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Microbe-Induced Degradation of Pesticides

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Editor

Microbe-Induced Degradation of Pesticides

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To parents in heavenly abode

Preface

Pesticides are usually referred as a broad range of insecticides, fungicides and herbicides. Presently, there are 900 pesticide products and 600 active pesticide ingredients available in the market. Although millions of tonnes of pesticides are applied in the agriculture and horticulture, less than 5 % of pesticides only reach to the targeted organisms and rest gets deposited on the soil and non-targeted organisms and also moves to water bodies and the atmosphere. The fate of these pesticides is governed by the abiotic factors (temperature, moisture, soil, pH, etc.) as well as biological and chemical reactors. Abiotic degradation of pesticides is mediated by oxidation, reduction, hydrolysis and photolysis and rearrangement, while biotic degradation is caused by both microbial communities (bacteria, fungi, etc.) and plant species.

In view of the above facts, the editor has compiled the latest developments on biodegradation of chemical pesticides used in agriculture in this edited volume contributed by Indian and foreign scientists which will serve as a ready reckoner not only to scientists, but also to policymakers, teachers, students and the farmers.

In this endeavour, I would like to thank all the contributors for their positive response and active participation by contributing the latest updates on the degradation of different chemical pesticides. I would like to thank my research scholars Ms. Nitanshi Jauhari and Mrs. Shweta Mishra for their academic and technical support. Besides, untiring support by Mr. Dilip Kumar Chakraborty in preparing the book manuscript is heartily acknowledged.

Lastly, I would like to thank my family members: Mrs. Manorama Singh (wife), Ragini (daughter) and her kids Antra and Avantika and Pritish (son) and Vishali (daughter-in-law) for their inspiration, endurance and moral support in this endeavour.

Lucknow, India — Shree Nath Singh

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Microbe-Assisted Degradation of Aldrin and Dieldrin

Adi Setyo Purnomo

1 Introduction

Environmental pollution is an inseparable evil associated with anthropogenic activities. Increasing human needs resulted in the growth of the industries which produce new products through modern technologies. Among the various kinds of environmental issues, synthetic pesticides, produced in the agricultural industry have become a serious environmental problem. Among the chemical pesticides, aldrin and dieldrin are chlorinated cyclodiene pesticides which are classified as persistent organic pollutants (POPs) that cause serious environmental problems. They are highly ecotoxic to higher organisms, because of their low solubility, their tendency to partition into the lipophilic phase, and also contain chlorine atoms (Foght et al. 2001). They cause numerous negative effects, including disruption of the endocrine system in birds and mammals, impairment of male reproductive ability, interference with sex hormones, eggshell thinning, and a carcinogen for humanbeings (WHO 1989). As a result of their chemical stability and lipophilicity, aldrin and dieldrin are extremely persistent in the soil and sediment environments, with a half-life of 1 year or more. Although these compounds have been prohibited over the past decades in most countries around the world, they are still found in the environment, especially in the soil in agricultural fields. In 2001, more than 100 countries signed the Stockholm Convention on Persistent Organic Pollutants (POPs), committing to eliminate the use of the 12 POPs of greatest concern, including aldrin and dieldrin (Xiao et al. 2011).

The removal of pollutions from contaminated waters and soils has become an environmental priority, and both physicochemical and biological remediation pro-

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cesses have been studied. Although chemical and physical treatments are more rapid than biological treatments, they are generally destructive and intrusive to affected soils, energy intensive and also more expensive than bioremediation (Foght et al. 2001). Biodegradation using microorganisms, including bacteria and fungi, has been found to be a cost-effective method of treating various pollutants including aldrin and dieldrin, which has been existing in the environment since late 1960s.

2 Microbial Degradation of Aldrin and Dieldrin

For several years, dieldrin residues were assumed to appear as a result of microbial epoxidation of aldrin in soil in areas, where dieldrin itself had never been used. The indirect evidence for the conversion of aldrin to dieldrin by epoxidation was provided by Lichtenstein and Schulz (1960). Aldrin was rapidly converted to dieldrin in non-sterile soil, while little conversion occurred in the sterile soil. Aldrin also disappeared more quickly in moist soil than in dry soil, as microbes are more active in moist soil. Subsequently, Lichtenstein et al. (1963, 1965) showed that the conversion of aldrin to dieldrin was inhibited by methylenedioxyphenyl synergists (sesamex, piperonyl cyelonene, piperonyl butoxide, sulfoxide and n-propyl isome). Sesamex was the best inhibitor for the conversion of aldrin to dieldrin compared to other methylenedioxyphenyl compounds. Sesamex was also found to reduce populations of microorganisms when added to pure cultures, soil suspension, or soils, which caused no dieldrin formation. A breakdown product of sesamex, i.e. sesamol, which is also known as antioxidant, caused no significant inhibition of dieldrin formation. It indicated that the conversion of aldrin to dieldrin in soil was inhibited by synergists at relatively high rates, due to their high toxicity to microorganism populations.

