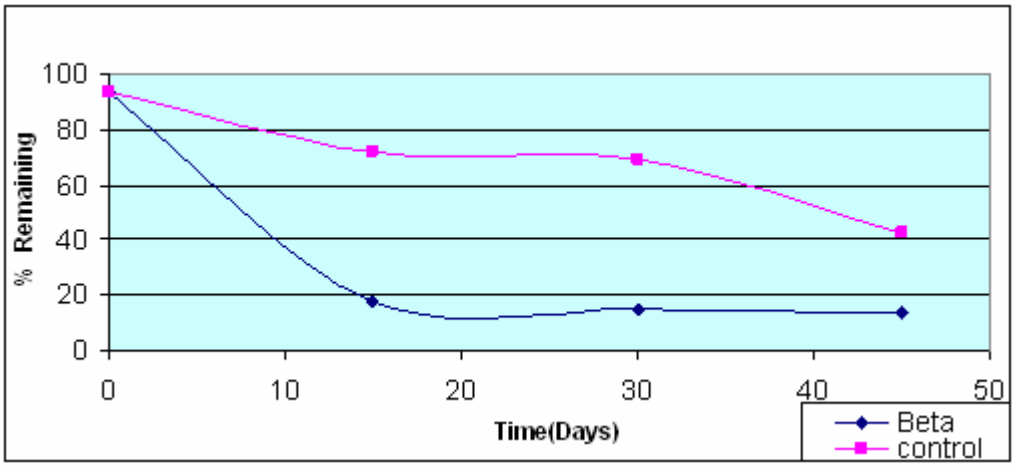


Fig. 16. The percentage of β-endosulfan remaining at various time intervals after inoculated with fungi isolated from Gorashi pesticide store soil.

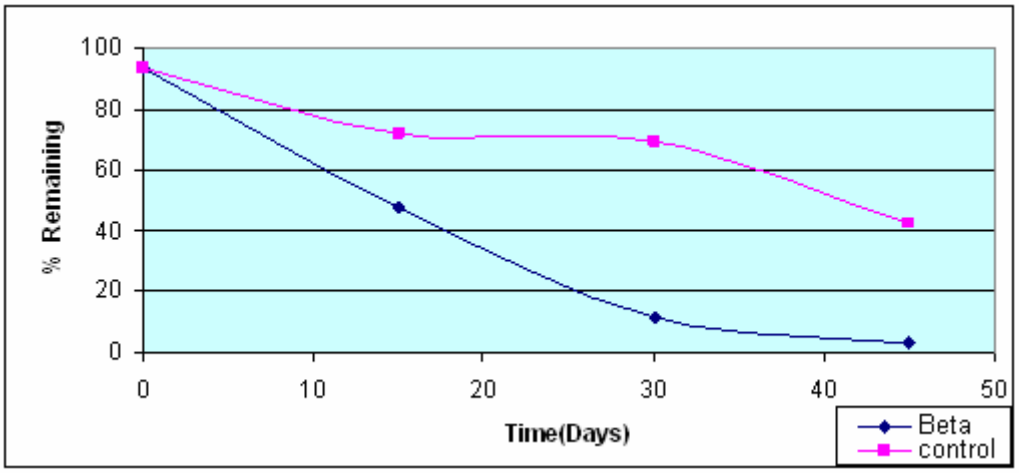


Fig. 17. The percentage of β-endosulfan remaining at various time intervals after inoculated with fungi isolated from Faw pesticide store soil.

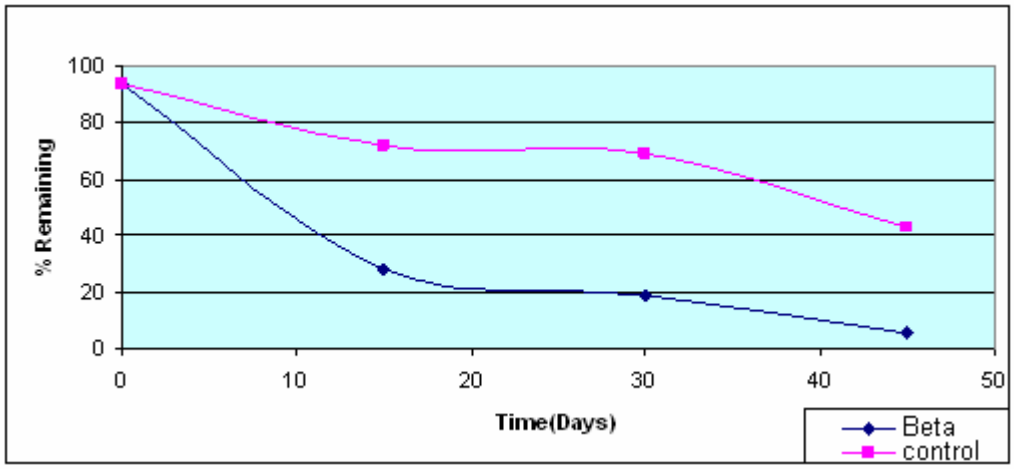


Fig. 18. The percentage of β-endosulfan remaining at various time intervals after inoculated with fungi isolated from Raselfeel cotton field soil.

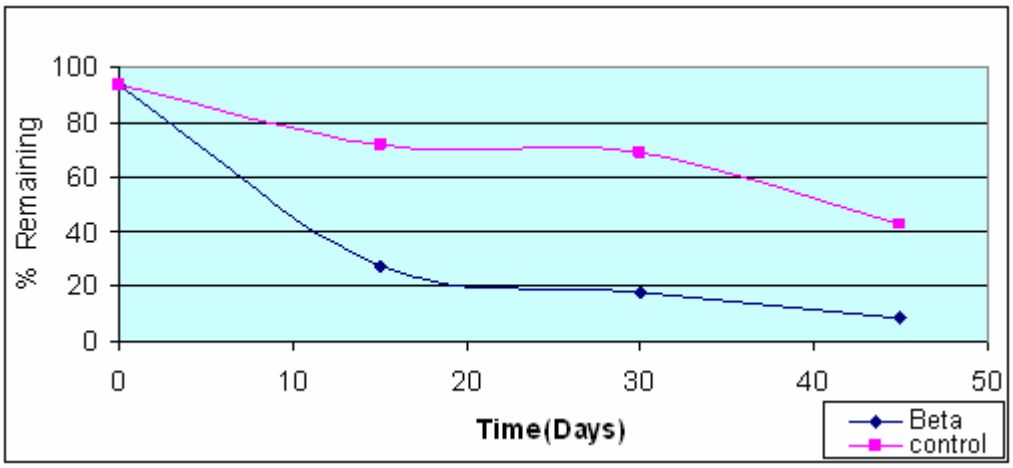


Fig. 19. The percentage of β-endosulfan remaining at various time intervals after inoculated with Inorganic Bacteria and actinomycetes isolated from Gorashi pesticide store soil.

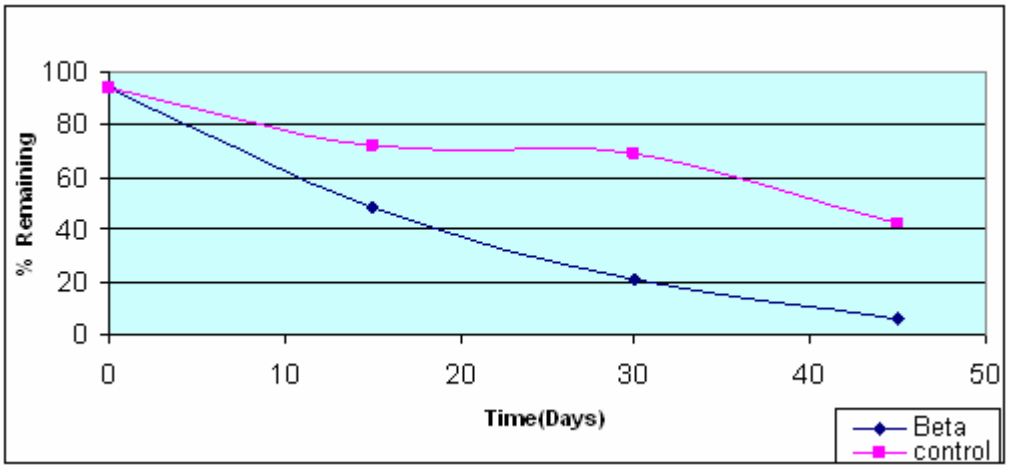


Fig. 20. The percentage of β-endosulfan remaining at various time intervals after inoculated with Inorganic bacteria and actinomycetes isolated from Faw pesticide store soil.

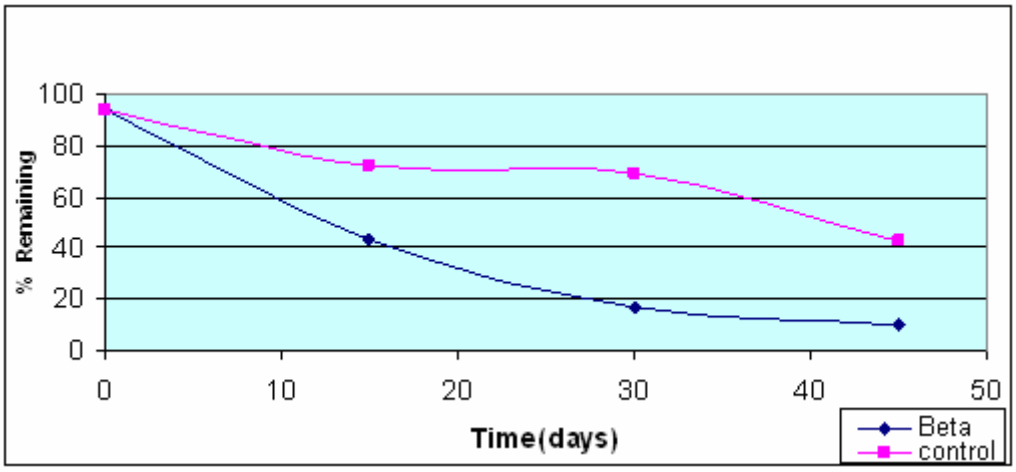


Fig. 21. The percentage of β-endosulfan remaining at various time intervals after inoculated with Inorganic bacteria and actinomycetes isolated from Faw cotton field soil.

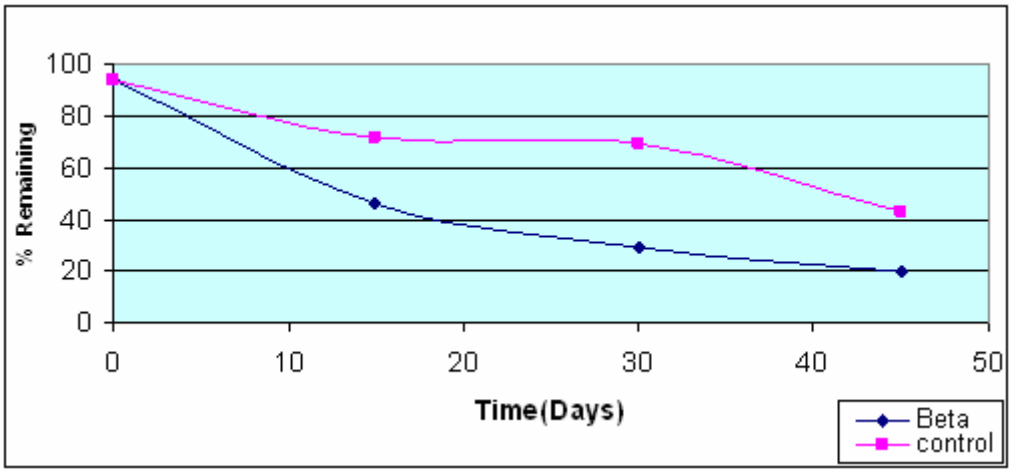


Fig. 22. The percentage of β-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Raselfeel pesticide store soil.

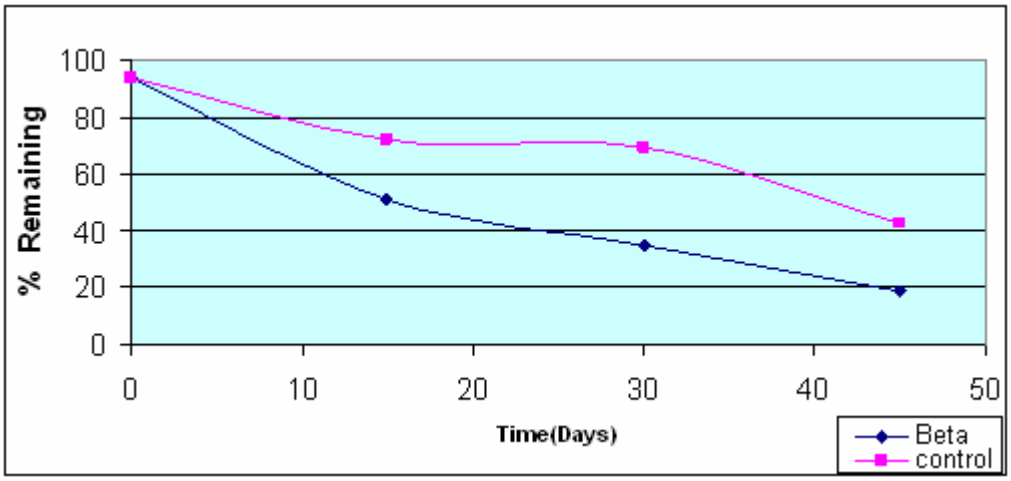


Fig. 23. The percentage of β-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Faw pesticide store soil.

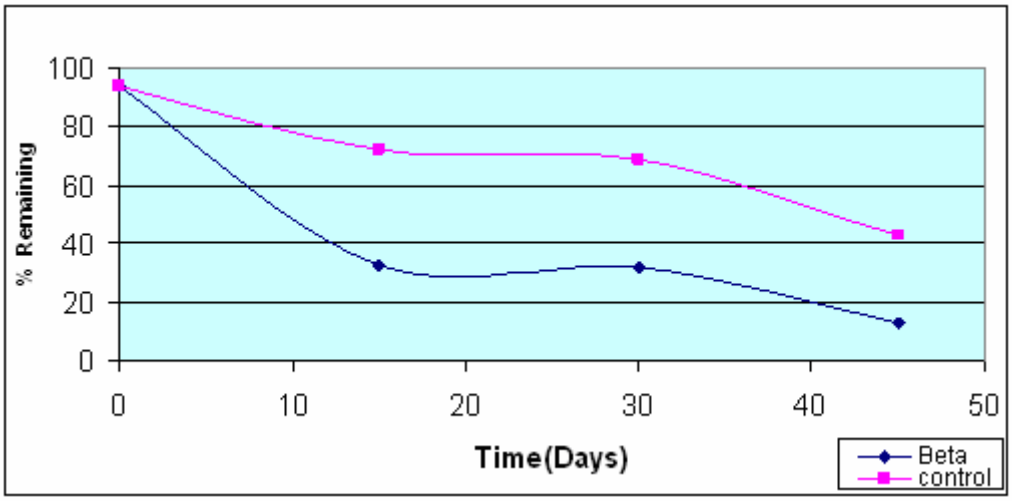
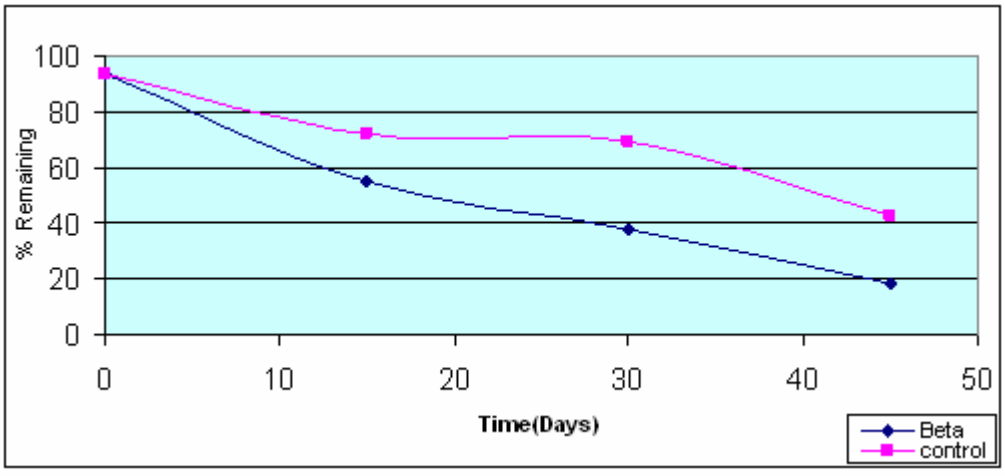


Fig. 24. The percentage of β-endosulfan remaining at various time intervals after inoculated with organic nitrogen bacteria isolated from Elfaw cotton field soil.



on β, actinomycetes and bacteria which live in poor media are more efficient in degrading β- endosulfan than others (Table 11).

