



BIOREMEDIATION OF ORGANOPHOSPHATE PESTICIDES : CURRENT STATUS AND FUTURE PROSPECTIVE

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Abstract

Pesticides are the chemicals that are used to control various kinds of household and agricultural pests that can otherwise damage the crops and livestock. These chemicals have become crucial part of modern agriculture. The major classes of pesticides are insecticides, herbicides, fungicides, rodenticides etc. Among these insecticides of several chemical moieties such as organochlorines, organophosphates, carbamates, pyrethroids etc. are used to control varied kinds of insects all over the world. Out of these, Organophosphates are the most widely used pesticides accounting for an estimated 36% of world-wide insecticide sales. Although there are numerous beneficial effects of organophosphate pesticides (OPPs) in controlling harmful agricultural and household pests, protection of food and prevention of various diseases, yet pollution of soil and water may takes place as a result of their careless handling during application in the fields, accidental discharge and widespread usages. The toxicity of these compounds is due to the ability to inhibit an enzyme, acetyl cholinesterase at cholinergic junctions of the nervous system. Several studies revealed that an extensive variety of land-dwelling and aquatic systems may be contaminated with OPPs. As these compounds possess high toxicity and it is therefore essential to eradicate them from the environment. Conventional approaches comprising chemical treatment, incineration, and landfills, are challenging due to the secondary risk of exposure and, in some cases, economically limiting. Therefore, there is an increasing interest in developing efficient and economically feasible methods for their degradation such as bioremediation. It is biotechnological approach to clean-up the environment. Considering research that is being carried out all over the world on the biodegradation of organophosphates, this review article mainly describes the usages, current pollution status, and major accomplishments towards bioremediation of some widely used OPPs such as chlorpyrifos, diazinon, dichlorvos, fenitrothion etc.

Key words: Organophosphates, Pesticide, Bioremediation, Biodegradation, Agriculture

Introduction

Enormous growth of population in the world necessitates high yields of crops from existing agricultural land. For high yield, protection of crops from pests is one of the major chores (Peshin and Zhang, 2014). That has made the usages of pesticides indispensable and currently is used in production of approximately one-third of the agricultural products (Zhang *et al.*, 2011). Because of excessive use, they are noticed in all environments, such as soil, water and air. This has led to increase in pollution and various kinds of diseases (Chen *et al.*, 2004; Pimentel, 2009; Liu *et al.*, 2008; Zhang *et al.*, 2011; Zhang and Liu, 2017; Zhang, 2018). Among pesticides, organophosphate pesticides make up for about 70% of the pesticides used

worldwide and has been estimated to be involved in around 3 million poisonings and 2 million deaths annually which accounted for 86.02% of the total cases (Zhang *et al.*, 2011). Some of the commonly used organophosphate pesticides are malathion, parathion, methyl parathion, monocrotophos, chlorpyrifos, dimethoate, phorate, dichlorvos etc. Chemically these are the esters of phosphoric acid and are used to control a variety of pests such as insects, mites, aphides etc. attacking crops like sugarcane, tobacco, cotton, peanuts, vegetables, fruits and ornamentals.

Widespread and excessive use of organophosphates over the years has led to the contamination of many environmental matrices throughout the world. This has raised the problems of their interaction with the living

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organisms in the environment. Their toxicity is due to their ability to inhibit an enzyme, acetyl cholinesterase at cholinergic junctions of the nervous system. Keeping in view the toxic effects of these pesticides, it is essential to remove these contaminants from the environment. Several microorganisms have proven of having potential of degradation of these pesticides. Thus, microbes can be used for active detoxification and degradation of these toxic compounds from contaminated environments. This is named as "Bioremediation" and has become the most effective management tool to clean-up the polluted environment and recover contaminated soil. In the bioremediation process, agricultural pesticides become available to varied kinds of microorganisms. Microorganisms use these pesticides in their metabolism by evolving in different ways. They use pesticides either as a principle source of carbon and energy, or consume them along with the other sources of carbon and energy. Now, it has been found that microbial metabolism is the only source of degradation of pesticides at most of the sites. There are different ways by which microbial degradation of pesticides takes place. Sometime pesticides enter into the cell via plasma membrane and metabolized by internal enzyme systems whereas some microbes produce enzymes outside their cell to predigest the enzymes. Thus, a proper interaction is needed between pesticide and microbes via enzymes (intracellular or extracellular). Thus, organophosphates can be removed from nature by the use of potential microbes. In this review article mainly the bioremediation of some widely used OPPs such as chlorpyrifos, diazinon, dichlorvos, fenitrothion etc. are discussed.