Many studies have shown the microbial transformation of aldrin and dieldrin to intermediate metabolites under aerobic conditions, but the metabolic pathways are still unclear. The investigation on biodegradation of aldrin by pure cultures of soil microorganisms had been reported by Tu et al. (1968). Ninety-two pure cultures of soil microorganism were screened for degrading aldrin of which a majority showed some ability for converting aldrin to dieldrin by epoxidation. Among the fungi, *Trichoderma, Fusarium,* and *Pinicillium* were the most active for aldrin transformation. Besides, *Actinomycetes* were also effective converters, with one exception, *Bacillus* sp. which was of less importance. *Fusarium* sp., the most active isolate, converted 9.2 % of the added aldrin to dieldrin during 6 weeks of incubation period.

In some instances, the production of dieldrin was linear in relation to time. Besides, some microorganisms transformed aldrin to products other than dieldrin. Moreover, dieldrin had been transformed into 6,7-trans-dihydroxydihydroaldrin (trans-aldrin diol), photodieldrin, and ketoaldrin (Matsumura and Boush 1967, 1968; Matsumura et al. 1970; Patil et al. 1970).

The ability to epoxidize aldrin to dieldrin is obviously a common trait widely distributed among soil bacteria. Ferguson and Korte (1977) have described a number of strains of gram-positive and gram-negative soil bacteria which produce

exclusively the exoisomer of dieldrin. Twenty-two strains of soil bacteria, including representatives of the genera *Bacillus, Micromonospora, Mycobacterium, Nocardia, Streptomyces, Thermoactinomyces* and *Pseudomonas* were found to degrade aldrin to its epoxide dieldrin. Previously, Tu et al. (1968) reported production of dieldrin in 30 g-positive isolates out of 45 tested, whereas Patil et al. (1970) found 11 g-negative and gram-positive soil bacteria with this trait.

Maule et al. (1987) reported that anaerobic microbial populations, developed from soil, freshwater mud, sheep rumen, and chicken litter, could transform dieldrin to monodechlorinated products. The freshwater mud microbial population was the most effective, and transformed 96 % dieldrin to the *syn-* and *anti-* monodechlorinated products in a culture, initially containing 10 μg ml^{-1} of dieldrin approximately for 7 days. Three isolates from this culture, classified as the genus *Clostridium*, were capable of dieldrin dehalogenation, although the dehalogenation rate by each isolate was much less than by the parent population. *Clostridium bifermentans*, *Clostridium glycolium*, and *Clostridium* sp. required 54, 87 and 95 days, respectively, to transform 80 % of the dieldrin in a culture.

On the other hand, degradation of aldrin and dieldrin was also achieved in free cell cultures of *Pseudomonas fluorescens* to the level of 94.8 % for initial concentration of 10 mg L^{-1} (Bandala et al. 2006). Sakakibara et al. (2011) also reported that strain KSF27 converted dieldrin to aldrin dicarboxyric acid via aldrin trans-diol (Fig. 1). Strain KSF27 exhibited a high sequence similarity to *Pseudonocardia* spp. Based upon the genetical and morphological characteristics, strain KSF27 was found to be a new species of the genus *Pseudonocardia*, designated as *Pseudonocardia* sp. strain KSF27. Although the endo-isomer of dieldrin is less stable than the exo-isomer, there is no reason to expect microbial epoxidation to lead to exo-dieldrin in every case. In fact, exo-isomer is only produced in dissimilar genera, such as *Pseudomonas*, *Bacillus*, and the members of *Actinomycetes*.

Recently, white rot fungi (WRF) was found to degrade lignin, a complex high-molecular-mass aromatic polymer, as well as a wide spectrum of recalcitrant organopollutans, including biphenyls, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (Kamei et al. 2010). WRF are generally more tolerant to high concentrations than bacteria (Xiao et al. 2011). Xiao et al. (2011) reported that 20 white rot fungi belonging to genus *Phlebia* were investigated for their ability to degrade dieldrin. In that experiment, screening of fungi was done with 50 μL (5 mmol L^{-1}) of dieldrin. Based on the screening results, *Phlebia acanthocystis*, *Phlebia brevispora*, and *Phlebia aurea* was evaluated for their degradation capacity and metabolic products of dieldrin and aldrin degradation.

As evident from Table 1 the degradation ability of three *Phlebia* fungi was to remove over 50 % of dieldrin in a low nitrogen (LN) medium, after 42-d of incubation. Three hydroxylated products were detected as metabolites of dieldrin, including 9-hydroxyaldrin and two carboxylic acid products. It suggested that in *Phlebia* strains, hydroxylation reaction might play an important role in the metabolism of dieldrin, in which methylene moiety of dieldrin molecules might be prone to enzymatic attack by white rot fungi.