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

4.5 Effect of fertilizers (activators) in enhancing the microbial degradation of endosulfan

4.5.1 Fungi

Table 12 shows the total counts of fungi in various treatments. All treatments were highly significantly different from the control. Generally the fungal counts decreased in the first 15 days following the treatment, increased during the next 15 and decreased again to level slightly higher than the first counts (15 days). Phosphate treatment caused the highist effects on fungal counts throughout the various time intervals. Other treatment caused variable effects through time, (Table 12).

Tables 19 and 20 shows the half lives of endosulfan α and β incubated for 45 days in soil treated with different fertilizer. Based on the percentage reduction in half lives it is clear that the range of half lives reduction 23.4 - 65.7 % in α-endosulfan compared to 19.7-73.6 % in β- isomer. The best promotion of fungal activity was obtained when the soil was treated with phosphate, 65 % for α 73% for β (Tables 19 and 20). Generation of sulfate from various treatments was also monitored threshold the exponent period. Generation of sulphate in the control treatment (activator free fungal inoculated soil) starts at 15^{th} days and steadily increased until the 30ist day thereafter it decreases at slow rate until 45^{th} day. (end of experiment). The level of sulphate reached 0.8 m Ml/l after 30 days and approaches anon-

detectable level of the 45 days (Fig 25). However fungal treatment (alone or combined with activator) induced significant change in the pattern of sulphate generation (Fig 25- 29), it starts before 15 days, reached its peak (1.0 mMl/l) at 30 days and decreased at faster rate to reach a non-detectable level after level 45 days.

4.5.2 Organic nitrogen bacteria

Table 13 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 was caused the highest increase in organic nitrogen bacteria counts thought the various time intervals. Other treatments caused variable effects throughout various time intervals (table 13).

Tables 21 and 22 shows the half live of endosulfan α and β incubated for a total of 45 days in soil treated with different fertilize. Based on the percentage redaction in half lives it is clear that 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 21). Which the best promotions of bacterial capability in degrading β- endosulfan was noticed in soil treated with urea (Table 22).

The generation of sulphate in the control treatment (Bacteria inoculated activator free soil) starts at 15^{th} days steadily increased until the 30 its and became non-detectable after decreased at slow rate after 45 days, the level

of sulphate reached 10 m Ml/l after 30 days and became non- date cable after 52 days (Fig 30). Generally the organic nitrogen bacterial treatments (alone or combined with activator), induces significant change in the pattern of sulphate generation curves (Fig. 30- 34).

4.5.3 Inorganic nitrogen bacteria and actinomycetes

Tables 14 and 15 show the total counts of inorganic nitrogen bacteria and actinomycets in various treatments. All treatments significantly enhanced the microbial counts throughout the various time intervals. Phosphate and phosphate + urea treatments caused the highest variance in the counts of inorganic nitrogen bacteria and actinomycetes throughout the various time intervals.

Tables 23 and 24 shows 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 α-endosulfan compared to
36.5 - 72.4 % in β- 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 (tables 23) and 72.4 in β-endosulfan (Table 24).

The generation of sulphate in the control treatment (bacteria and actinomycetes activator free soil) starts at the 15 day, steadily increased until the 30 ist day and thereafter decreased at slow rate become non-detectable after 40 days. The level of sulphate reached 0.6 mMl/l after 30 days (Fig. 35). However in organic nitrogen bacteria and actinomycetes treatments induces significant change in the pattern of sulphate generation (Fig 4- 44),

it starts from the first day and reach its peak (0.9 m Ml/l) at the 30 days and decreased at a fasten rater and become non-detectable after 45 days.

4.5.4 Bacteria and actinomycetes which is live in poor media

Tables 16, 17 and 18 show the counts of bacteria and actinomycetes which livers in poor media. All treatment were highly significantly different from the control. All treatments enhanced the microbial count throughout the various time intervals. A phosphate treatment caused the highest increase in counts of bacteria and actinomycetes which lives in poor media through out the various time intervals.

Tables 25 and 26 shows the half lives of endosulfan α and ß incubated with Bacteria and Actinomycetes which lives in poor media for 45 days in soil treated with different fertilizers.

The reduction of half lives ranged between 46.5 -61.4% in α- endosulfan compared to 31.7 - 68.1% in B isomer. The best promotion in the activity of such microorganism in degrading endosulfan was obtained in soil treated with urea; 68.1 for α- endosulfan (table 25)and soil treated with urea+ phosphate, 62.2% for β isomer (table 26).

The generation of sulphate in the control treatment (bacteria and actinomycetes which lives in poor media inoculated activator free soil) start from the first days, steadily increased until 30 ist and there after decreased at slow rate until became non – detectable after 60 days. The level of sulphate reached 0.2 Mml/l. after 30 days (Fig 35). However bacteria and actinomycetes which liver in poor media induces significant change in the pattern of sulphate generation.

Sulphate generations in treatments starts at day zero day and approaches higher levels (1.2 m Ml/ l) with a clear belt shaped curve (Fig 35–39).

4.6 Microbial degradation high endosulfan concentration in carbon free media

Tables 27 and 28 show the half lives of endosulfan α and β-incubated for a total of 30 days in carbon free media treated with elevated endosulfan concentration (500 mg/l). The rate of reduction in half lives ranges between 62.3 - 72.9% for α- endosulfan and 51.5 - 71.3% for β-endosulfan. Results (Tables 11 and 28) indicated that high concentration of β-endosulfan caused reduction in the microbial capability of degrading this chemical. By comparing results from this Table (28) and Table 11 the rate of reduction in capability of various groups ranged between 2 - 12% the higher reduction occurred in the fungal activity while the lowest reduction was noting in the activity of in organic nitrogen bacteria and a ctinomycetes. Mixing the various groups of microorganism together died not cause much improvement in their activity.

The generation of sulphate (Figs 46- 55) was monitored for 30 days. Sulphate was slowly generated from the microbial treatments reaching maximum after 20 days (0.2 m Ml/l), thereafter the Sulphate level slowly decline and became non- detectable after thirty days. On the other hand the Sulphate level in the control gradually in creased at but faster rate and apparently did not decline even after 30 days (when the experiments was terminated). The higher level of sulphate generated was 0.2 m Ml/l .

Table 12. Average count of fungi per gram of fertilizer treated soils

Fertilizer	Time (Days)		
	15	30	45
Control	0.2×10^4	0.4×10^4	0.1×10^3
Urea	0.3×10^3	1.2×10^4	0.1×10^4
Urea + phosphate	0.8×10^3	0.6×10^4	0.3×10^3
Phosphate	0.9×10^3	2.4×10^4	1.2×10^4
Organic fertilizer	0.7×10^3	0.3×10^4	0.2×10^4
Grand means	0.4×10^4	4.8×10^4	1.6×10^4
SE ±	30.6^{**}	109.1^{**}	76.6^{**}
C.V.	1.2	0.4	0.8

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil were not treated with fertilizers.

SE = Standard Error

C.V. = Coefficient of Variation

Table 13. Average count of organic nitrogen bacteria per gram of fertilizer treated soils

Fertilizer	Time (Days)		
	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^{**}
C.V.	0.8	0.1	0.03

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 14. Average count of inorganic nitrogen bacteria per gram of fertilizer treated soils

Fertilizer	Time (Days)		
	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^{**}
C.V.	0.01	0.01	0.02

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 15. Average count of inorganic nitrogen actinomycetes per gram of fertilizer treated soils

Fertilizer	Time (Days)		
	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^{**}
C.V.	0.08	0.02	0.04

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 16. Average count of bacteria and actinomycetes which live in poor media per gram of fertilizer treated soils after 15 days.

Fertilizer	Actinomycetes/g soil			Bacteria /g soil		Total
	Nocardia	*Bactoderma*	*Micromonospora*	*Arthrobacterium*	*Mycobaterium*	
Control	4.5×10^3	1.1×10^3	5.7×10^3	1.4×10^3	6.8×10^3	19.5×10^3
Urea	3.9×10^3	0.8×10^3	6.0×10^3	2.0×10^3	5.9×10^3	18.6×10^3
Urea + phosphate	8.9×10^3	4.7×10^3	8.0×10^3	3.3×10^3	5.0×10^3	29.9×10^3
Phosphate	6.5×10^3	10.9×10^3	16.2×10^3	2.9×10^3	17.3×10^3	53.8×10^3
Organic fertilizer	12.6×10^3	4.5×10^3	11.9×10^3	4.9×10^3	15.3×10^3	49.2×10^3
Grand means	36.4×10^3	22.0×10^3	47.8×10^3	14.5×10^3	50.3×10^3	171.0×10^3
SE ±	579.7^{**}	568.9^{**}	434.3^{**}	616.9^{**}	1426.1^{**}	-
C.V.	2.2	4.5	1.5	6.4	4.9	-

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 17. Average count of bacteria and actinomycetes which live in poor media per gram of fertilizer treated soils, after 30 days.

Fertilizer	Actinomycetes/g soil			Bacteria /g soil		Total
	Nocardia	*Bactoderma*	*Micromonospora*	*Arthrobacterium*	*Mycobaterium*	
Control	5.9×10^3	1.4×10^3	4.7×10^3	1.7×10^3	5.7×10^3	19.4×10^3
Urea	8.2×10^3	7.9×10^3	4.2×10^3	0.7×10^3	7.0×10^3	28.0×10^3
Urea + phosphate	6.5×10^3	17.0×10^3	9.7×10^3	11.5×10^3	8.3×10^3	53.0×10^3
Phosphate	11.8×10^3	20.3×10^3	17.2×10^3	11.1×10^3	24.6×10^3	85.0×10^3
Organic fertilizer	10.5×10^3	6.2×10^3	16.5×10^3	8.2×10^3	18.4×10^3	59.8×10^3
Grand means	42.9×10^3	52.7×10^3	52.4×10^3	33.0×10^3	63.9×10^3	244.9×10^3
SE ±	789.1^{**}	447.3^{**}	744.2^{**}	537.9^{**}	445.4^{**}	-
C.V.	3.2	1.4	2.5	2.8	1.2	-

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 18. Average count of bacteria and actinomycetes which live in poor media per gram of fertilizer treated soils, after 45 days.

Fertilizer	Actinomycetes/g soil			Bacteria /g soil		Total
	Nocardia	*Bactoderma*	*Micromonospora*	*Arthrobacterium*	*Mycobaterium*	
Control	1.9×10^3	6.7×10^3	5.2×10^3	2.8×10^3	2.0×10^3	18.6×10^3
Urea	1.7×10^3	1.2×10^3	4.4×10^3	2.4×10^3	6.7×10^3	16.4×10^3
Urea + phosphate	2.3×10^3	9.0×10^3	6.5×10^3	4.0×10^3	10.2×10^3	32.0×10^3
Phosphate	3.9×10^3	10.3×10^3	8.3×10^3	8.3×10^3	9.6×10^3	40.4×10^3
Organic fertilizer	7.3×10^3	9.9×10^3	3.1×10^3	0.5×10^3	4.7×10^3	25.5×10^3
Grand means	17.1×10^3	37.1×10^3	27.5×10^3	18.0×10^3	33.2×10^3	132.9×10^3
SE ±	130.7^*	198.8^*	279.3^*	114.3^*	192.1^*	-
C.V.	1.3	0.9	1.8	1.1	1.0	-

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

Control = Soil was not treated with fertilizers.

SE = Standard Error

C.V. =Coefficient of Variation

Table 19. Half lives, percentage reduction in half lives and percentage of degradation after 45 days of α – endosulfan incubated with fungi in fertilizer treated soil.

Treatment	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Fertilizer-free soil	1.77	0.8671	20.2	43.9	45.4
Urea treated soil	2.30	0.9667	27.6	23.4	100
Urea + Phosphate treated soil	2.19	0.8575	15.3	57.5	100
Phosphate treated soil	2.08	0.7311	12.3	65.7	100
Organic fertilizer treated soil	2.28	0.9476	21.0	41.7	96.8
Sterilized fertilizer-free soil	1.63	0.9099	36.0	00.0	66.2

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 20. Half lives, percentage reduction in half lives and percentage of degradation after 45 days of β– endosulfan incubated with fungi in fertilizer treated soil.

Treatment	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Fertilizer-free soil	1.59	0.9284	32.4	19.7	70.6
Urea treated soil	2.23	0.8951	16.4	59.5	100
Urea + Phosphate treated soil	2.21	0.8892	15.9	60.1	100
Phosphate treated soil	1.98	0.7047	10.9	73.1	100
Organic fertilizer treated soil	2.08	0.8475	14.2	64.8	100
Sterilized fertilizer-free soil	1.38	0.9363	40.4	00.0	57.5

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 21. 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.

Treatment	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
Urea + Phosphate treated soil	2.03	0.7283	12.9	63.9	100
Phosphate treated soil	2.18	0.6742	11.2	69.0	100
Organic fertilizer treated soil	2.07	0.8630	15.5	56.9	100
Sterilized fertilizer-free soil	1.63	0.9099	36.0	00.0	66.2

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 22. 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.

Treatment	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
Urea + Phosphate treated soil	2.14	0.8572	14.5	63.9	100
Phosphate treated soil	2.15	0.8622	14.7	63.6	100
Organic fertilizer treated soil	2.19	0.9258	19.5	52.8	100
Sterilized fertilizer-free soil	1.38	0.9363	40.4	00.0	57.5

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 23. 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.

Treatment	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
Urea + Phosphate treated soil	2.27	0.8181	13.7	62.2	100
Phosphate treated soil	2.18	0.8244	14.3	60.2	100
Organic fertilizer treated soil	2.03	0.6899	14.1	60.1	100
Sterilized fertilizer-free soil	1.63	0.9099	36.0	00.0	66.2

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 24. Half lives, percentage reduction in half lives and percentage of degradation after 45 days of ß –endosulfan incubated with inorganic nitrogen bacteria and actinomycetes in fertilizer treated soil.

Treatment	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
Urea + Phosphate treated soil	2.99	0.7192	11.2	72.4	100
Phosphate treated soil	2.12	0.8303	13.7	66.2	100
Organic fertilizer treated soil	2.47	0.8366	22.1	45.3	100
Sterilized fertilizer-free soil	1.38	0.9363	40.4	00.0	66.2

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 25. Half lives, percentage reduction in half lives and percentage of degradation after 45 days of α – endosulfan incubated with actinomycetes and Bacteria which lives in poor media in fertilizer treated soil.

Treatment	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Fertilizer-free soil	1.78	0.6689	14.3	60.2	89.7
Urea treated soil	2.22	0.8854	16.2	55.2	100
Urea + Phosphate treated soil	2.12	0.7978	13.9	61.4	100
Phosphate treated soil	2.22	0.8852	15.3	57.4	100
Organic fertilizer treated soil	2.19	0.9417	19.3	46.5	98.4
Sterilized fertilizer-free soil	1.63	0.9099	36.0	00.0	66.2

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 26. Half lives, percentage reduction in half lives and percentage of degradation after 45 days of ß–endosulfan incubated with actinomycetes and Bacteria which lives in poor media in fertilizer treated soil.