Application practices and pollution prominence in India

Organophosphate (OP) pesticides since their introduction in India in 1960s have contributed greatly in the control of pests and increase in the agricultural yield (Akhtar *et al.*, 2009). The authority responsible for pesticides registration for use on crops in India is the Central Insecticides Board and Registration Committee (CIBRC). It falls under the Ministry of Agriculture and Farmer Welfare. Here pesticides have been registered under Section 9(3) of the Insecticides Act, 1968. To date several hundreds of organophosphate compounds have been registered and successfully marketed in India (Kumar *et al.*, 2010). Among them some commonly used are malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, fenitrothion, phorate and monocrotophos. According to a survey, Government of India during 2005-2010, the decreasing order of consumption of these compounds is Phorate, Methyl parathion, Monocrotophos,

Chlorpyrifos, Malathion, Quinalphos, Dichlorvos. Although these pesticides have contributed much in controlling different kinds of pests and increasing crop yield, but their extensive and non-regulated use have proven them a major threat to our environment and life.

Earlier organochlorine pesticides such as DDT, aldrin, dieldrin were more in use as compared to OPPs. But now OPPs have become more popular due to their characteristics such as less persistence and more degradability in soil, water and food. However, problems are also associated with OPPs such as high toxicity may be due to their more solubility in water. Even more, these can easily enter into the living organisms via several routes, such as inhalation, ingestion, and dermal absorption. According to reports, residues of OPPs have been detected all over in India table1.

Metabolism/degradation of OPPs

Common ways of degradation of OPPs in the environment are abiotic or biotic or both. Under abiotic degradation chemical and physical transformations of the pesticide takes place by processes such as oxidation, reduction, photolysis, hydrolysis, and rearrangements etc. It occurs mainly when pesticides become biologically unavailable because of compartmentalization, due to adsorption to soil and soil colloids without alteration in the chemical structure of the original molecule. According to reports, OPPs degrade rapidly by hydrolysis on exposure to sunlight in the presence of air (Dhas and Srivastava 2010). The overall efficiency of degradation depends upon many factors such as pesticide characteristics (stability, hydrophilicity, etc.), environmental factors (temperature, moisture, pH, aeration etc.), microbial or plant activities (or sometimes both) etc. Complete metabolism of pesticides takes place in three-steps (Shimabukuro, 1985; Hatzios, 1991). Initially, compounds are transformed into a more water-soluble and less toxic products than the parent. Thereafter, pesticide metabolites conjugates with any other compound such as a sugar, amino acid etc. which increases further, water solubility and reduces toxicity. Finally converted into secondary conjugates, which are nontoxic (Hatzios, 1991).

Biodegradation of OPPs

Application of pesticides in agriculture for the control of pests is a routine practice of modern agriculture. Microorganisms present in the soil can easily get expose to these pesticides. Most of these organisms die due to the toxic effects of pesticides but few of them evolve to use pesticides in cellular metabolism. Various microorganisms such as bacteria, fungi, actinomycetes etc. have

been reported of being capable of degrading OPPs. In most of the isolated microbial species either of the two modes of degradation *i.e.* catabolic (use directly as a source of carbon and energy) and co-metabolic (consume the pesticides along with other sources of food or energy) have been observed. Biodegradation of some of the important OPPs may be summarized as follows:

Chlorpyrifos

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is one of the most extensively used OPPs. It belongs to the class II of pesticides that mainly comprises moderately toxic compounds. The fate of chlorpyrifos in environment has been broadly studied (Kim and Ahn, 2009). It has polluted air, ground water, rivers and lakes due to extensive usages. Its presence has even been detected up to 24 km away from the region of application (Bhagobaty *et al.*, 2007). It produce adverse effect on public health and environment due to its long persistence period in the environment (Mohan *et al.*, 2004). Therefore, degradation or removal from contaminated sites have been paid close attention (Racke *et al.*, 1988; Yang *et al.*, 2005). Abiotic as well as biotic degradation of this pesticide has been reported in the soil. Many chlorpyrifos-degrading microorganisms have been isolated in the laboratories but have not been still applied for commercial purpose (Bhagobaty and Malik,