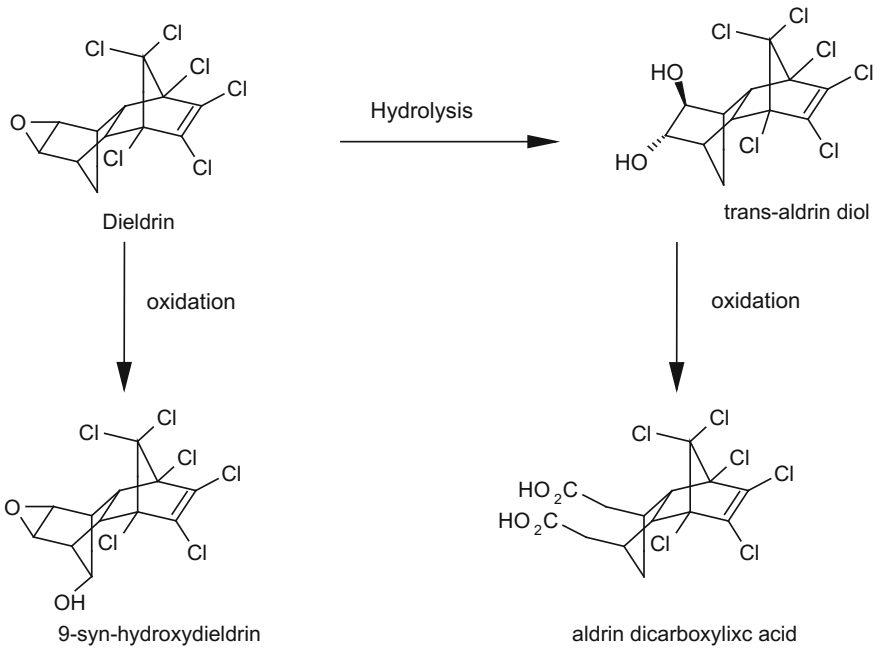

Fig. 1 Proposed metabolic pathway of dieldrin transformation by strain KSF27 (Sakakibara et al. 2011)

Kataoka et al. (2010) reported that an aerobic dieldrin-degrading fungus, *Mucor racemosus* strain DDF was isolated from a soil to which endosulfan had been annually applied for more than 10 years until 2008. Strain DDF degraded dieldrin to 1.01 mM from 14.3 mM during 10 days incubation at 25 °C. Approximately, 0.15 mM (9 %) of aldrin trans-diol was generated from the dieldrin degradation after a 1 day incubation. The degradation of dieldrin by strain DDF was detected over a broad range of pH and concentrations of glucose and nitrogen sources.

Kamei et al. (2010) reported thirty-four isolates of wood-rotting fungi were investigated for their ability to degrade dieldrin. Among these fungal isolates, *Phlebia* sp. YK543 degraded 20 % of dieldrin during the initial 7 days and then

Table 1 Degradation rate of aldrin and dieldrin by *Phlebia* fungi in low-nitrogen (LN) medium during 42-d incubation period

Phlebia fungi	Substrate	Degradation (%)
P. acanthocystis	Aldrin	96.0
	Dieldrin	56.0
P. brevispora	Aldrin	97.6
	Dieldrin	51.6
P. aurea	Aldrin	96.4
	Dieldrin	54.0

39.1 % of dieldrin during 30 days of incubation period in LN medium. 9-Hydroxylation was detected as a metabolite in the cultures of *Phlebia* sp. YK543.

On the other hand, Birolli et al. (2015) isolated marine-derived fungi *Aspergillus sydowii* CBMAI 935, *A. sydowii* CBMAI 933, *Penicillium miczynskii* CBMAI 930 and *Trichoderma sp*. CBMAAI 932 from the marine sponges *Geodia corticostylifera* and *Chelonaplysylla erecta*. In degradation studies, *P. miczynskii* CBMAI 930 showed the highest tolerance to dieldrin and catalyzed the biotransformation of dieldrin (50 mg L^{-1}) with high conversion rates (90 %) after 14 days in liquid medium. The organochlorine compounds were identified in the biodegradation reaction as endrin, endrin ketone and cyclopentene.

Matsumura and Boush (1968) reported that *Trichodermaviride*, isolated from soil heavily contaminated with various insecticides, had the ability to degrade dieldrin in liquid medium after 30 days of incubation without shaking and identified aldrin, dieldrin aldehyde, ketoaldrin, and photoisomer of ketoaldrin as metabolic products of the degradation of dieldrin in soil by microorganisms. Yamazaki et al. (2014) observed that *Mucor racemosus* strain DDF could decrease dieldrin levels with simultaneous production of a small amount of aldrin-trans-diol. The degradation was performed by adding 50 μL of an aldrin-trans-diol stock solution (1000 mg L^{-1}) and incubated for 14 days at 25 °C in the absence of light.

3 Involvement of Enzymes in the Degradation Process

Organochlorine pesticides (OCPs) act as prooxidant stressors and increase the intracellular generation of reactive oxygen species (ROS) and oxidative conditions, which, in turn, modulate levels and function of antioxidants enzymes (Osburn and Kensler 2008). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the first line of defence against ROS and other free radicals while glutathione S-transferases (GSTs) are phase II enzymes providing defence against the toxicity cause by ROS. The cytochrome P450 dependent monooxygenase (MO) and glutathione S-transferase (GST) are among the most widely studied enzymes involved in the metabolism of xenobiotics (Jensen et al. 1991). Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase in the form of serum are capable of degrading aldrin and dieldrin found in the animal body (Luckens and Phelps 1969).