Treatment	Slope	R^2	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	Degradation % after 45 days
Fertilizer-free soil	1.71	0.9712	27.6	31.7	85.1
Urea treated soil	2.06	0.7963	12.9	68.1	100
Urea + Phosphate treated soil	2.26	0.8997	17.0	57.8	100
Phosphate treated soil	2.20	0.8862	17.1	57.5	100
Organic fertilizer treated soil	2.02	0.9751	19.6	51.6	96.3
Sterilized fertilizer-free soil	1.38	0.9363	40.4	00.0	57.5

All fertilizers in various treatments were applied at a rate of one gram per 50 g soil

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Fig. 25. Effect of fungi in degradation of endosulfan (α & β) and generation of sulphate in soil

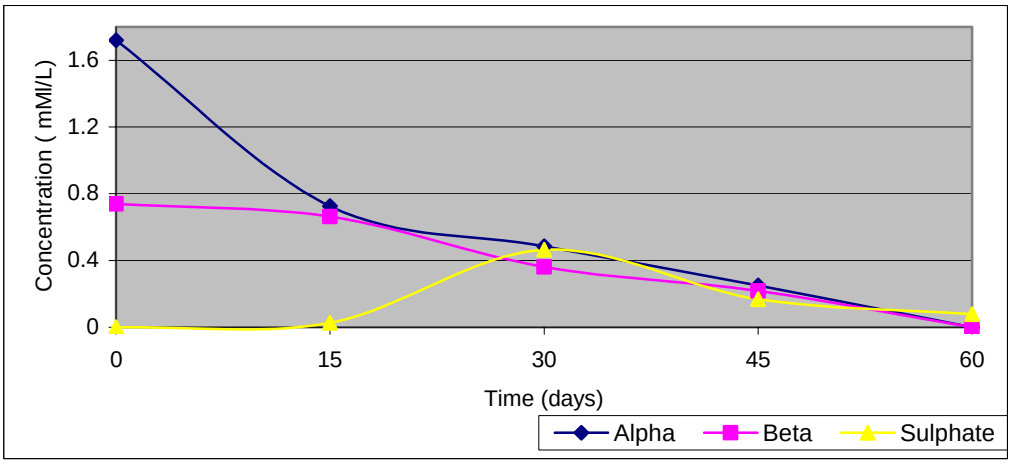


Fig. 26. Effect of fungi in degradation of endosulfan (α & β) and generation of sulphate in urea treated soil

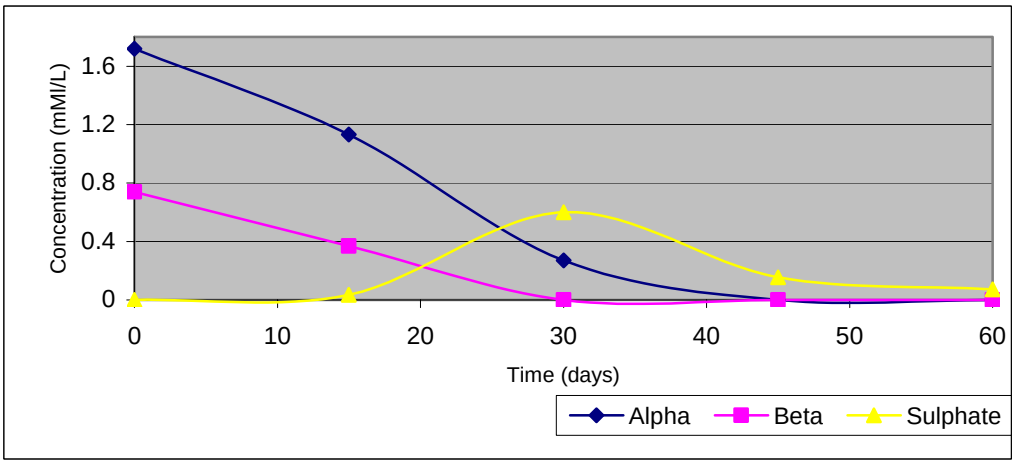


Fig. 27. Effect of fungi in degradation of endosulfan (α & β) and generation of sulphate in urea and phosphate treated soil

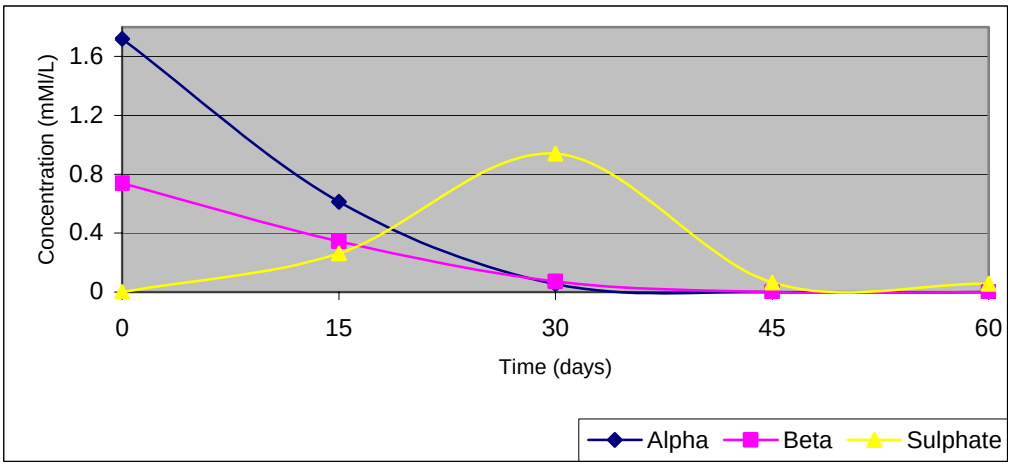


Fig. 28. Effect of fungi in degradation of endosulfan (α & β) and generation of sulphate in phosphate treated soil.

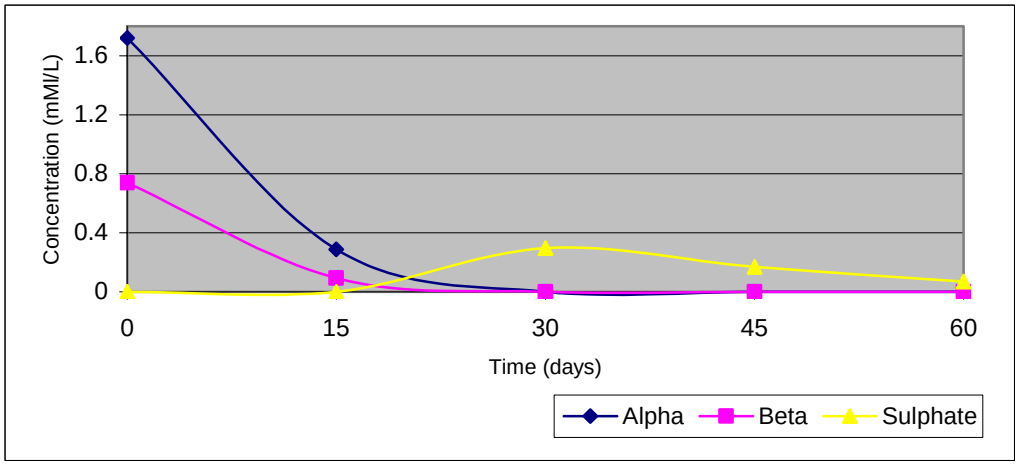


Fig. 29. Effect of fungi in degradation of endosulfan (α & β) and generation of sulphate in cow manure treated soil

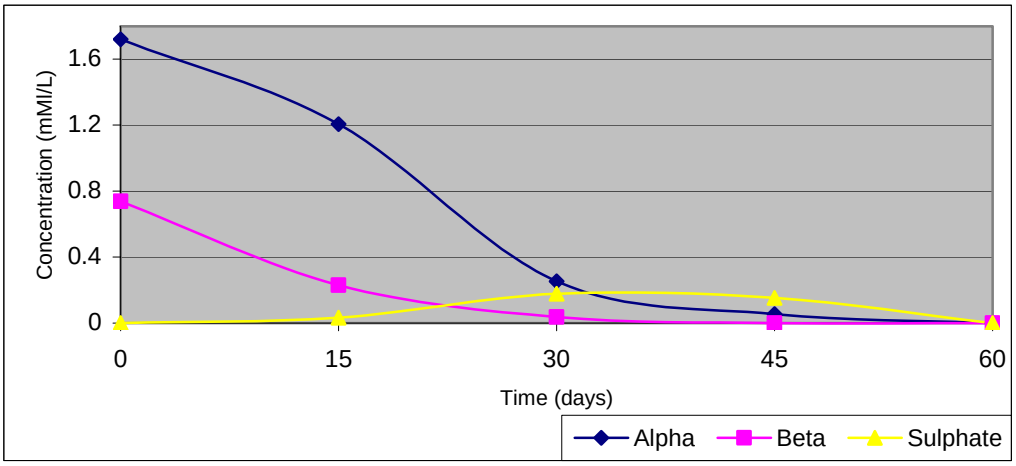


Fig. 30. Effect of nitrogen bacteria in degradation of endosulfan (α & β) and generation of sulphate in soil

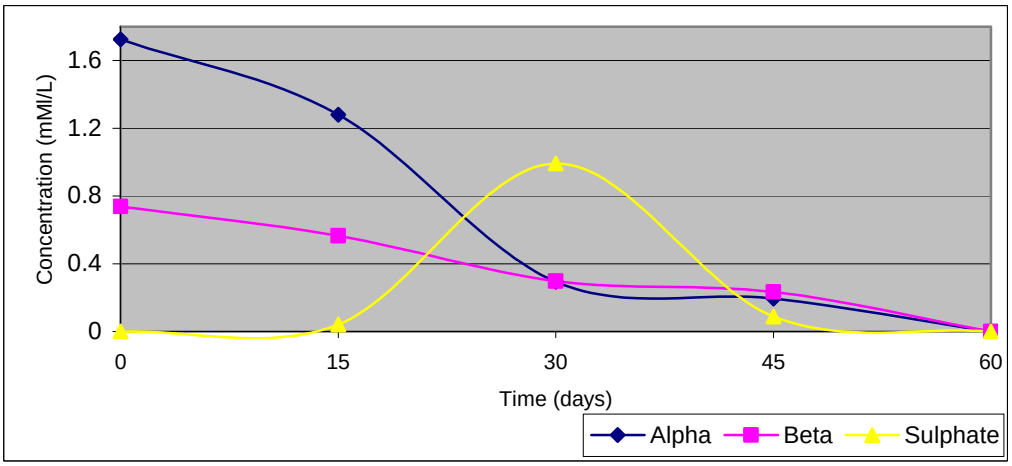


Fig. 31. Effect of nitrogen bacteria in degradation of endosulfan (α & β) and generation of sulphate in urea treated soil

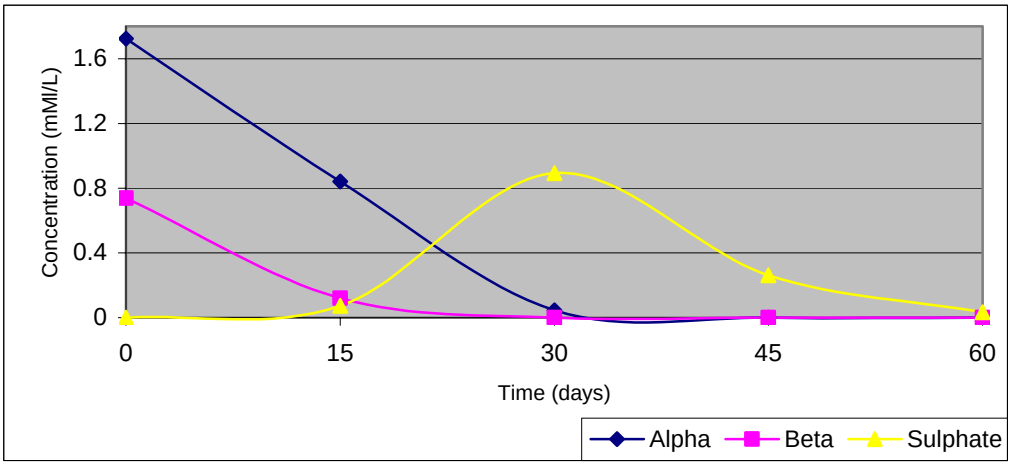


Fig. 32. Effect of nitrogen bacteria in degradation of endosulfan (α & β) and generation of sulphate in urea and phosphate treated soil

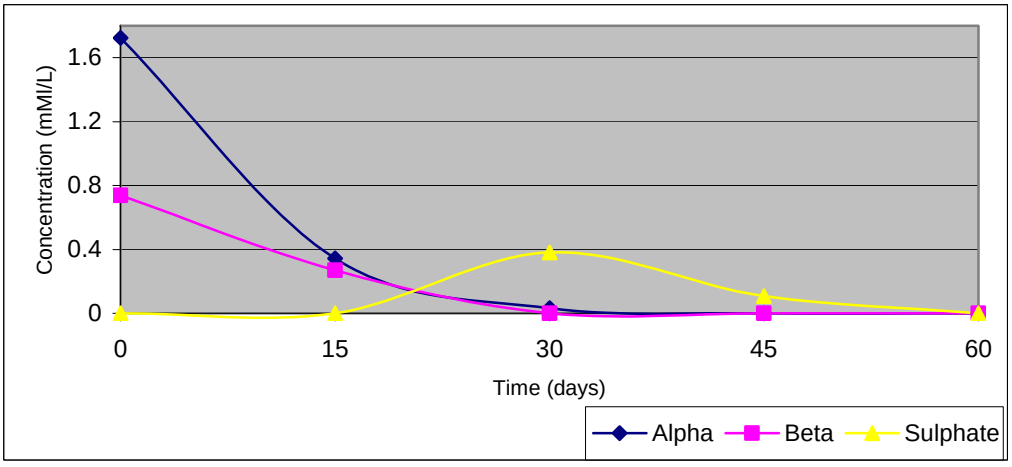


Fig. 33. Effect of nitrogen bacteria in degradation of endosulfan (α & β) and generation of sulphate in phosphate treated soil

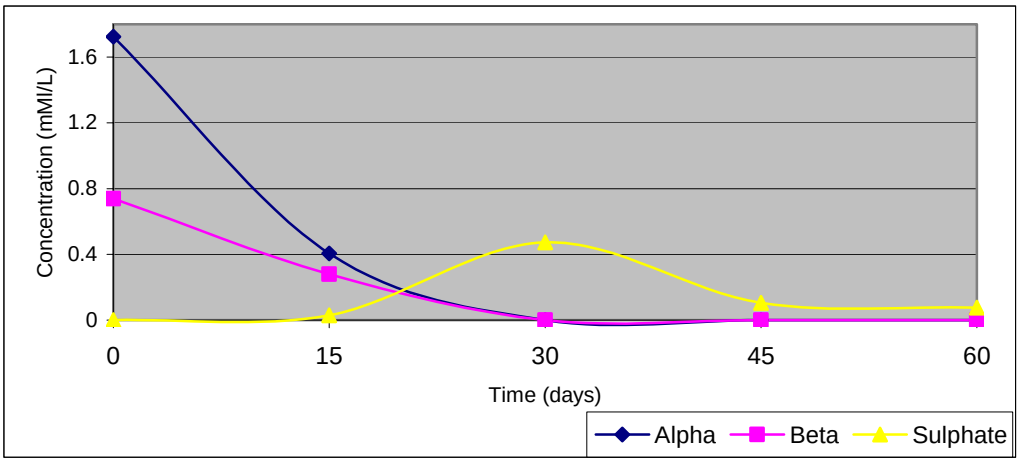


Fig. 34. Effect of nitrogen bacteria in degradation of endosulfan (α & β) and generation of sulphate in cow manure treated soil