2008; Awad *et al.*, 2011). Various species of *Pseudomonas*, including *P. aeruginosa*, *P. stutzeri*, *P. putida* etc. isolated from different regions have been confirmed to be effective in the biodegradation of chlorpyrifos (Bhagobaty and Malik, 2008; Vidya Lakshmi *et al.*, 2008; Awad *et al.*, 2011; Maya *et al.*, 2011; Briceño *et al.*, 2012; Latifi *et al.*, 2012; Sasikala *et al.*, 2012). Pailan *et al.*, (2015) isolated chlorpyrifos degrading bacterial sp. *Bacillus aryabhatai* from agricultural soil of West Bengal, India. *Stenotrophomonas* sp. was isolated from an industrial sludge in China degraded 63% of chlorpyrifos within 24 h with an initial concentration of 50 mg mL⁻¹. Cho *et al.*, 2009 isolated some strains of lactic acid bacteria such as *Leuconostoc mesenteroides*, *LactoBacillus brevis*, *Plantarum* L. and *Sakei* L. from kimchi which were found to be capable of utilizing chlorpyrifos as the only source of carbon and energy. Jones and Hastings (1981) reported the metabolism of 50 ppm chlorpyrifos in cultures of several forest fungi (*Trichoderma harzianum*, *Penicillium vermiculatum*, and *Mucor* sp. Lal and Lal (1987) studied degradation of chlorpyrifos by the yeast *Saccharomyces cerevisiae* in liquid medium and recovered only half the initial level of chlorpyrifos after 12 h of inoculation in the medium containing 1-10 ppm. Anwar *et al.*, (2009) isolated a bacterial strain *Bacillus pumilus* C2A1 from soil which was highly effective in

Table 1: Detected contamination with residues of OPPs.

Detected Pesticide	Reference/s
Chlorpyrifos	Amaraneni and Pillala 2001; Singh and Gupta 2002; Mukharjee 2003; Sanghi 2003; Kumari <i>et al.</i> , 2003; Kumari <i>et al.</i> 2004; Kumari <i>et al.</i> 2005; Bhanti and Taneja 2007; Kumari <i>et al.</i> 2008; Singh <i>et al.</i> 2008; Bishnu <i>et al.</i> , 2009; Mandal and Singh 2010; Sinha <i>et al.</i> , 2011; Anand and Somasekhar 2012; Sinha <i>et al.</i> , 2012; Chandra <i>et al.</i> , 2014; Harinathareddy <i>et al.</i> , 2014; Ramesh and Selvanayagam 2015.
Chlorpyrifos ethyl	Mukharjee 2003; Pujeri <i>et al.</i> 2011
Phorate	Lari <i>et al.</i> 2014
Malathion	Amaraneni and Pillala 2001; Mukharjee 2003; Kumari <i>et al.</i> , 2003; Sanghi 2003; Bhanti and Taneja 2007; Choudhary and Sharma 2009; Harinathareddy <i>et al.</i> , 2014
Ethion	Bishnu <i>et al.</i> , 2009; Sinha <i>et al.</i> , 2012
Quinalphos	Singh and Gupta 2002; Mukharjee 2003; Kumari <i>et al.</i> , 2003; Kumari <i>et al.</i> , 2004; Choudhary and Sharma, 2009; Sinha <i>et al.</i> , 2012; Mandal and Singh 2010; Harinathareddy <i>et al.</i> , 2014
Dichlorovos	Srivastava <i>et al.</i> , 2011
Methyl-parathion	Mukharjee 2003; Sanghi 2003; Bhanti and Taneja 2007; Srivastava <i>et al.</i> , 2008
Profenofos	Anand and Somasekhar 2012; Mandal and Singh 2010; Harinathareddy <i>et al.</i> , 2014
Monocrotophos	Singh and Gupta 2002; Kumari <i>et al.</i> , 2003; Kumari <i>et al.</i> , 2004; Sinha <i>et al.</i> , 2012; Chandra <i>et al.</i> , 2014
Dimethoate	Singh and Gupta 2002; Kumari <i>et al.</i> , 2003; Choudhary and Sharma, 2009; Srivastava <i>et al.</i> , 2011; Harinathareddy <i>et al.</i> , 2014
Fenitrothion	Kumari <i>et al.</i> , 2003; Sinha <i>et al.</i> , 2012
Phosphamidon	Kumari <i>et al.</i> , 2003
Acephate	Mandal and Singh 2010
Diazinon	Srivastava <i>et al.</i> , 2011; Sinha <i>et al.</i> , 2012

degrading chlorpyrifos and its first hydrolysis metabolite TCP. Vidya Lakshmi *et al.*, (2008) detected 75%–87% degradation of chlorpyrifos with 3, 5, 6-trichloro-2-pyridinol (TCP) as a product by *P. fluorescence*, *Brucella melitensis*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella* sp., *Serratia marcescens* and *P. aeruginosa*. Similar studies were also done by Mallick *et al.*, (1999), who isolated *Arthrobacter* sp. from Indian agricultural soil samples. Kim and Ahn (2009) also isolated *Burkholderia cepacia* from paddy field soil in Korea capable of degrading chlorpyrifos-methyl to TCP.