In the rat, dieldrin is metabolized through the three different degradation routes. Judging from the *in vitro* studies alone, the major metabolite of dieldrin, produced in the liver, is conjugated with glucuronic acid. This metabolite is probably excreted through urine and feaces mediated by β-glucorinadase (Matthews and Matsumura 1969). Aldrin and dieldrin are substrates specific to purified armyworm midgut enzyme epoxide hydrolase. The purified armyworm enzyme is able to hydrate mono-substituted epoxides faster than 1,2-disubtituted epoxides and these, in turn,

are cleaved faster than trisubstituted substrates. Active site of enzyme may be located within restricted pit or cleft in the enzyme surface which allows ready access to the monosubstituted epoxides. However, cis-1,2-disubstituted epoxides, which remained unhindered, imposed increasingly severe steric constrains on the ingress of more bulky substrates. Perhaps, only those substrates substitute one side of oxine ring having sufficient conformational elasticity to gain access to the depression and may interact successfully with active site. Strict regioselectivity has been established for the catalytic center of rat liver epoxide hydrolase (Mullin and Wilkinson 1980). The present investigation indicates that any alteration in the activities of acid phosphatase (ACPase) and alkaline phosphatase (ALPase) creates disturbances in the normal functioning of the various tissues of *M. monoceros* after addition of aldrin, an organochlorine insecticide (Reddy and Jayaprada 1991).

Since microsomonal aldrin epoxidase of the strain housefly was not due to a single chromosome, the involvement of a regulatory mechanism was considered. It was reasoned that if the substrains were treated with an inducing agent, the substrains carry the structural gene for high epoxidase activity as the Fe parent. Heptachlor was used instead of aldrin as the enzyme substrate in order to learn whether the substrains differed in their epoxidase activities toward the two cyclodienes. Epoxidase activities of all the substrain were increased five to seven folds by treatment with phenobarbital, but none reached the level of induced Fe strain. It was interesting to note that the amount of induced enzyme in the parent strain was greater than that in any of the substrain (Schonbord et al. 1973).

The effect of cyclodiene insecticides on O-demethylase activity was studied in two strains of houseflies. An increase in microsomonal demethylase activity was observed when houseflies were treated with aldrin, dieldrin, heptachlor or heptachlor epoxide at the doses required for maximum induction of epoxide (Yu and Terriere 1972).

A reliable, cheap and highly sensitive biosensor has been developed for the near-real time monitoring of aldrin and dieldrin. The basic sensor operating mechanism relies on the inhibition of acetylcholinessterase (AchE) enzyme by organophosphate and carbamate pesticides. The degree of enzyme inhibition, which is related to the amount of pesticide present, is determined potentiometrically by measuring changes in proton activity in the locality of the enzyme immobilized working electrode. The unique characteristic of the sensor resides in the fact that stable and repeatable enzyme immobilization onto the indicator electrode was achieved within a polyacrylamide gel matrix by in situ polymerization. This novel technique gives consistent results with good correlation with samples of known concentration and provides a novel, generic approach to reliable immobilization of labile enzyme and other biological molecules. This sensor may be used in the future for the direct determination of pesticides in real samples, such as soil extracts, horticultural produce, milk and potable water, as well as for more general environmental and Hazard Analysis and Critical Control Point (HACCP) applications.

Monooxygenase activities and the corresponding half-lives of foreign compounds metabolized by this system have been studied in a range of vertebrata.

A similar relationship was found between plasma antipyrine half-lives in the dog and rabbit and the corresponding hepatic microsomonal monooxygenase activities (antipyrine as substrate with the dog, aniline and ethyl morphine with rabbit). Since most liposoluble foreign compounds must be metabolized before they can be excreted to an extent, excretion rates as well as half-lives may be related to the activity of monooxygenase and/or detoxifying enzymes. It seems likely that the rate of metabolism will be important in determining the excretion rate where conversion is slow, assuming that availability is not a limiting factor in the case of the organochlorine insecticide i.e. dieldrin. Animals, which are deficient in monooxygenase or other key detoxifying enzyme, should be identified, and should receive close attention in monitoring program for pollutants. A pesticide is likely to be efficiently accumulated by species that are deficient in the detoxifying enzymes responsible for its degradation. Finally, this approach is also helpful in the extrapolation of toxicological data to man. It can indicate which species are most likely to resemble man with regard to the metabolism of particular drug (Walker 1978). Undoubtedly, cytochrome P450 monooxygenase plays some roles in the metabolism of organochlorine pesticide, such as dieldrin and aldrin (Xiao et al. 2011).

4 Mechanism and Pathways of Degradation

Although metabolism of dieldrin and aldrin by some microorganisms has been reported, the detailed metabolic pathways were not still well understood. A number of studies have described the metabolic products of dieldrin degraded by bacteria and fungi.