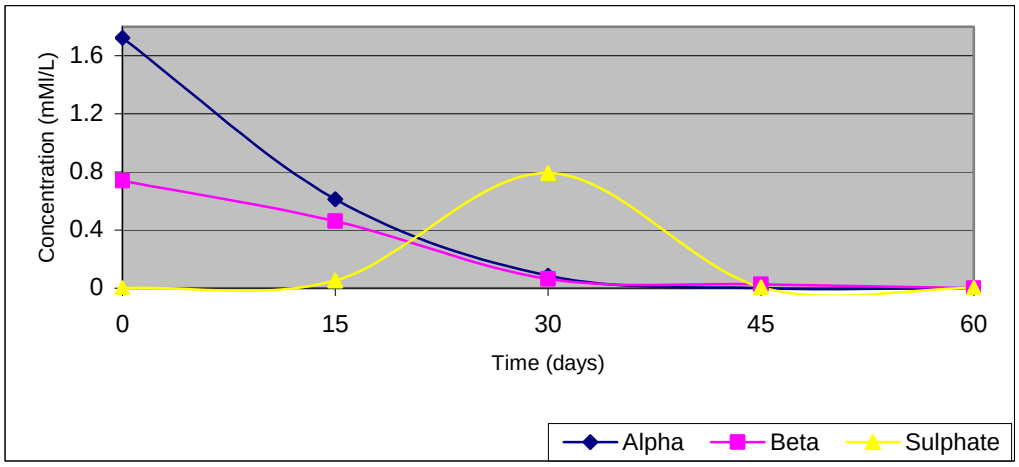


Fig.35. Effect of bacteria and actinomycetes which live in poor media in degradation of endosulfan (α & β) and generation of sulphate in soil

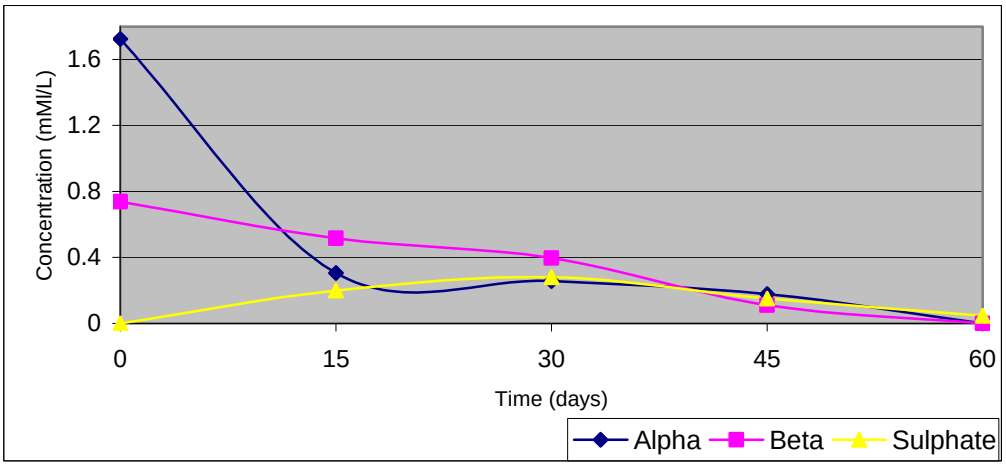


Fig. 36. Effect of bacteria and actinomycetes which live in poor media in degradation of endosulfan (α & β) and generation of sulphate urea treated soil.

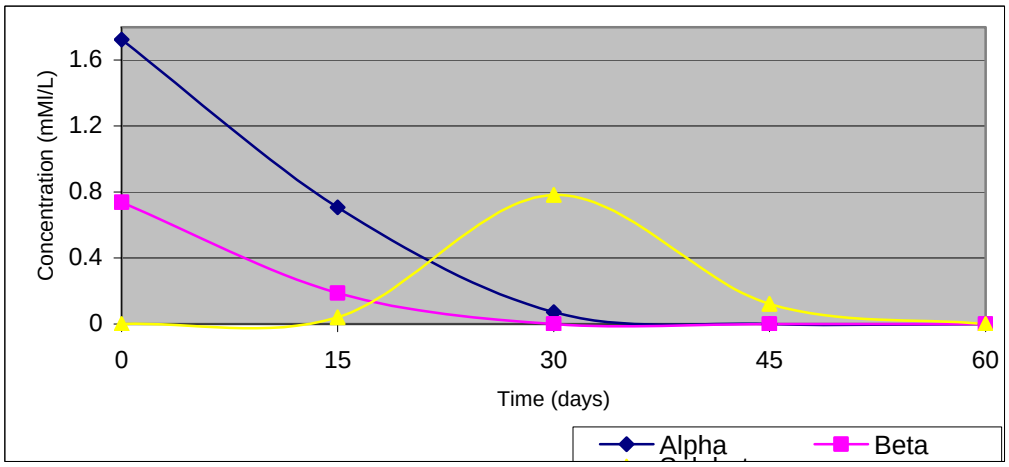


Fig. 37. Effect of bacteria and actinomycetes which live in poor media in degradation of endosulfan (α & β) and generation of sulphate in urea and phosphate treated soil.

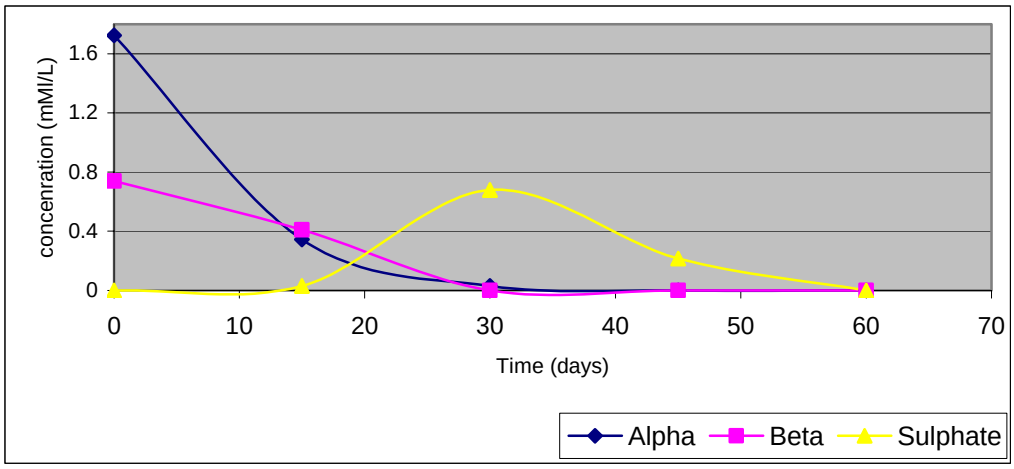


Fig. 38. Effect of bacteria and actinomycetes which live in poor media in degradation of endosulfan (α & β) and generation of sulphate in phosphate treated soil.

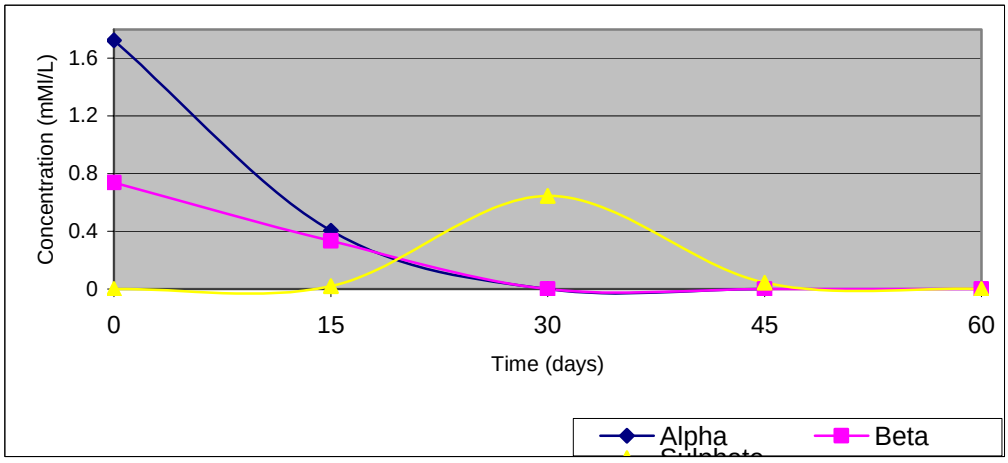


Fig. 39. Effect of bacteria and actinomycetes which live in poor media in degradation of endosulfan (α & β) and generation of sulphate in cow manure treated soil.

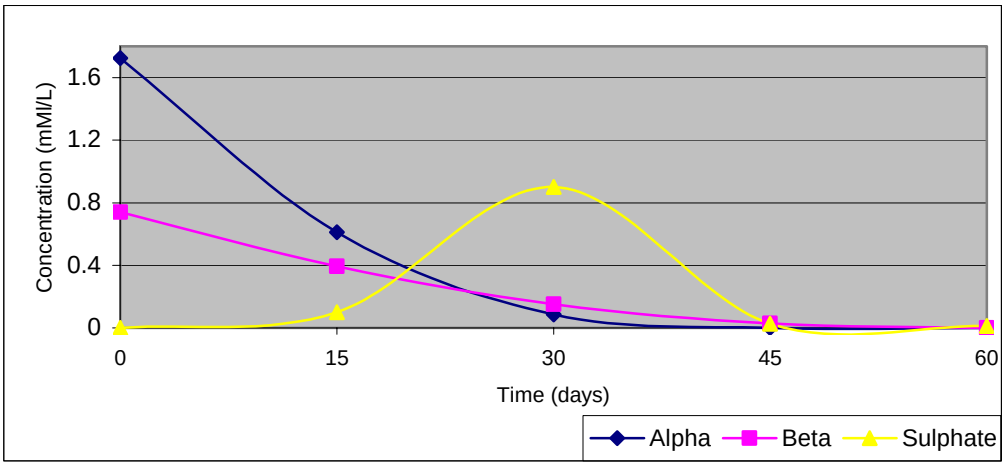


Fig. 40. Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α & β) and generation of sulphate in soil

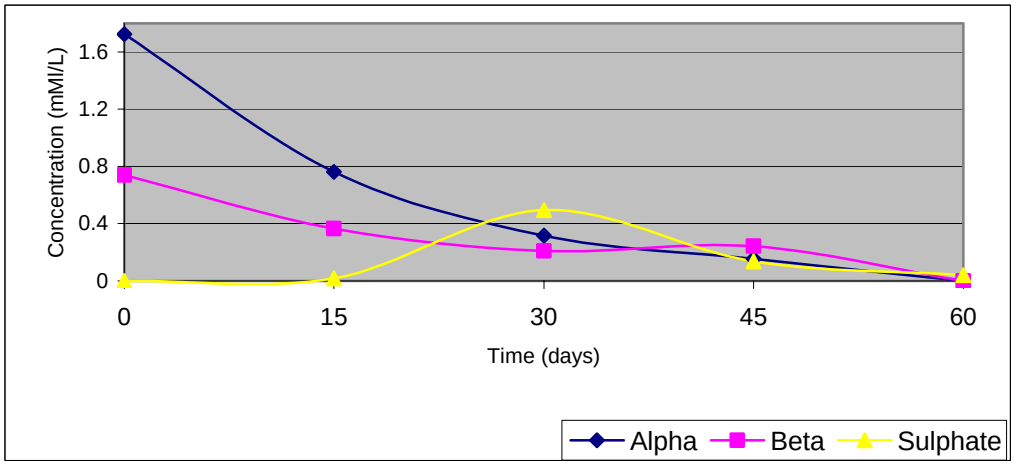


Fig. 41. Effect of inorganic nitrogen bacteria and actinomycetes indegradation of endosulfan (α & β) and generation of sulphate in urea treated soil.

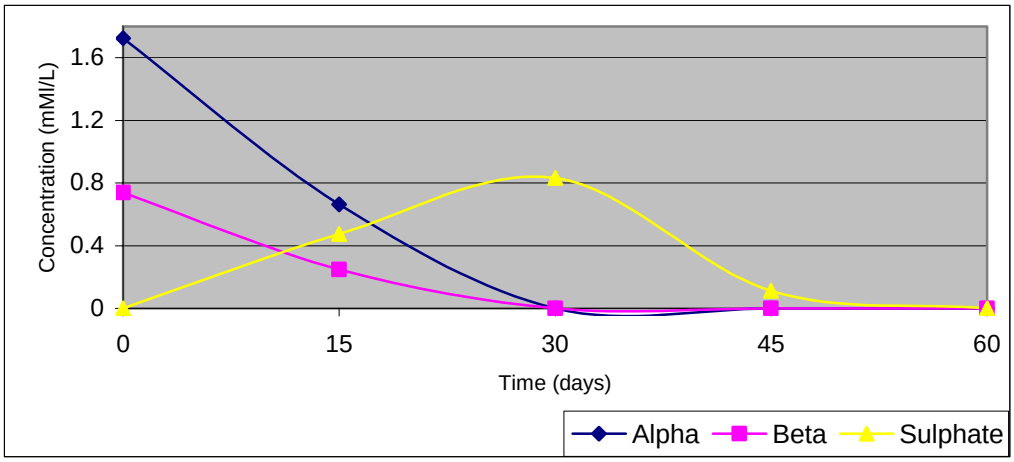


Fig. 42. Effect of inorganic nitrogen bacteria and actinomycetes indegradation of endosulfan (α & β) and generation of sulphate in urea and phosphate treated soil.

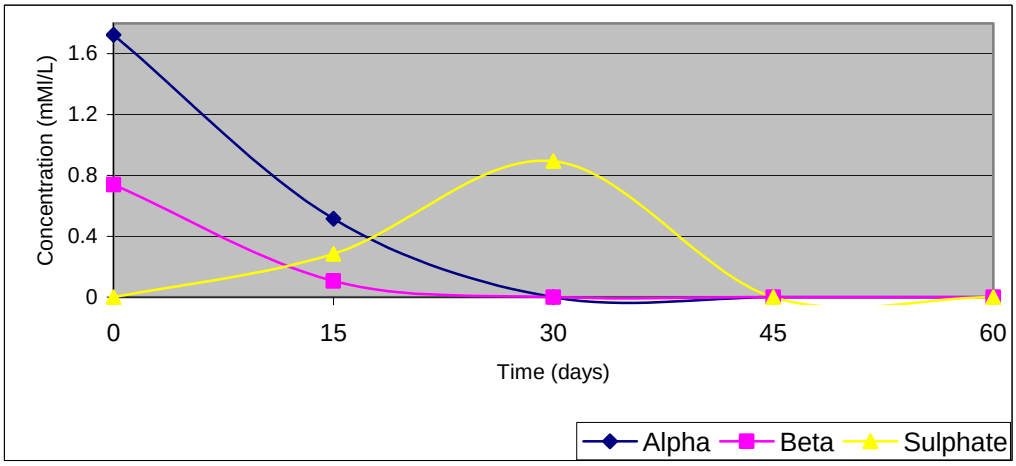


Fig. 43. Effect of inorganic nitrogen bacteria and actinomycetes indegradation of endosulfan (α & β) and generation of sulphate in phosphate treated soil.

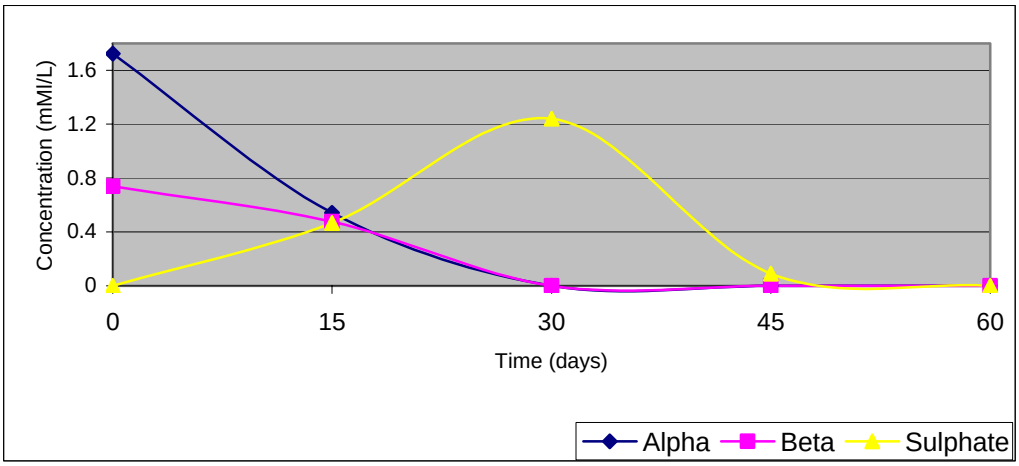


Fig. 44. Effect of inorganic nitrogen bacteria and actinomycetes in degradation of endosulfan (α & β) and generation of sulphate in cow manure treated soil.