Parathion

Parathion (O, O-diethyl - O - p - nitrophenyl phosphorothioate) was introduced in the year 1947. It belongs to the hazard 'class Ia' according to WHO (2009). It is probably a potential carcinogen. Due to its extreme toxicity it has resulted in numerous human and other non-target organisms' deaths in the developing countries (McConnell *et al.*, 1999). It has half-life of 12-58 days in the soil that can vary with the environmental conditions. It is rapidly degraded in soil having rich microbial populations. Several species of bacteria capable of hydrolyzing parathion have been isolated from varied regions (Munnecke *et al.*, 1982; Kertesz *et al.*, 1994; Racke *et al.*, 1996). *Flavobacterium* sp., that could degrade parathion and diazinon was isolated by Sethunathan & Yoshida in 1973. Similarly, Siddaramappa and his co-workers in the same year isolated a *Pseudomonas* sp. that was able to hydrolyze parathion and utilized the hydrolysis product p-nitrophenol as a carbon or nitrogen source. Later on, *P. stutzeri* (Daughton & Hsieh, 1977); *Arthrobacter* strains (Nelson, 1982); *Pseudomonas* sp. and a *Xanthomonas* sp. (Tchelet *et al.*, 1993) were also isolated which were having the capability of degrading parathion. Another study reported hydrolysis of parathion to paraoxon by mixed bacterial culture (Tomlin, 2000). Deng and his coworkers in 2015, isolated *Stenotrophomonas* sp. from industrial sludge sample in China. *Serratia marcescens* was isolated from agricultural soil sample in Poland. Cycoń and his coworkers in 2013 found *Leuconostoc mesenteroides*, *Brevis* L., *Plantarum* L., *Sakei* L. from Kimchi in Korea during fermentation.

Diazinon

Diazinon (O, O - diethyl O - [6 - methyl - 2 - (1 - methylethyl - 4 - pyrimidinyl)] ester) insecticide, was first time introduced in 1953. It is generally used to control ants, cockroaches, silverfish, and fleas etc. in residential areas, food industries and especially in farms to control leaf-eating insects (Grube *et al.*, 2011). It is hazardous, moderately toxic and included in class II by WHO (2009).

The half-life of diazinon in soil is 40 days which is variable depending upon temperature, pH, moisture and soil characteristics (Kegley *et al.*, 2014). To date, several microorganisms have been documented having capability of degrading diazinon. *Serratia marcescens*, a member of enterobacteriaceae was isolated from agricultural soil sample of Saudi Arabia and was found to completely degraded 50 mg L⁻¹ diazinon in the laboratory conditions within 11 days (Abo-Amer, 2011). Some lactic acid bacteria such as *LactoBacillus brevis*, *Plantarum* L., and *Sakei* L. were found to be efficient in the degradation of diazinon when provided as a sole source of carbon and phosphorus (Cho *et al.*, 2009; Zhang *et al.*, 2014). Biodegradation of this pesticide was also studied by Cycoń *et al.* (2009) using *Serratia liquefaciens*, *S. marcescens* and *Pseudomonas* sp. isolated from polluted agricultural soil samples from Poland. *Arthrobacter* sp. and *Mycobacterium* sp. capable of metabolizing diazinon were also isolated by Seo *et al.*, (2007) from petroleum-contaminated soil of Hawaii, USA. Another study also reported that the *Stenotrophomonas* sp. isolated from industrial sludge in China might be an excellent candidate for using in remediating the pollution of diazinon and other organophosphate pesticides, with a removal efficiency of nearly 100% (Deng *et al.*, 2015).