A new dieldrin-degrading bacterium was isolated from the soil. Sakakibara et al. (2011) reported that *Pseudonocardia* spp. strain KSF27 converted dieldrin to aldrin dicarboxyric acid via aldrin trans-diol (Fig. 1). Although the endo-isomer of dieldrin is less stable than the exo-isomer, there is no reason to expect microbial epoxidation to lead to exo-dieldrin in every case. The exo-isomer is only produced in different genera such as *Pseudomonas*, *Bacillus*, and the members of *Actinomycetes*. The proposed pathway of metabolism of dieldrin via aldrin *trans*-diol has been shown in Fig. 1 (Sakakibara et al. 2011).

Photodieldrin is the main metabolite from dieldrin, and a small amount of diol and other unidentified metabolites were also found in some cases. Dieldrin and trans-aldrin diol are the metabolic products of aldrin (Patil et al. 1972). Although no photolytic reaction could be observed in any of the control tubes which were illuminated in the absence of microorganisms, there is a possibility that a photo-sensitizing substance is present among the microbial products and the reaction is of photochemical-biochemical nature.

This conversion could be catalyzed by epoxide hydrolase, although there have been no studies focusing on the enzyme responsible for this conversion. Patil et al.

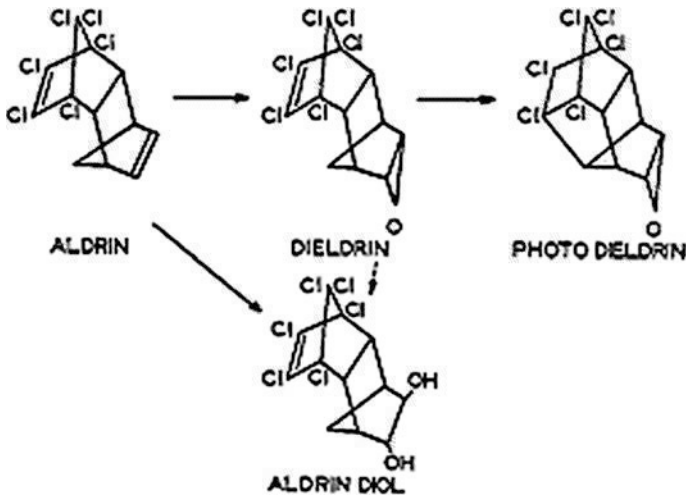

Fig. 2 Metabolite pathway of aldrin and dieldrin under oceanic conditions (Patil et al. 1972)

(1972) reported that in the metabolic transformation of dieldrin by marine microorganisms, photodieldrin was found as the major metabolic product of dieldrin (Fig. 2).

Several white rot fungi of *Phlebia* genus were able to degrade dieldrin. However, its metabolism differs from bacteria to bacteria. In this pathway, a large amount of 9-hydroxydieldrin was observed, suggest that the hydroxylation of dieldrin at the nine position is the major metabolic reaction, and the initial metabolic reaction of dieldrin seems to be similar to that in mammals. 9-hydroxydieldrin is further metabolized to dihydroxydieldrin, which hydroxylation reactions might play an important role in the metabolism of dieldrin, although the complete pathway of dieldrin is still unclear. The product monohydroxy 6,7-dihydroxydihydroaldrin could be produced from two alternate pathways: by hydroxylation of 6,7-dihydroxydihydroaldrin or by hydrolysis of monohydroxy-dieldrin at the epoxy ring. However, no trace of 6,7-dihydroxydihydroaldrin was observed in the present study. Thus, it seems that hydrolysis occurs at the epoxy ring of 9-hydroxydieldrin to produce a diol compound, which was probably 9-hydroxy-6,7-dihydroxydihydroaldrin. As no other monohydroxylated dieldrin was detected, only 9-hydroxydieldrin was found as main product of dieldrin from the cultures of three fungi (Fig. 3). Therefore, the methylene moiety of the dieldrin molecule seems to be the prior site for enzymatic attack in selected *Phlebia* species (Xiao et al. 2011).

It is generally known that aldrin is easily metabolized to dieldrin by multi-function oxidase of microbes or mammalians under aerobic conditions. It has been demonstrated that aldrin was initially branched to different metabolic routes, i.e. epoxidation, hydroxylation and oxidation, even though a large amounts of dieldrin were observed in selected fungal cultures with aldrin. This observation was