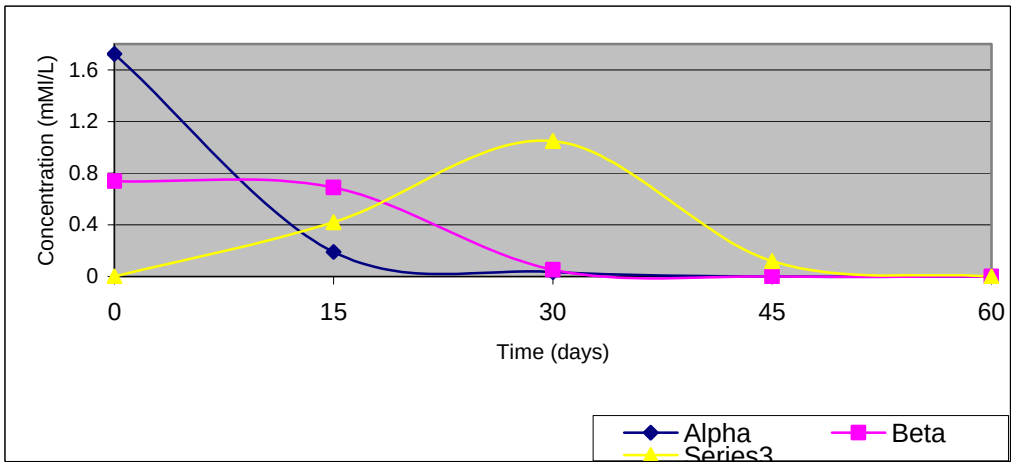


Fig.45. Degradation of endosulfan (α & β) and generation of sulphate in sterilized fertilizer-free soil (Control)

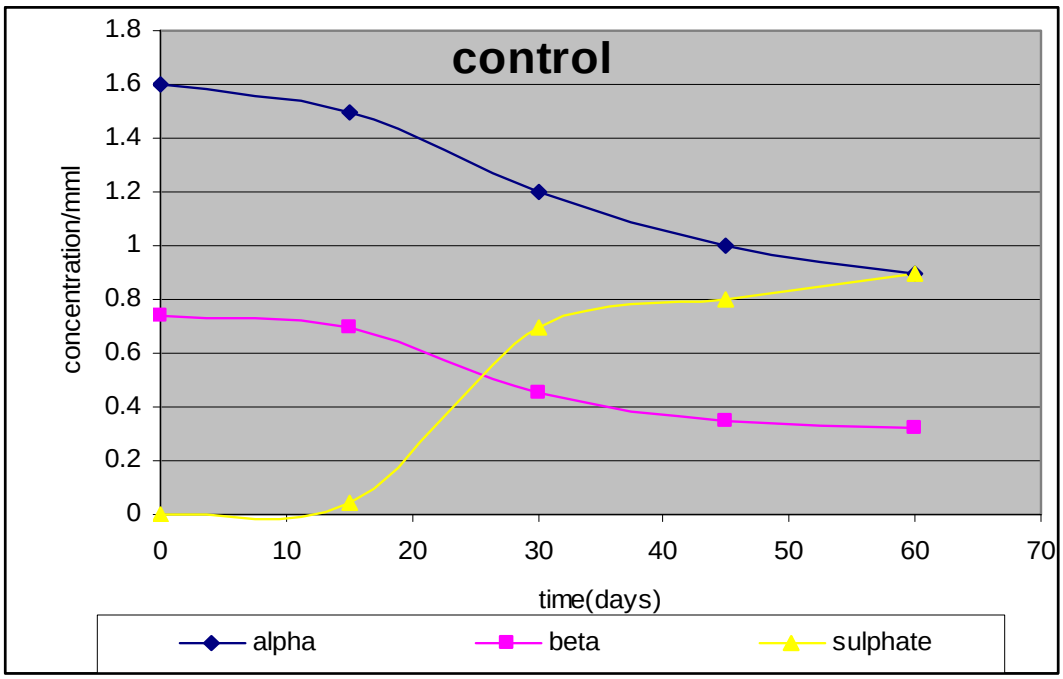


Table 27. Half live (days) and percentage reduction in half live of α-endosulfan incubated with isolated soil microorganisms in carbon-free media containing endosulfan (500 mg/l)

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %
Fungi	0.9484	2.4027	20.8	64.7
Inorganic nitrogen Actinomycetes and Bacteria	0.8162	2.3128	17.3	70.7
Actinomycetes and Bacteria which lives in poor media	0.8742	2.2362	17.6	70.2
Organic nitrogen bacteria	0.8672	2.4364	15.9	72.9
Nocardia	0.8819	2.8089	18.3	68.9
Bactoderma	0.9301	2.8761	18.2	69.1
Micromonospora	0.7510	1.8446	20.0	66.1
Arthrobacter	0.9324	1.9497	22.2	62.3
Mixture	0.9443	2.2989	20.9	64.4
Controls	0.9851	0.8257	58.9	00.0

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 28. Half live (days) and percentage reduction in half live of β-endosulfan incubated with isolated soil microorganisms in carbon-free media containing endosulfan (500 mg/l)

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %
Fungi	0.9992	3.2821	15.1	55.5
Inorganic nitrogen Actinomycetes and Bacteria	0.7324	2.5339	11.9	64.9
Actinomycetes and Bacteria which lives in poor media	0.8604	2.8620	12.1	63.5
Organic nitrogen bacteria	0.8292	3.2839	09.8	71.3
Nocardia	0.9759	3.3449	12.8	62.4
Bactoderma	0.8750	2.7381	13.2	61.2
Micromonospora	0.7490	2.2501	14.4	57.5
Arthrobacter	0.9185	2.4329	16.5	51.5
Mixture	0.9893	3.4039	14.5	57.4
Controls	0.9786	1.3445	33.9	00.0

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Fig. 46.Degradation of α & β-endosulfan and sulphate generation by soil fungi exposed to endosulfan (500 mg/l) in carbon-free media.

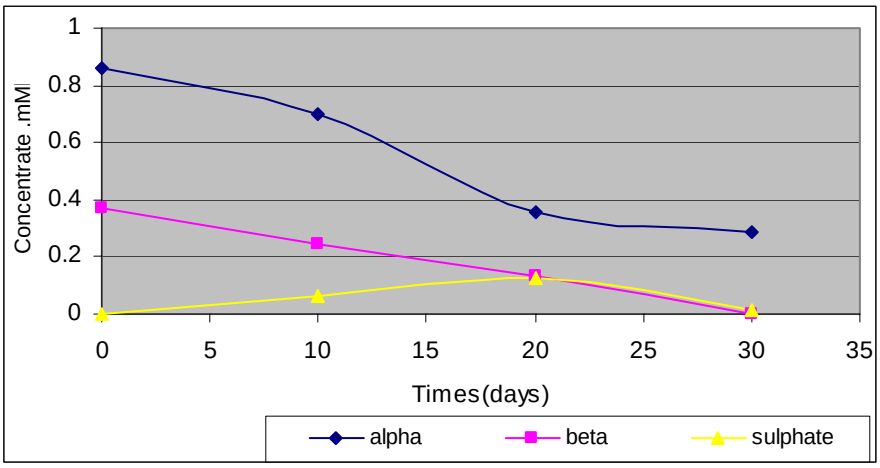


Fig. 47.Degradation of α & β-endosulfan and Sulphate generation by soil inorganic nitrogen `actinomycetes and bacteria. exposed to endosulfan (500 mg/l) in carbon-free media.

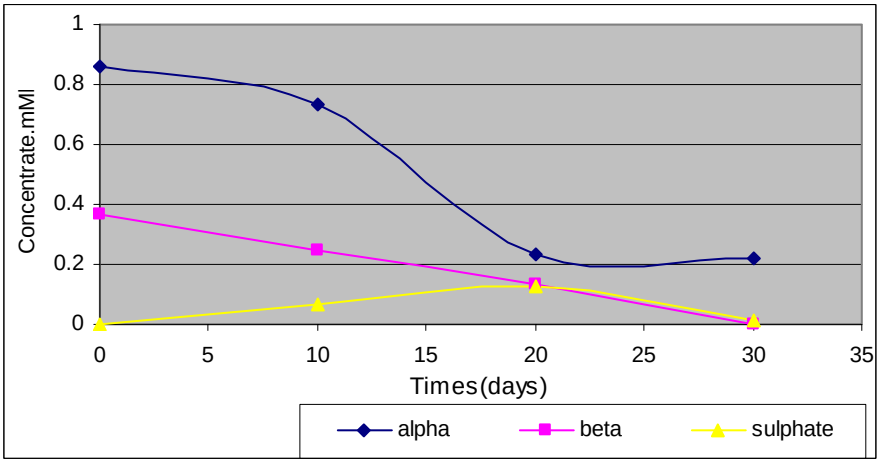


Fig. 48.Degradation of α & β-endosulfan and Sulphate generation by soil bacteria and actinomycetes which lives in poor media. exposed to endosulfan (500 mg/l) in carbon-free media.

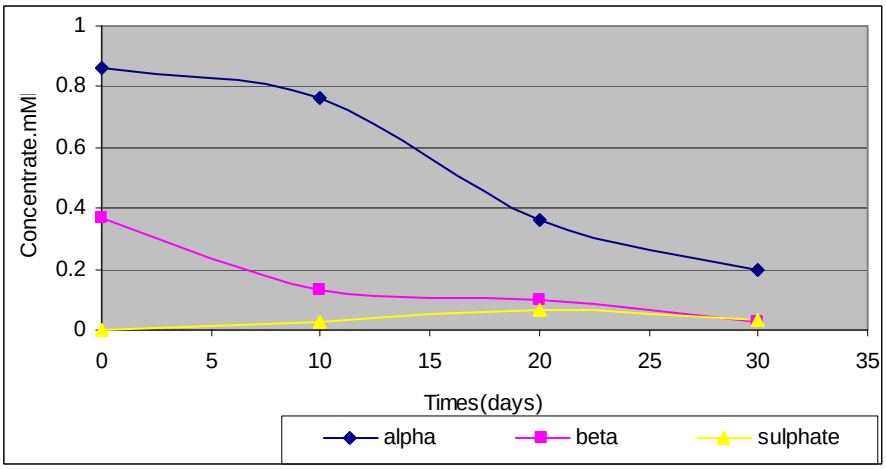


Fig. 49.Degradation of α & β-endosulfan and sulphate generation by soil organic nitrogen Bacteria. exposed to endosulfan (500 mg/l) in carbon-free media.

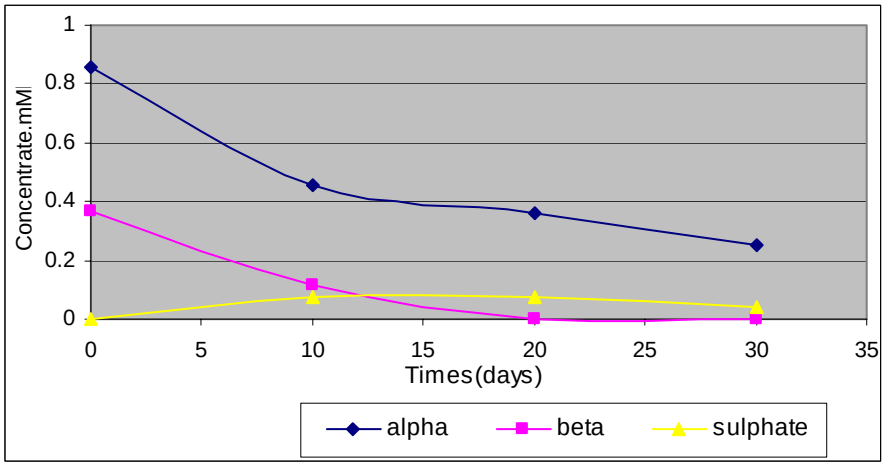


Fig. 50.Degradation of α & β-endosulfan and sulphate generation by soil *Nocardia* exposed to endosulfan (500 mg/l) in carbon-free media.

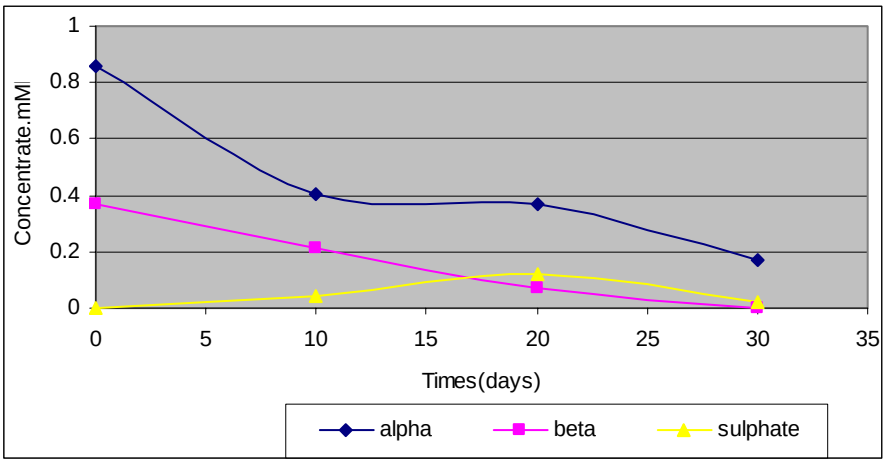


Fig. 51.Degradation of α & β-endosulfan and sulphate generation by soil *Bactoderma* exposed endosulfan (500 mg/l) in carbon-free media.

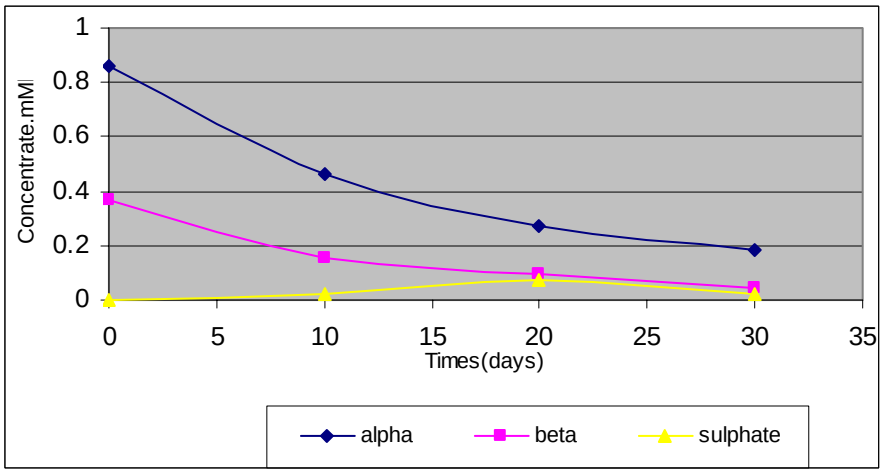


Fig. 52.Degradation of α & β-endosulfan and Sulphate generation by soil *Micromonospora* exposed to endosulfan (500 mg/l) in carbon- free media.

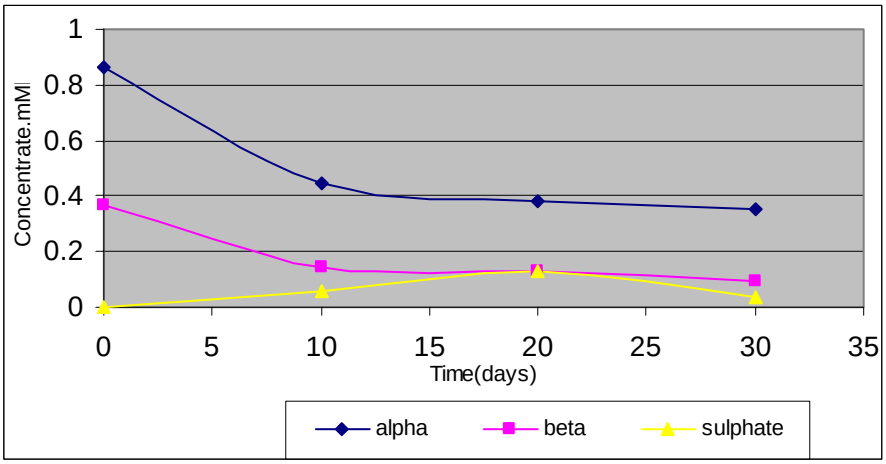


Fig. 53.Degradation of α & β-endosulfan and sulphate generation by soil Arthrobacterium exposed to endosulfan (500 mg/l) in carbon- free media.