Dichlorvos

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) is a moderately toxic insecticide of class II of hazardous compounds (WHO, 2009). Its biodegradation in the environment have been reported by many researchers. They have isolated various microbial strains capable of degrading dichlorvos. *Ochrobacterium* sp. was isolated by Zhang and his coworkers in 2006 from wastewater sludge from Jining, China, which was found to be able to utilize dichlorvos as sole carbon source. *Bacillus* sp. and *Pseudomonas* sp. were isolated from soil samples taken from groundnut field of Andhra Pradesh, India by Madhuri and Rangaswamy in 2009. Similarly, *Bacillus* sp. was also isolated from soil sample taken from grape wine yard located in Maharashtra, India by Pawar and Mali, 2014. Several potential degraders of dichlorvos were also isolated from the agricultural farm of Nigeria, and were identified as *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp. and *Acinetobacter* sp. (Agarry *et al.*, 2013). Improvement in the degradation potential of earlier isolated microbial strain *Trichoderma atroviride* was achieved by genetic engineering by Tang and his coworkers in 2009.

Fenamiphos

Fenamiphos (ethyl 4 - methylthio - m - tolyl isopropylphosphoramidate) is a nematicide which is extensively used for the control of nematodes. It was

introduced in the year 1967. It belongs to the hazard class Ib of hazardous compounds (WHO, 2009). Various biodegradation and persistence studies have been carried out on fenamiphos. The half-life of fenamiphos varies from 30- 90 days in soil (Johnson, 1998). Ou & Thomas (1994) isolated the first microbial consortium comprising six different bacterial species that degraded fenamiphos in the liquid culture under laboratory conditions. Megharaj and his co-workers in 2003 isolated *Brevibacterium* sp. which hydrolyzed fenamiphos as well as its hydrolysis products. Similarly, *Pseudomonas putida* and *Acinetobacter rhizosphaerae* were isolated from soil taken from banana field located in Eastern Crete, Greece (Chanika *et al.*, 2011). In another study, *Microbacterium esteraromaticum* was isolated from Turf green soil taken from South Australia (Caceres *et al.*, 2008). *Pseudomonas* spp., *Flavobacterium* sp. and *Caulobacter crescentus* were also isolated from soil samples taken from UK and Australia (Singh *et al.*, 2003).

Fenitrothion

Fenitrothion (O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate) pesticide was introduced in the year 1959. It is moderately toxic in nature and belongs to the hazard class II of hazardous compounds (WHO, 2009). It exerts its effect by inhibiting the enzyme acetylcholinesterase. Its half-life in soil is 2.7 days (Kegley *et al.*, 2014). Due to excessive usages it has caused pollution of soil and water and has been proven to be potential hazard for human health and environment (Tago *et al.*, 2006; Kim *et al.*, 2009). Its major hydrolysis product 3-methyl-4-nitrophenol has also been found to be hazardous for life. Microbial degradation of this pesticide has been studied by several researchers. Adhya and his co-workers in 1981 isolated *Flavobacterium* sp. that was found to degrade fenitrothion along with the other sources of carbon and energy. Later on, *Arthrobacter aurescenes* TW17 was reported to be using it as a principle source of energy (Ohshiro *et al.*, 1996). Similarly, *Burkholderia* sp. NF100 was also isolated (Hayatsu *et al.*, 2000). *Ralstonia* sp. SJ98 was reported to be capable of degrading 3-methyl-4-nitrophenol and to utilize it as a source of carbon (Bhushan *et al.*, 2000). In other studies, *Burkholderia* sp. was isolated from wastewater sludge from China (Zhang Z.H. *et al.*, 2006), *Corynebacterium* and *Arthrobacter* species were reported in the degradation (Kim *et al.*, 2009), *Serratia marcescens* was isolated from agricultural soil taken from Poland by Cycoń and his co-workers in 2013 and *LactoBacillus* sp. was isolated by Zhang and his co-workers in 2014.

Concluding remarks

Usages of pesticides in agriculture have become

avoidable practice due to increase in population and food demands. Among pesticides, OPP group is most widely used group. Most of these have been found to be biodegradable. Currently, biodegradation or bioremediation has been established as a potent reducer of organophosphate pesticide pollution of the environment. Up to date much of studies have already been done on isolation and characterization of microorganisms capable of degrading OPPs and their removal from contaminated sites. Among these, chlorpyrifos, diazinon, dichlorvos, fenitrothion and others have been removed effectively and efficiently from the environment by microbial degradation. Further, more research is recommended to be needed in this context. More work is also needed in generation of genetically improved microbial strains to enhance their ability to decontaminate the environment from toxic OPPs.

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