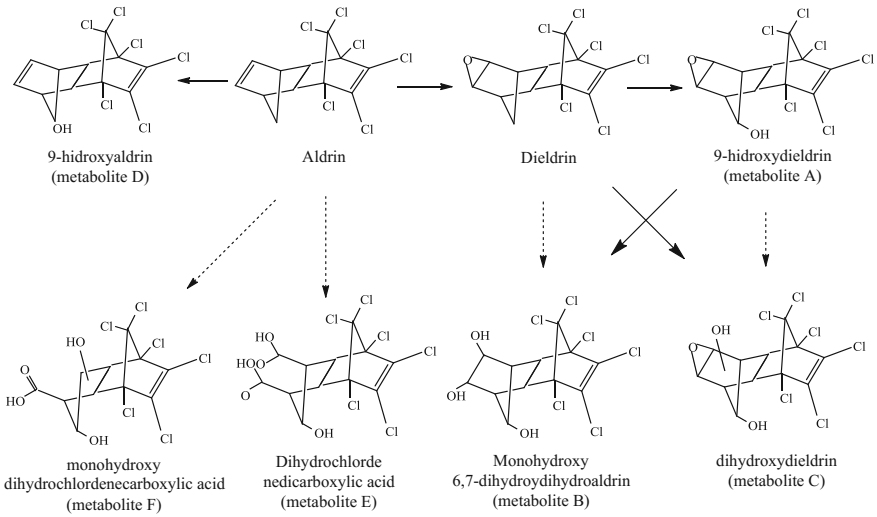

Fig. 3 Proposed pathway for the metabolism of aldrin and dieldrin by selected *Phlebia* species. The *broken arrows* indicate involvement of more than one step and the *dotted arrows* represent possible metabolic routes (Xiao et al. 2011)

supported by the result that 9-hydroxyaldrin and two carboxylic acid products were not detected from fungal cultures with dieldrin (Xiao et al. 2011).

The degradation pathway of aldrin and dieldrin has been shown in Fig. 3. A novel finding was the presence of 9-hydroxyaldrin, which was considered to be produced by hydroxylation at methylene of the aldrin molecule as dieldrin. The finding of 9-hydroxyaldrin further supports the hypothesis that the methylene bridge carbon of aldrin and dieldrin molecules is prone to enzymatic attack in selected WRF. Additionally, two carboxylated products were detected from these fungal cultures with aldrin. The dihydrochlordenedicarboxylic acid, a product resulting from oxidative ring cleavage of aldrin, has also been observed in some plants and soils (Klein et al. 1973; Kohli et al. 1973; Scheunert et al. 1977; Stewart and Gaul 1977), but it has not been detected in isolated bacteria and fungi. The formation of dihydrochlordenedicarboxylic acid suggests the ability of fungi to degrade aldrin by successive oxidation reactions.

However, Birolli et al. (2015) did not detect intermediate product that could be related to the degradation of dieldrin, suggesting that the dieldrin might be mineralized or transformed in polar xenobiotic compounds or CO_2 by conjugation reaction (Fig. 4). This is an important result for bioremediation purpose, since complete pesticide degradation or conjugation is desirable avoiding the presence of toxic or long-lasting products of biotransformation. The biotransformation, in the present of hydrogen peroxide, suggests the involvement of oxidoreductase in the pesticide degradation.

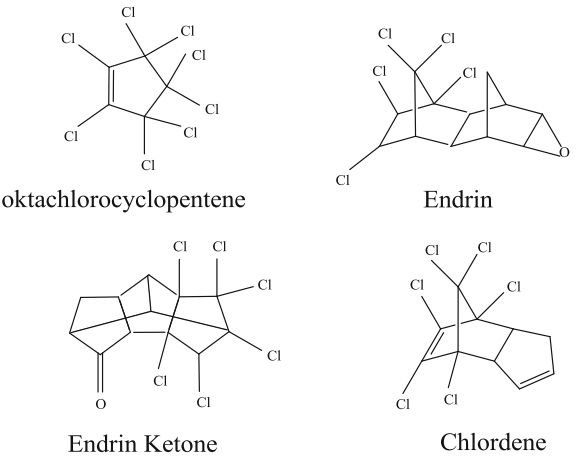

Fig. 4 Organochlorine compound identified by GC-MS in the sample of dieldrin biodegradation (Birolli et al. 2015)

5 Factors Effecting Degradation Process of Aldrin and Dieldrin

The efficiency of degrading microorganisms introduced into contaminated sites depends on many factors. In particular, the pollutant characteristics (e.g., concentration, bioavailability and microbial toxicity), the physicochemical characteristics of the environment, microbial ecology (e.g., predatory and competition), the characteristics of the degrading microorganisms and methodology for site remediation are dominant factors (Matsumoto et al. 2008, 2009). Therefore, it is important to understand the characteristics of the microorganisms and appropriate environmental conditions to achieve optimal degradation ability. Furthermore, it is necessary to isolate new competitive microorganisms that can degrade aldrin and dieldrin efficiently in natural environments as well as in the laboratory.

5.1 Effect of PH

The degradation of dieldrin was investigated at pH 4.0, 6.0, and 8.0 using soil fungus *Mucor racemous* strain DDF (Kataoka et al. 2010). About 90 % of dieldrin was degraded at all three pH values (Fig. 5). In the remediation study, *M. racemosus* strain DDF was found to degrade dieldrin under a wide range of pH. It is also advantageous that strain DDF was capable of growing rapidly in the soil compared to white rot fungi. Besides, Ferguson and Korte (1977) had reported that *Bacillus cereus*, *Bacillus subtilis* and *Nocardia* sp. degraded aldrin to exo-dieldrin, between pH range 5 and 9.5.