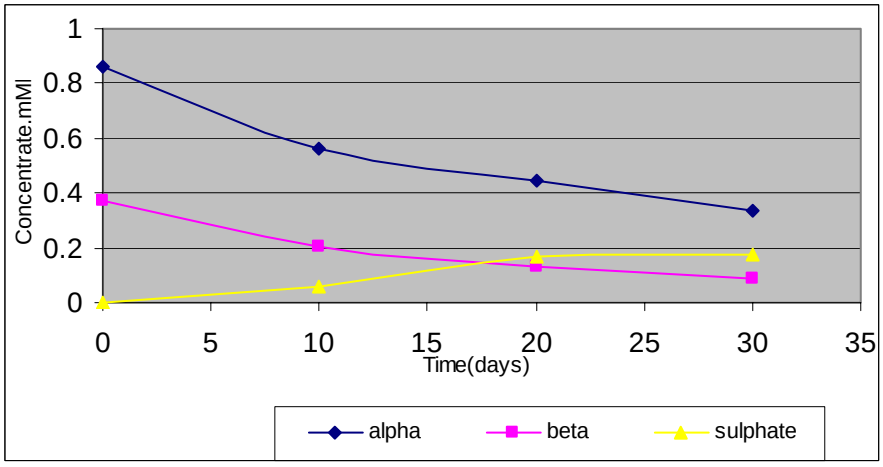


Fig. 54.Degradation of α & β-endosulfan and sulphate generation by soil Mixture Microorganism exposed toendosulfan (500 mg/l) in carbon- free media.

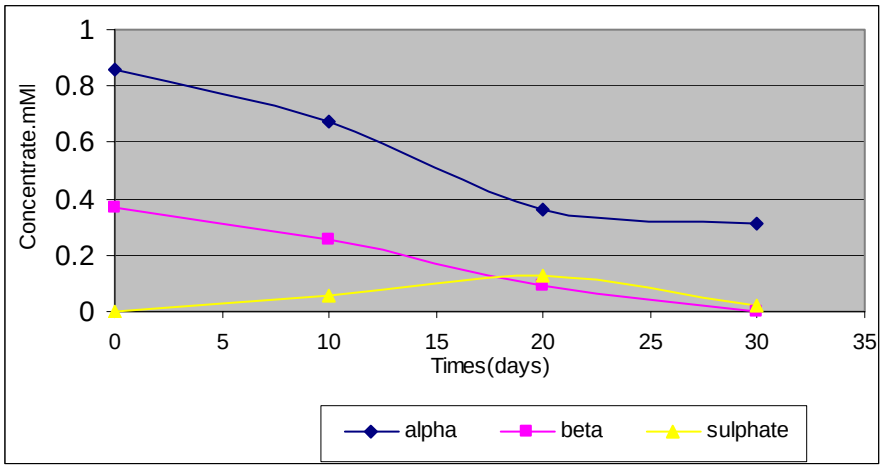
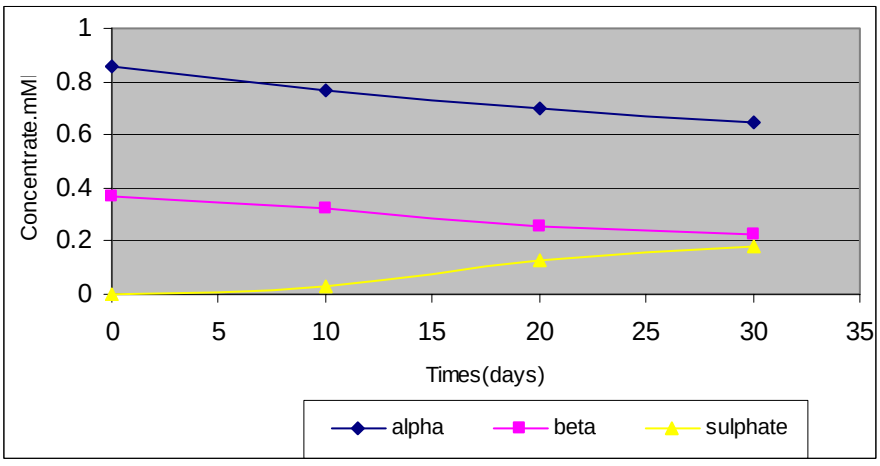


Fig. 55. Degradation of α & β-endosulfan and sulphate generation in sterilized treated soil (control)



4.7 comparative degradation of endosulfan by tolerant microorganism and their parents

4.7.1 Identification of tolerant strains capable of growing at elevated level of endosulfan.

Fungal and organic nitrogen bacteria from stock culture of selected soil types (Ras Alfeel pesticide store) were exposed to elevated concentration (200, 400, 600, 1000 mg/l) of endosulfan in carbon free media. The general growth, counts and shape of colonies observed.

4.7.2 Identification of organic nitrogen bacteria tolerate to high concentration of endosulfan

The organic nitrogen bacteria were cultured according to the methods described before (Brough, 1999).

Results of various steps of culturing leading to the identification as *Bacillus sp*. was summarized in table 29.

Table 29: Identification tests for tolerant organic nitrogen bacteria.

Test sequence	Culture test	Observations
I st test	Culture in nutrient agar media	Dry, white and creamy colonies
2 nd test	Gram stain	Gram positive rod with central and terminal to sub terminal spores.
3 rd test	Inoculation in Manito salt agar.	Yellow, flat dry colonies.

4.7.2.2 Identification of tolerant fungi

The fungi tolerant to elevated level of endosulfan was identified following the methods of Brough.(1999). Summary of the test done and main observation leading to the identification of the fungus as *Aspergillums fumigatus* in table 30.

Table 30. Identification test for tolerant fungi and main observation.

Test sequence	test done	Main observations
Ist lest	Inoculation in PDA	In face green-yellowing hyphen
2 nd. test	Lacto phenol cotton blue stain	Septet. Hyphen. Phased containing conidia spores.

Less tolerates fungal, types*; Mocur sp* and *Aspergillus niger* were tentatively identified based on the color of the hyphae (white hyphae for *Mocur sp*. and Black hyphae for *Aspnegillus niger*).

4.7.3 Counts of Identified tolerant microorganism

Results of counts of identifies tolerant types are summarized in table 31.
It is clear that the various types of fungi have different tolerance to elevated level of endosulfan; *Mocur sp*. can tolerate up to 400 mg/L, *Aspregillus niger* can tolerate up to 600 mg/l *Aspergillums fumigatus* can tolerate up to 1000 mg/l. Counts generally decrease with increasing the concentration of endosulfan in the media.

However the only bacteria tolerant to the highest level of endosulfan (1000 mg/l) was *Bacillus sp*.

Other unidentified types of bacteria were observed at lower concentration.

Bacterial counts also seen to decrease when the concentration of endosulfan in the media increased.

4.7.4 Comparative degradation of endosulfan by tolerant strains and their parents.

4.7.4.1 Degradation in soil

Results in Tables 32 and 33 show the half lives of endosulfan and the counts of microorganism. The parent strains of both bacteria and fungi (from stock culture) were the most effective in reducing the half lives of both α and β-endosulfan. The parent bacteria strains caused 83% reduction in half lives of Both α and β- endosulfan while the parent fungi were capable of causing up to 69 and 78% reduction in half lies of α and β-endosulfan respectively.

The general count of parent isolates of bacteria and fungi were high (80.3×10^4 for bacteria and 0.3×10^4 for fungi) compared the general counts of the most tolerate strains (0.9×10^4 for bacteria and 0.01×10^4 for fungi).

Generation of sulphate from degradation of endosulfan was monitored for a total of 60 days. In the control sets sulphate starts appear before 10th day and gradually increases with time and did not appear to decrease until 60 days.

The highest level of sulphate observed was 0.2 mMl/l. However incubating endosulfan with various isolates of tolerant strains caused a clear change in the level and fate of sulphate. The level of sulphate recorded was 0.2 mM l/L

observed after 60 days of incubation with fungi isolate from 600mg/l endosulfan (Fig. 62)

4.7.4. 2 degradation in carbon free media

Results in Table 34 and 35 show the half lives of endosulfan and the general counts of microorganism. The parent strains of both bacteria and fungi (from stock culture) were most effective in the reduction of half lives of both α and $\beta-$ endosulfan. The parent bacterial strains caused up to 61.2 and 83% reduction in half lives of both α and β- endosulfan respectively. While the parent fungi were capable of causing up to 55 and 77%. reduction in half lives of α and β-endosulfan respectively. The parent bacteria counts and fungi were high ($63.2x10^4$ for bacteria and $0.1x10^4$ for fungi) compared to counts of the most tolerate strains ($0.6x\ 10^4$ for bacteria and $0.01x\ 10^4$ for fungi).

Generation of sulphate from degradation of endosulfan was monitored for a total of 60 days. In control set sulphate starts to appear before 10^{th} day and gradually increases with time and did not appear to decrease until 60 days. The highest level of sulphate observed was 0.2 mM l/l.

Tolerant strains caused a clear change in the level and fate of sulphate generated (Fig 64). The level of sulphate recorded was 0.09 mMl/l observed after 45 days of incubation with bacteria isolated from 600 mg/l eudosulfan (Fig 69)

Table31. General counts of Fungi & Organic Nitrogen bacteria tolerant to the highest concentration of endosulfan (1000 mg/l).

Types of microorganisms		Concentration (mg/l)					
		Zero	200	400	600	800	*1000*
Fungi	*Mocur*	+ + +	+ +	+	-	-	-
	Aspregillus Niger	+ + +	+ + +	+ +	+	-	-
	Aspregillus fugamugatus	+ + +	+ + +	+ + +	+ +	+ +	+
Bacteria	*Bacillus Sp.*	+ + +	+ + +	+ + +	+ +	+ +	+

+ = Growth covering less than 25 % of plate

++ = Growth covering between 25 – 50 % of the plate

+++ = Growth covering above 50 % of the plate

- = no growth observed

Table 32. Half live (days) and percentage reduction in half live of α-endosulfan incubated with microorganisms isolated from elevated concentration of endosulfan in soil.

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	No. of Cells per gm soil
Organic Nitrogen Bacteria from stock culture	0.7173	1.5	14.4	82.7	80.3×10^4
Organic Nitrogen Bacteria isolated from 200 mg/l endosulfan	0.8947	0.99	39.7	52.2	4.9×10^5
Organic Nitrogen Bacteria isolated from 600 mg/l endosulfan	0.8097	1.2	28.7	65.4	6.7×10^4
Organic Nitrogen Bacteria isolated from 1000 mg/lendosulfan	0.8862	1.4	33.3	59.9	0.9×10^4
Fungi from stock culture	0.9800	1.6	26.0	68.7	0.3×10^4
Fungi isolated from 200 mg/l endosulfan	0.9687	1.5	29.6	64.4	0.1×10^4
Fungi isolated from 600 mg/lendosulfan	0.7671	1.6	16.6	79.9	0.1×10^4
Fungi isolated from 1000 mg/l endosulfan	0.8775	1.2	30.9	62.7	0.1×10^4
Controls	0.9529	0.54	82.9	0.0	0.0

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 33. Half live (days) and percentage reduction in half live of β-endosulfan incubated with microorganisms isolated from elevated concentration of endosulfan in soil.

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	No. of Cells per gm soil
Organic Nitrogen Bacteria from stock culture	0.7753	1.54	15.4	82.5	8.0×10^5
Organic Nitrogen Bacteria isolated from 200 mg/l endosulfan	0.8331	0.89	35.5	59.6	4.9×10^5
Organic Nitrogen Bacteria isolated from 600 mg/l endosulfan	0.8754	1.54	18.1	78.9	6.7×10^4
Organic Nitrogen Bacteria isolated from 1000 mg/L endosulfan	0.9737	1.69	30.4	65.4	0.9×10^4
Fungi from stock culture	0.8910	1.61	19.4	77.9	0.3×10^4
Fungi isolated from 200 mg/l endosulfan	0.7900	1.74	20.6	76.6	0.1×10^4
Fungi isolated from 600 mg/lendosulfan	0.9223	1.59	22.2	74.8	0.1×10^4
Fungi isolated from 1000 mg/l endosulfan	0.8403	1.82	30.7	65.1	0.01×10^4
Controls	0.9921	0.53	87.9	0.0	0.0

R^2 = Determination coefficient
$\tau_{½}$ = Half lives

Table 34. Half live (days) and percentage reduction in half live of α-endosulfan incubated with microorganisms isolated from elevated concentration of endosulfan in Carbon-free media.

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	No. of Cells per ml
Organic Nitrogen Bacteria from stock culture	0.7535	0.91	35.4	61.2	6.3×10^5
Organic Nitrogen Bacteria isolated from 200 mg/l endosulfan	0.7026	0.81	39.9	56.2	3.5×10^5
Organic Nitrogen Bacteria isolated from 600 mg/lendosulfan	0.8909	0.76	63.2	30.6	4.9×10^4
Organic Nitrogen Bacteria isolated from 1000 mg/l endosulfan	0.9333	1.1	41.6	54.3	0.6×10^4
Fungi from stock culture	0.9402	1.1	41.0	54.9	0.1×10^4
Fungi isolated from 200 mg/l endosulfan	0.7014	0.93	31.9	64.9	0.4×10^3
Fungi isolated from 600 mg/l endosulfan	0.9673	1.1	47.8	47.5	0.4×10^3
Fungi isolated from 1000 mg/l endosulfan	0.9231	0.68	62.9	27.4	0.1×10^3
Controls	0.9266	0.49	91.1	0.0	0.0

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Table 35. Half live (days) and percentage reduction in half live of β-endosulfan incubated with microorganisms isolated from elevated concentration of endosulfan in Carbon-free media.

Microorganisms	R2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %	No. of Cells per ml
Organic Nitrogen Bacteria from stock culture	0.7698	1.6	16.8	82.5	6.3×10^{5}
Organic Nitrogen Bacteria isolated from 200 mg/l endosulfan	0.8862	1.6	21.1	77.9	3.5×10^{5}
Organic Nitrogen Bacteria isolated from 600 mg/l endosulfan	0.8346	1.4	37.5	60.8	4.9×10^{4}
Organic Nitrogen Bacteria isolated from 1000 mg/l endosulfan	0.8137	1.6	17.6	81.7	0.6×10^{4}
Fungi from stock culture	0.9323	1.8	25.9	73.0	0.1×10^{4}
Fungi isolated from 200 mg/l endosulfan	0.7883	1.4	21.7	77.3	0.4×10^{3}
Fungi isolated from 600 mg/l endosulfan	0.9245	1.5	33.2	65.4	0.4×10^{3}
Fungi isolated from 1000 mg/l endosulfan	0.8499	0.72	55.9	41.7	0.1×10^{3}
Controls	0.9936	0.50	95.8	0.0	0.0

R^2 = Determination coefficient

$\tau_{½}$ = Half lives

Fig. 56 Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (Isolated from stock culture free from endosulfan) in soil.

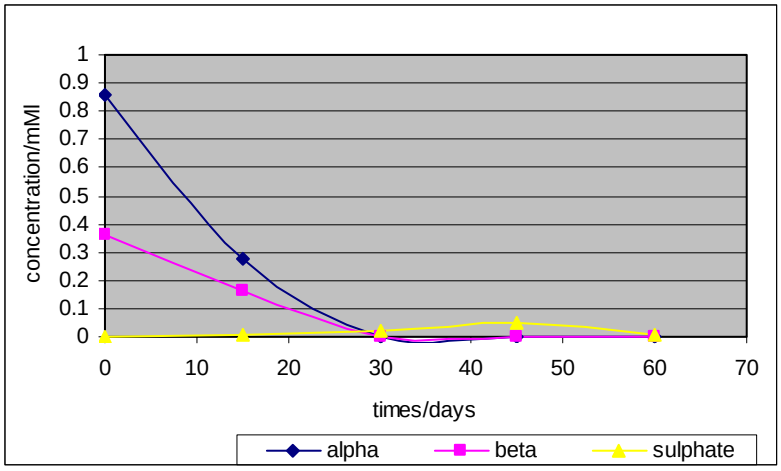


Fig. 57 Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 200 mg/l of endosulfan) in soil.

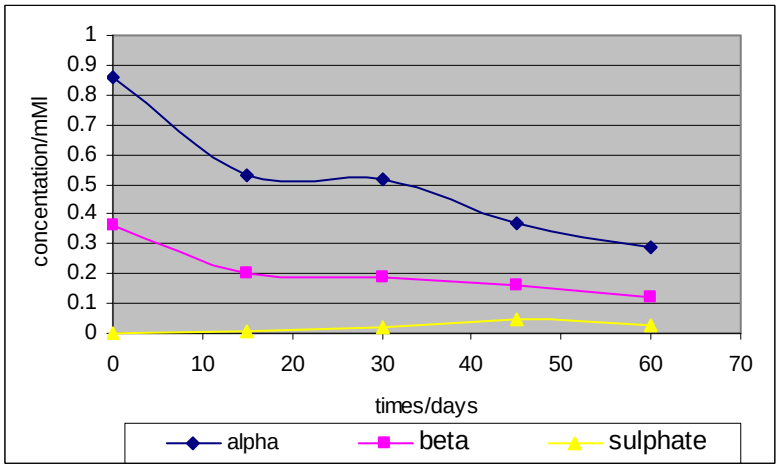


Fig. 58. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 600 mg/l of endosulfan) in soil.

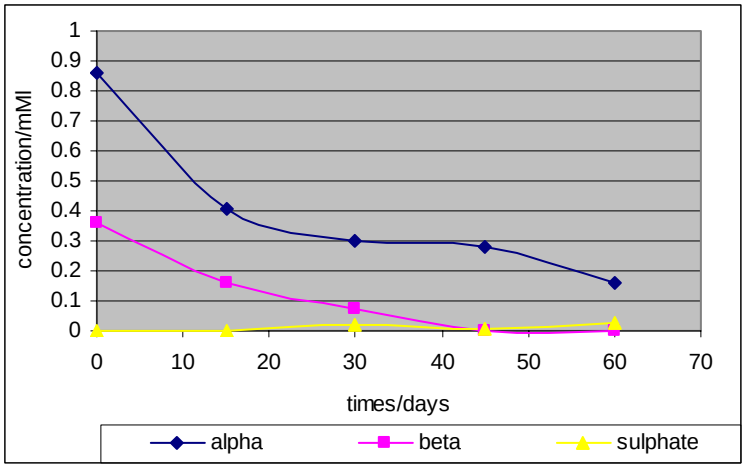


Fig. 59. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 1000 mg/l of endosulfan) in soil.

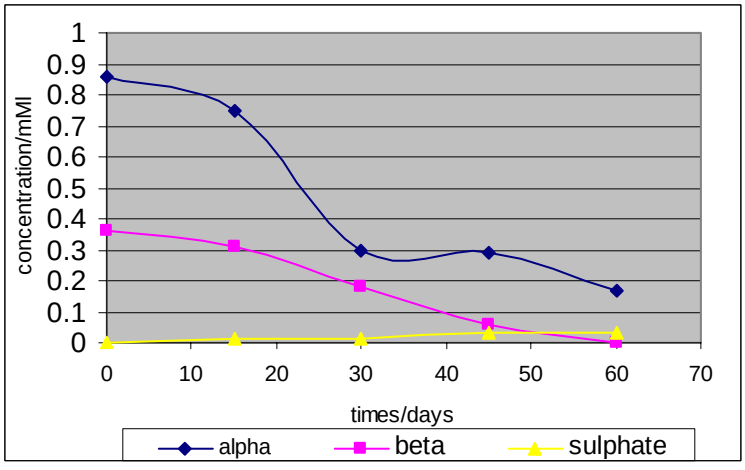


Fig. 60. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (Isolated from stock culture free from endosulfan) in soil.

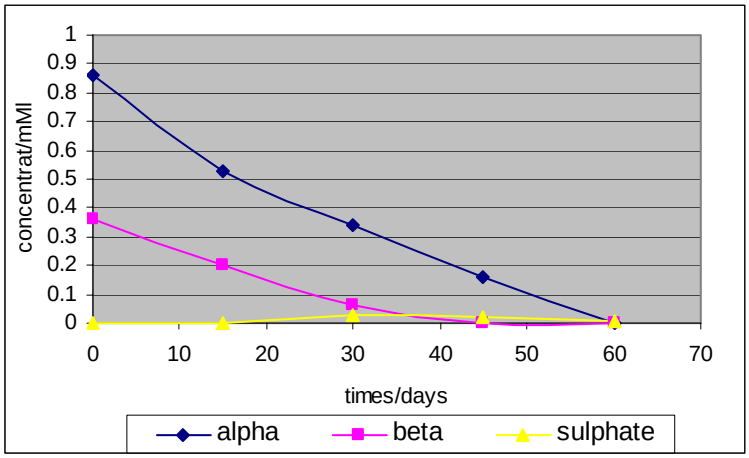


Fig. 61. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposed to 200 mg/l of endosulfan) in soil.

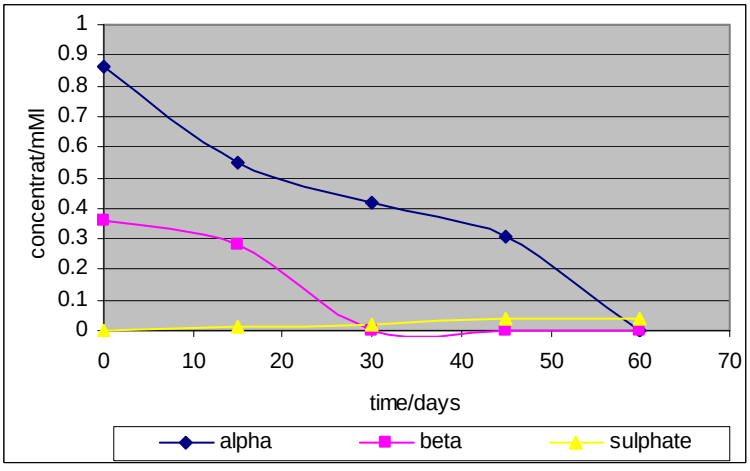


Fig. 62. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposed to 600 mg/l of endosulfan) in soil.

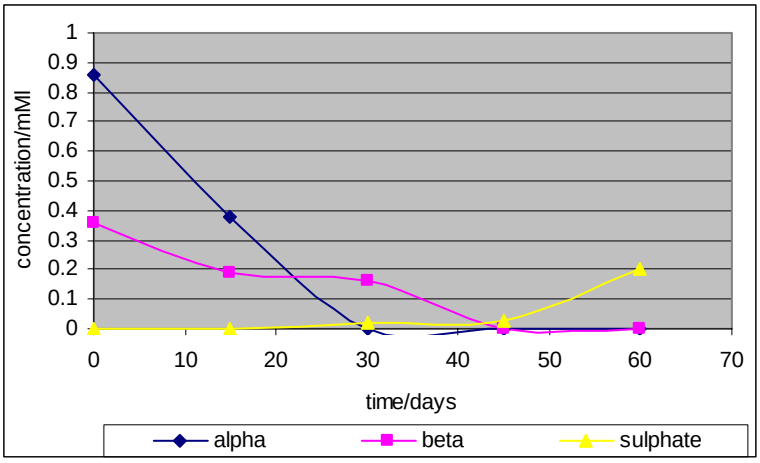


Fig. 63. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposed to 1000 mg/l of endosulfan) in soil.

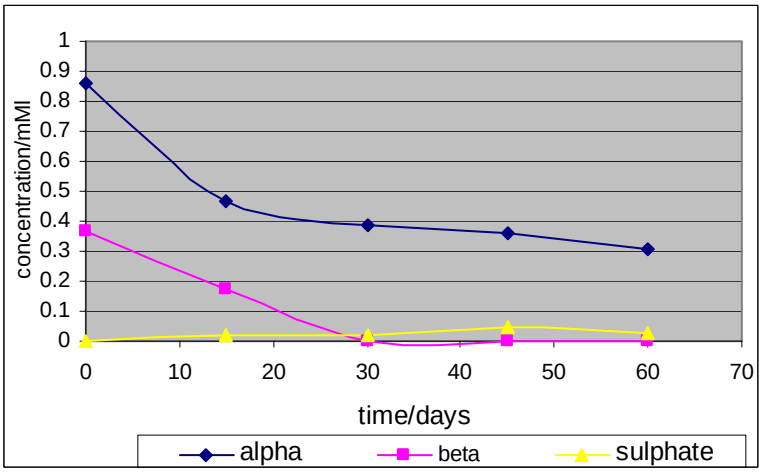


Fig. 64. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (Isolated from stock culture free from endosulfan) in carbon-free media.

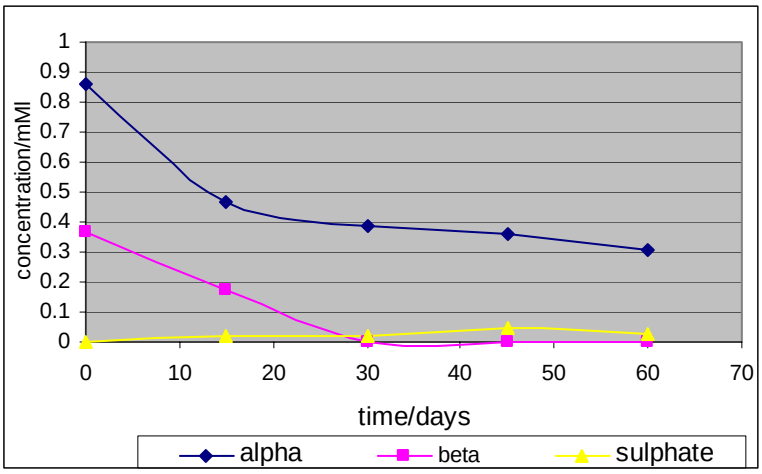


Fig. 65. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 200 mg/l of endosulfan) in Carbon-free media.

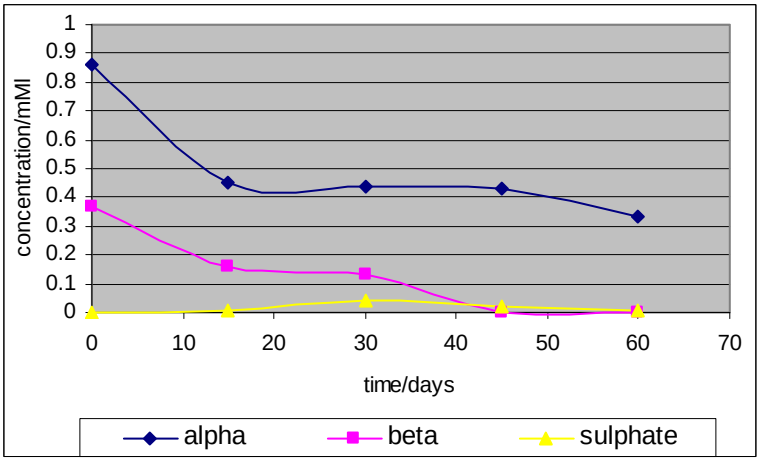


Fig. 66. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 600 mg/l of endosulfan) in Carbon-free media.

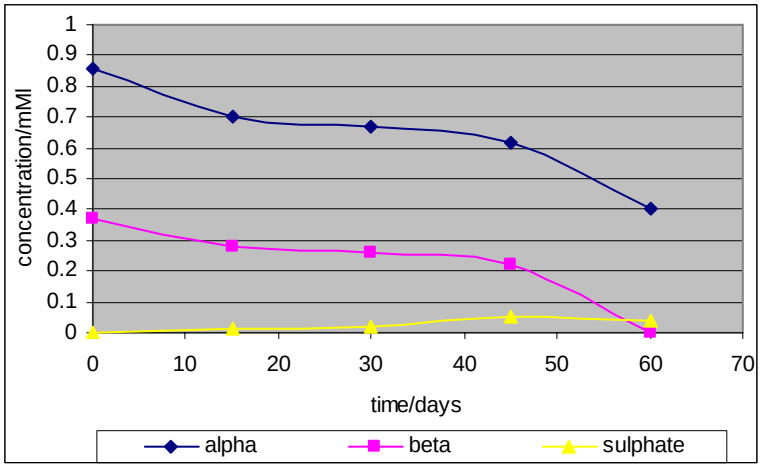


Fig. 67. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of organic nitrogen bacteria (exposed to 1000 mg/l of endosulfan) in carbon-free media.

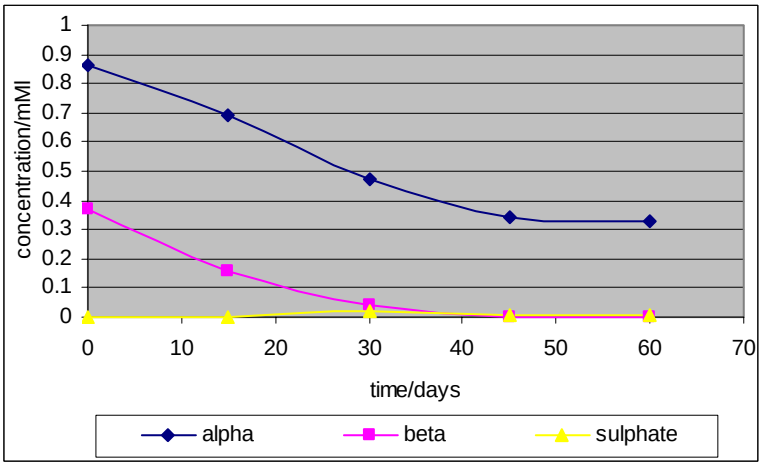


Fig. 68. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (Isolated from stock culture free from endosulfan) in carbon-free media.

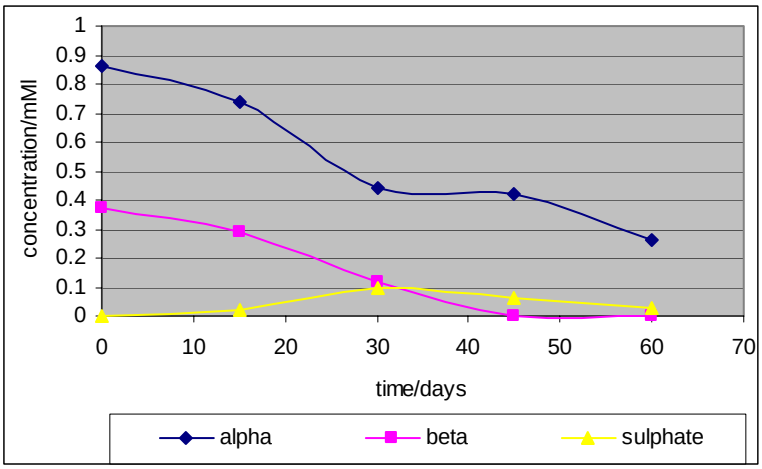


Fig. 69. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposedto 200 mg/L of endosulfan) in carbon-free media.