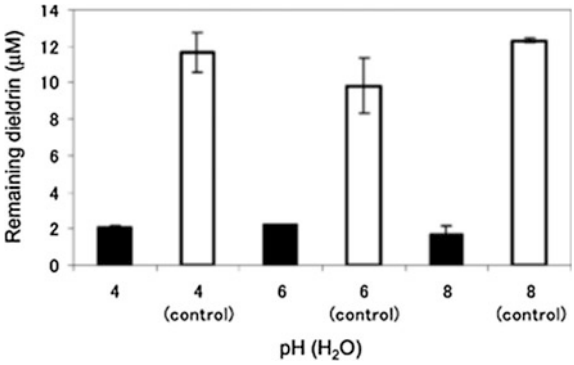

Fig. 5 Effect of pH of the medium on dieldrin degradation by *M. racemous* strain DDF

5.2 *Effect of Carbon and Nitrogen Concentrations*

The maximum degradation of dieldrin by *M. racemous* strain DDF was achieved up to 89.8 % at glucose concentration of 1.0 % (Kataoka et al. 2010). However, there was significant difference in degradation of dieldrin after addition of glucose concentrations of 0.5, 1.0, and 2.0 %. In contrast, lower dieldrin degradation was observed at addition of glucose concentrations of 0.1 and 10 % (Fig. 6).

Dieldrin was degraded over the range of added nitrogen concentrations, as reflected in Fig. 7. However, there were no significant differences among 0.5-, 1-, and 2-folds variations in nitrogen concentrations.

The results clearly indicated that *M. racemous* strain DDF degraded dieldrin under a wide range of carbon and nitrogen source concentrations. At low concentrations of nitrogen or carbon sources, a low degradation capability of strain DDF was observed (Figs. 6, 7). The hyphal growth was poor at the low concentrations of glucose and nitrogen, probably due to nutrient deficiency, and at 10 % glucose due to high osmotic pressure (data not shown). These results indicate that strain DDF needs a suitable growth situation to degrade dieldrin (Kataoka et al. 2010).

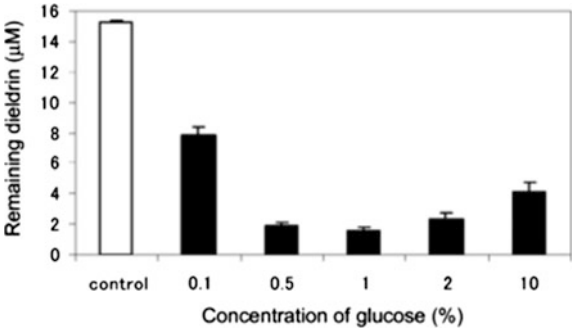

Fig. 6 Effect of carbon in the medium on dieldrin degradation by *M. racemous* strain DDF

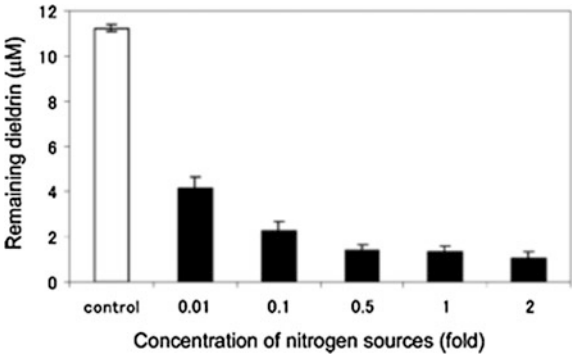

Fig. 7 Effect of nitrogen in the medium on dieldrin degradation by *M. racemous* strain DDF

Huggenholtz and MacRae (1990) investigated the effect of carbon amendments and analogues on the disappearance of aldrin and dieldrin residues from the soil. Soil samples, supplemented with xylose and inositol, showed a marked decrease in detectable concentrations of aldrin (84–96 %) and dieldrin (50–79 %), after 60 days, relative to untreated and zero time controls. Decalin, borneol and isoborneol treated soils produced a statistically significant decrease in aldrin concentration (27–74 %) in the same period of time relative to zero time control and untreated soil control. The remaining amendments had no appreciable effect on the levels of insecticide residues in the soil (Table 2). Biodegradation of aldrin and dieldrin in the test soil appears to be stimulated by the addition of xylose and inositol. These amendments may be acting as growth substrates for the co-metabolism of aldrin and dieldrin by the soil microflora. Growth substrates perhaps, provide co-factors or metabolites necessary for the catalytic activity of enzymes (usually non-specific) capable of transforming xenobiotics (Janke and Fritsche 1985).

5.3 *Effect of the Extracellular Fluid (Involvement of Epoxide Hydrolase Enzyme)*

Kataoka et al. (2010) used the extracellular fluid of the spent broth culture for the dieldrin degradation experiments. First, the extracellular fluid was concentrated by ultrafiltration, and the ultrafiltrate was further fractionated with ammonium sulfate. The ultra filtrate degraded dieldrin by 46 % compared to control. Besides, degradation of dieldrin was observed in the fraction corresponding to 55–75 % saturation of ammonium sulfate, whereas almost no degradation was observed in the fraction corresponding to 35–45 % saturation. The highest degradation was observed in the 55 % fraction (Fig. 8). *Aspergillus niger* is known to produce epoxide hydrolase (Faber et al. 1996). This enzyme involves in the reaction and causes opening of the epoxide, leading to the formation of the trans-diol. Thus, it is evident that epoxide hydrolase was involved in the transformation of dieldrin to aldrin trans-diol.

Table 2 Effect of selected amendments on the detectable aldrin and dieldrin in soil over a 60 days incubation period at 28 °C (Huggenholtz and MacRae 1990)

Amendment	Incubation time (days)	Average concentration in soil (ng g^{-1})	
		Aldrin	Dieldrin
Non	0 15 60	7025 ± 399 7348 ± 818 7578 ± 2198	3763 ± 362 3662 ± 489 3385 ± 1677
Amino acids	0 15 60	6495 ± 351 7944 ± 1754 7490 ± 3303	3638 ± 540 3140 ± 588 4236 ± 2836
Glucose	0 15 60	5735 ± 723 6789 ± 1391 4255 ± 4088	3210 ± 66 4088 ± 1490 2453 ± 1235
Inositol	0 15 60	6954 ± 2448 6085 ± 1846 548 ± 175	3530 ± 518 2952 ± 880 1156 ± 204
Xylose	0 15 60	8221 ± 657 7684 ± 5085 535 ± 155	4112 ± 305 3378 ± 1916 1296 ± 386
CHC	0 15 60	7659 ± 138 5624 ± 809 7041 ± 1360	5212 ± 1252 3239 ± 448 4571 ± 1260
DCCH	0 15 60	7436 ± 177 8852 ± 2670 6314 ± 1796	4834 ± 1786 6420 ± 3159 3636 ± 821
Decalin	0 15 60	7338 ± 37 6127 ± 962 3781 ± 1367	4046 ± 671 2417 ± 576 2918 ± 628
Borneol	0 15 60	7485 ± 171 7103 ± 2875 3673 ± 1661	4250 ± 382 3680 ± 1110 633 ± 1289
Isoborneol	0 15 60	7393 ± 301 7450 ± 2240 3630 ± 833	4007 ± 38 4040 ± 1860 2757 ± 849

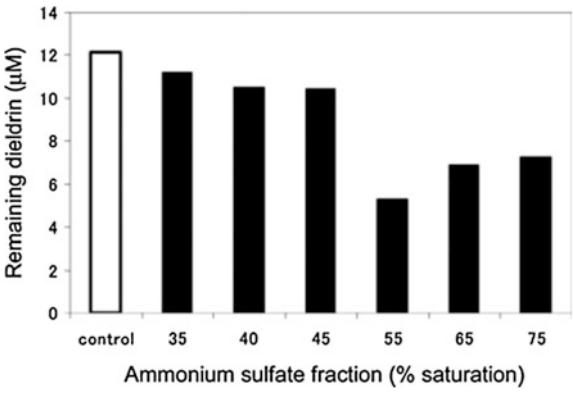

Fig. 8 Degradation of dieldrin by the extracellular fluid from *M. racemous* strain DDF

5.4 Effects of Variations in Light on Growth and Conversion

Brain and Lines (1982) used *Phaseolus vulgaris* cell culture to degrade aldrin and dieldrin. Effect of variations in light was investigated in cultures fed with 10 mg [^{14}C] aldrin or dieldrin, keeping under light or dark conditions throughout the culture period (Table 3). In case of aldrin, both treatments resulted in the production of dieldrin, aldrin-trans-dihydrodiol and polar material, but no photodieldrin and polar material were produced. However, uptake and conversion of aldrin was slow in the dark-grown cultures. It was noted that the dieldrin-treated cultures, grown in the dark, appeared considerably darker than those treated with aldrin, or left untreated, and kept in the dark. Photodieldrin was only inside the cells. These results confirmed that production of photodieldrin took place only after uptake and its production was independent of the presence of light. Thus photodieldrin is a true metabolite of dieldrin in the plant system.

5.5 Effect of Variation in Hormonal Levels

10 mg [^{14}C] aldrin and [^{14}C] dieldrin were added to cultures in seven closely related well-defined media based on a commercial formulation and supplemented

Table 3 Uptake and metabolism of [^{14}C] aldrin and dieldrin by suspension cultures from *Phaseolus vulgaris* root under light and dark conditions

		Light (%)	Dark (%)
Aldrin added			
Growth inhibition		10	10
Aldrin	Medium	34	37
	Cells	11	10
	total	45	47
Dieldrin	Medium	11	9
	Cells	26	31
	total	37	40
Aldrin-trans-diol		4	4
Polar metabolites		3	4
Total conversion		43	48
Dieldrin added			
Growth inhibition		42	46
Dieldrin	Medium	46	75
	Cells	36	11
	total	82	87
Photodieldrin		6	4
Polar metabolites		6	3
Total conversion		12	7