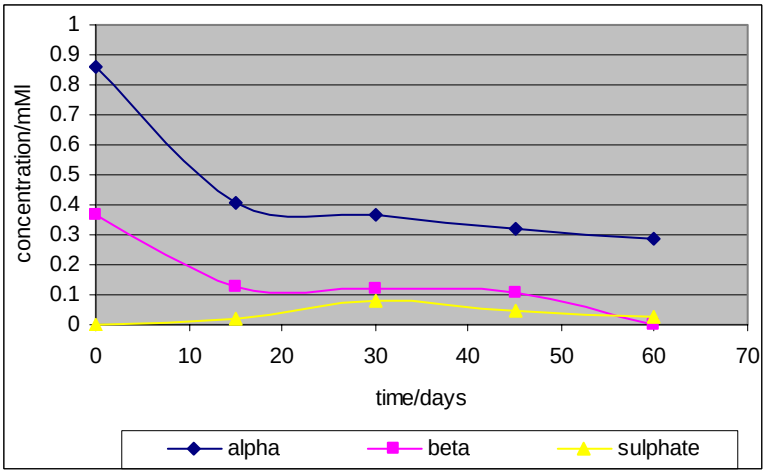


Fig. 70. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposed to 600 mg/l of endosulfan) in carbon-free media.

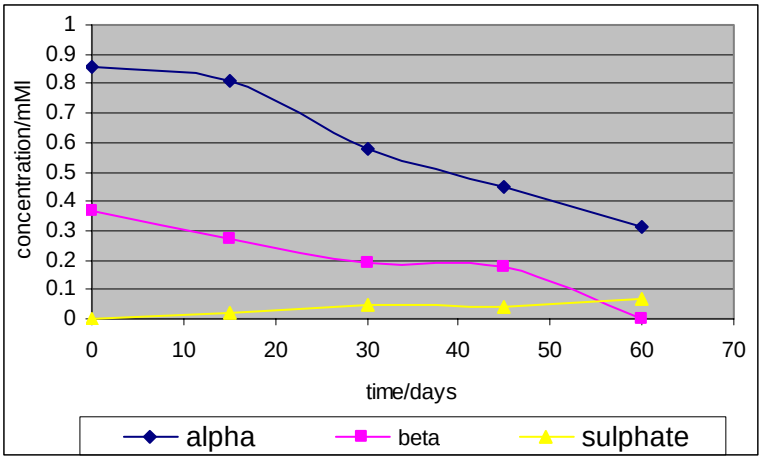


Fig. 71. Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) with parent strain of fungi (exposed to 1000 mg/l of endosulfan) in carbon-free media.

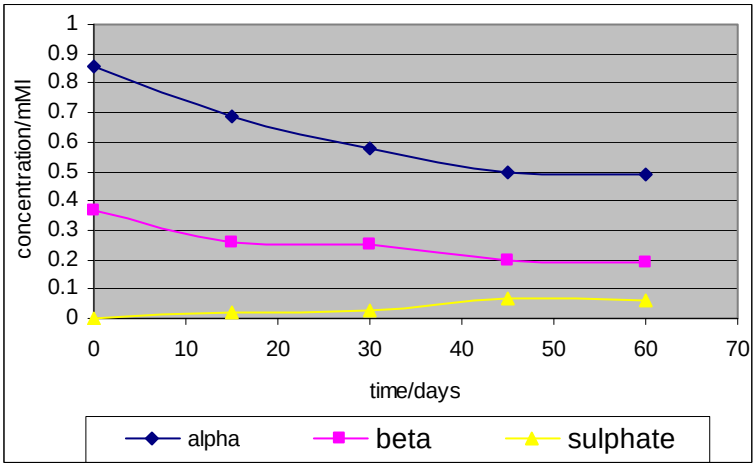


Fig. 72 Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) in sterilized soil (control) .

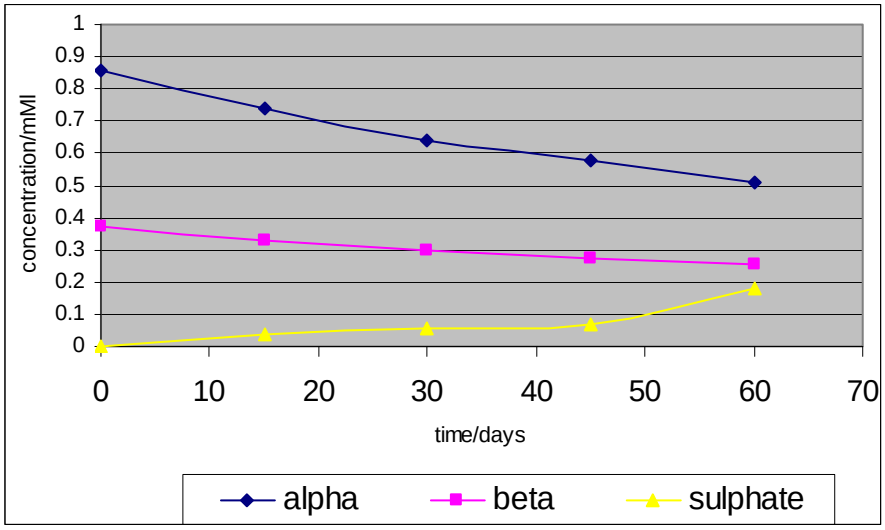
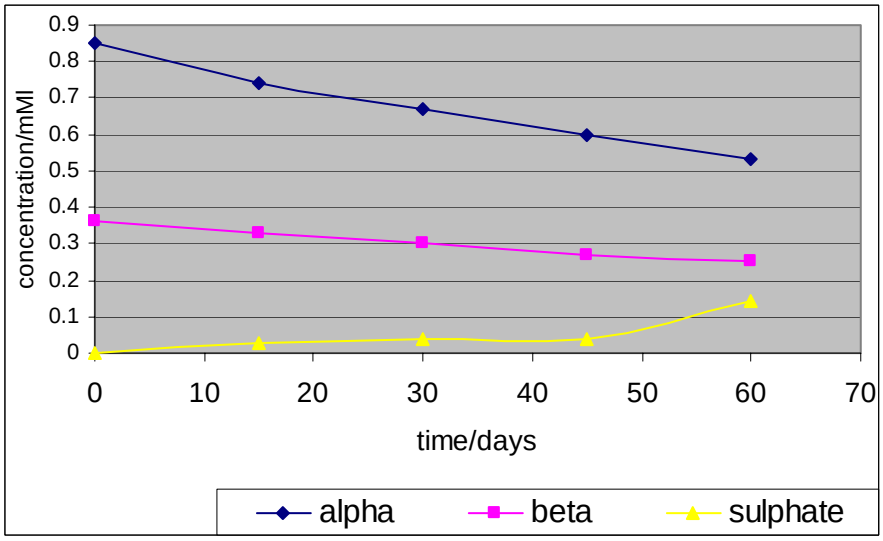


Fig. 73.Degradation of Endosulfan (α, β) and generation of sulphate upon incubation of the chemical (100 mg/l) in sterilized carbon-free media(control) .



CHAPTER FIVE

Discussion

The 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 sources or farming activities) and their staffs were less trained in store management. The poor storage facilities and management practices in Sudan have 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 soil scattered over 43 major and minor sites in the country (Butrous, 1999). The previous and current effort is Sudan was directed towards estimation of quantities and how to get rid of them. Nothing was done towards treatment of affected sites. As obvious soil is heavy and difficult to transport abroad for decontamination. Further more horizontal and vertical movement of contaminants complicated the problem (Babiker, 1998). Therefore, in situe treatments of affected sites appeared more attractive, suitable and could be feasible. In preliminary reports of Almahi, 1996, Abdelbagi, *et al*., 2000; 2003 argued the potential use of endogenous soil microorganisms in cleaning highly polluted soil and dump sites. Ali, (2005) conducted preliminary investigation on the potential role of endogenous microorganism in degradation of endosulfan and γHCH. The results indicated a promising role of such bioagents.

Seven Sets of experiments were executed as follows: survey of naturally occurring soil microorganism , Scanning of the capability of isolated microorganism in degrading α and β- endosulfan under conditions of selective media , degradation of α and β- endosulfan by selected isolates of

microorganism in selective and carbon free media , impact of fertilizer activators in enhancing the microbial degradation of endosulfan in endosulfan treated soil , microbial degradation under condition of elevated endosulfan concentration in carbon free media and comparative degradation of endosulfan by tolerant microorganism and their parents.

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 (Peterson and Batley, 1993). Previous studies in Sudan (Kabbashi, *1999*) 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 (AlHindi, 1994). Endosulfan also was reported to constitute a major fraction of obsolete pesticides stocks in Sudan (Butrous, 1999).

Soil samples representing the various degrees of contamination by pesticides (pesticides stores for high level, cotton soil for intermediates level residential areas for low level) and variable history of pesticide use (Gezira , Managil and Rahad) were chosen as inoculum sources for the present study . Therefore nine soil samples representing the sites mentioned above were collected.

A General survey of four groups of soil microorganisms was studied in the collected soil samples using four selective media ; Meat peptone agar for organic nitrogen bacteria , chebeck media for fungi , starch amino agar for

inorganic nitrogen bacteria and actinomycetes and nitrate for bacteria and actinomycetes which lives in poor media (Tepper *et al*., 1994) .

The results of the general survey indicated that the organic nitrogen bacteria is more prevalent and found in all types of soil followed by inorganic nitrogen bacteria and actinomycetes, fungi and lastly the bacteria and actinomycetes which live in poor media. The level of organic nitrogen bacteria was higher in cotton growing fields followed by residential areas (presumably least exposed to pesticide application) and in pesticide store soil. The counts of fungi and inorganic nitrogen bacteria and actinomycetes followed similar trend. However bacteria and actinomycetes which lives in poor media was higher in soil of cotton field followed by soil of pesticide stores and less in the soil of residential areas .Previous work by Ali (2005) reported the presence of organic nitrogen bacteria, inorganic nitrogen bacteria and actinomycetes and bacteria and actinomycetes which lives in poor media in three types of Sudanese soil (Gorashi pesticide store, cotton field near Hasahissa and residential soil from Hasahissa town).

The cotton soil score the highest counts of all the four groups and this expected as cotton soil is an agricultural soil which is expected to have the highest level of microorganism and probably sustain a relatively better condition for the growth of the various groups of microorganism (Alexander, 1965). The residential areas rank the second in microbial count of the first three groups and this could be attributed to its less degree of exposures to pesticides and its relatively lower level of organic matter other factors needed to support microbial growth, pesticides application can affects the counts of microorganisms as reported by Alexander (1965).

The store soil harbor the least counts of the first three groups as it is expected to have lower optimum conditions including higher level of

contamination by pesticides and lower level of organic matter Alexander (1965). On the other hand the count of bacteria and actinomycetes which lives in poor media is higher in store soil compared to residential ears. It is worth to mention that the increases in the level of this group in store soil is due to the increase in the counts of *Mycobacterium* and *Bactoderma.* These microorganisms were known to have better adaptability to highly polluted soils Ali, 2005

Soil microorganism showed great potential in degrading endosulfan in selective media. Results of Round I (first 15 days incubation with inoculums from stock culture) and Round II (15 days incubation with inoculums from Round I) followed a similar trend, except that levels of degradations were higher in round II . Significant differences were noticed among various soil types. Degradation of endosulfan by microorganism isolated from soil was studied by many authors (Guerin and Kennedy 1999, Katayama and Matsumura 1993, Lee *et al.* 1995, Mukherjec and Gopal 1994, Shetty *et al.*2000, Sutherland *et al.* 2000, Kwon *et al.* 2005 and Kumar and Philip 2006).

Generally the result indicated that microorganisms isolated from highly contaminated soils (stores and cotton soils) showed superior capability in degrading the two isomers of endosulfan this results is in conformity with the report of Awasthi, (1997) who mentioned that microorganism isolated from contaminated areas are relatively more adapted and consequently have a greater potential for cleaning highly polluted soil. Similar conclusions were reported by other author (Tariq, *et al*. 2000, Alli 2005, and Shivaramaiah and Kennedy 2006).

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 authers (Lee *et al.*, 1995, Guerin and kennedy, 1999; Tariq *et a*l. 2000, , Shetty *et al.*, 2000 Alli 2005, and Al-Hassan *et at.*, 2004).

Reduction in half lives was greater in α-endosulfan compared to β-endosulfan this results is in conformity with the results of Tara (2000) 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 1965 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 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 (1997) who described endosulfan as a poor biological energy source.

The effect of fertilizers activators on microbial growth and capability in degrading α and β- endosulfan was studied by incubating the four 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. The enhancement of counts and activity of microorganism in soil as a result of addition of fertilizer was previously reported by Alexander (1965), Cook (1982), ELagib (1997) , Mohamed (1990) 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 non – doubt promote their capability in degrading endosulfan in soil as reported by Tariq *et al*. (2000) 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.

Microbial degradation at elevated concentration of endosulfan in media free from carbon sources was studied. The results showed that there were no significant differences in the reduction of half lives between high (500 mg/l) and low (100 mg/l) concentration. Although there is no significance in half lives but looking the cures in figures 1-24 and 46-54 one could conclude that clear but delayed affects were noticeable. Such delayed effects can not easily be observed by examining half lives, since half lives were computed assuming a first order rate. This delayed effect can be explained by the assumption that microorganism slowly adapted themselves to live in such higher level and after the adaptation periods they became highly capable of degrading the pesticides, evident by the delayed sharp drop in degradation rate.

Tolerant strains of bacteria and fungi from the soil of Ras Elfeel pesticide store were isolated through consecutive exposure to elevated concentration of endosulfan. Following the criteria listed in Brough (1999) the most tolerant fungi (can tolerate up to 1000 mg/l) was identified as *Aspergillus fumigatus*, while the most tolerant Bacteria was identified as *Bacillus sp.* Other fungi tolerant to lower concentration of endosulfan were tentatively identified as *Asperegillus niger* (600 mg/l) and *Mocur sp.* (up to 400 mg/l). The tentative identification was based on partial fulfillment of the criteria listed by Brough (1999).

The comparative degradation of endosulfan by tolerant strains and their parents was studied in both soil and carbon free media. Results indicated that parents' strains (present in more number) caused faster decrease in half lives compared to tolerant strains (found in lower number). Although the most tolerant isolates appear relatively less efficient, (compared to parent) but relating the counts with the capability in reducing half lives it appeared that they can be of a great potential if they had a chance to propagate in massive numbers. This explanation could be supported by the work of Tariq *et al.* (2000) who indicated that increasing the number of microorganism caused better activity and more degradation.

The sulphate was generated more under soil conditions compared that of carbon free media. Various factors may affected the generation of sulphate under soil conditions specially the rate of oxygen diffusion. Such condition may not be available in carbon free media.

The Sudanese isolates of microorganism could be of great potential in reducing the level of endosulfan in highly polluted storage soils. The use of microorganism for bioremediation requires better and more understanding of all the physiological and biochemical aspects involved in chemical

transformations. This work is attempt to put a corner stone for some aspects needed for bioremediation of polluted sites. However further studies are needed prior to start any bioremediation process in such sites.

Conclusions and Recommendations

(1) Survey of naturally occurring soil microorganism in different the tested soil types showed that the organic nitrogen bacteria was more prevalent in all soil types however the bacteria *Mycobacterium* and the actionmycetes *Bactoderm* were associated with highly polluted soils.

(2) Microorganism isolated from highly polluted soil had a great potential in degrading endosulfan.

(3) The degradation rate was relatively higher in selective media compared to carbon free media

(4) Addition of fertilizers significantly enhanced the microbial growth and accelerated the degradation rate of endosulfan.The enhancing of degradation caused by addition of fertilizer was higher under soil condition compared to carbon free media, However there is grater sulphate generation under soil condition.

(5) The fungus *Aspergillus fumigatus* and bacteria *Bacillus sp.* were the most tolerant strains to highest concentration tested (1000 mg/l) while *Aspergillus niger* and *Mocur sp.* were found tolerant to low concentration 600 mg/l and 400 mg/l respectively.

(6) The tolerant strains were apparently less efficient in shorting the half lives of endosulfan however when

comparing their numbers to those of the parents they may be of great potential.

The suggested future studies include, further screening of tolerant and capable strains of microorganism, identification and characterized of various products generated from degradation trails, enzymes responsible for degradation should be isolated, characterized and evaluated for capability of degradation under both laboratory and field conditions and a secondary tests for future validation should be followed by field confirmatory trials.

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