Int. J. Pure Appl. Sci. Technol., 20(2) (2014), pp. 79-94

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Review Paper

Biodegradation and Analytical Methods for Detection of Organophosphorous Pesticide: Chlorpyrifos

Nancy George[1], Prakram Singh Chauhan[1], Sonica Sondhi[1], Shilpa Saini[1], Neena Puri[2] and Naveen Gupta[1,*]

[1] Department of Microbiology, Panjab University, Chandigarh, India

[2] Department of Microbiology, Guru Nanak Khalsa College, Yamunanagar, Haryana, India

* Corresponding author, e-mail: (ng.puchd@gmail.com)

(Received: 13-12-13; Accepted: 8-2-14)

Abstract: *Chlorpyrifos is a broad spectrum moderately toxic organo-phosphorous insecticide. It is widely used in agriculture for pest control and in households as a termicide. However, exposure to chlorpyrifos and its metabolites can affect the public health resulting from its long residual periods in soil and water. Thus, there is an essential need to develop sensitive method for the detection of chlorpyrifos in environmental samples. Emerging technologies like development of biosensor has gained enormous attention in this area. Apart from detection, worldwide efforts are going on to develop efficient and cost effective methods for its degradation. Chlorpyrifos previously shown to be immune to biodegradation has now been proved to undergo enhanced microbe mediated decay into less harmful and non-toxic metabolites. Now-a-days, research activity in this area has shown that a diverse range of microorganisms are responsible for chlorpyrifos degradation. This article therefore aims at giving an overview of the present status of research in analytical detection and biodegradation of chlorpyrifos.*

Keywords: Chlorpyrifos, Organophosphorus compounds, Biodegradation, Biosensor.

1. Introduction

Pesticides were introduced in agriculture to fulfil the increased food needs of growing population. But now the use of pesticides has become a necessary evil. Residues of applied pesticides stay in the environment (air, soil, ground and surface water) for variable periods of times [1]. Due to the long persistence of organochlorines (lindane, heptachlor, dichlorodiphenyltrichloroethane [DDT] etc.),

their tendency to bioaccumulate and their potential toxicity towards non-target organisms, this group of pesticides was replaced by relatively less persistent and yet effective organophosphorus (OP) compounds [2].

There are about 100 types of organophosphate compounds, out of them many have been banned because of their high toxicity. Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is still one of the most widely used OP pesticides effective against a broad spectrum of insect pests of economically important crops [3]. Chlorpyrifos, first introduced into the market place in 1965, has been widely used globally as an insecticide to control crop pests in agriculture, reduce household pests such as termites, reduce insect damage to turf on lawns and golf courses, and for mosquito control. Chlorpyrifos shows a wide spectrum of biological activity and is used to control wide range insect pests as well as soil dwelling grubs, rootworms, borers and subterranean termites. It is available in a variety of formulations, such as granules, wettable powder, dustable powder and emulsifiable concentrate [4].

Commercially, it is available in different brand names like Dursban, Lorsban, Agromil, Dhanwan, Dorson, Omexan etc. However extensive use of chlorpyrifos contaminates air, ground water, rivers, lakes, rainwater and fog water. The contamination has been found up to about 24 kilometres from the site of application. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions [5].

Chlorpyrifos interferes with the normal functioning of the central nervous system, including the brain. The extensive usage of organophosphates pesticide particularly chlorpyrifos for pest control and the potential toxicity in human body have raised serious public concerns in regard of human health, environment and food safety [6]. Hence, the methods for detection and degradation of pesticide residues in various environmental matrices such as soil, air and water is necessary for solving various environmental and biological problems [7].

Initially, degradation of pesticide was observed in alkaline soils and phenomenon was related to its hydrolysis at high pH. However, hydrolysis of chlorpyrifos was not observed in high pH sterile soils, which indicated the involvement of soil microorganisms in its degradation [8,9]. This article aims at summarizing the mode of action of chlorpyrifos, proposed pathway of its degradation and to throw light on the research efforts undertaken worldwide to develop sensitive methods for its detection and degradation of chlorpyrifos.

2. Mechanism of Action of Chlorpyrifos and its Effects

The mechanism of action for the toxic effects of chlorpyrifos is related to the ability of the oxon metabolite of chlorpyrifos to bind to and irreversibly inhibit acetylcholinesterase (AChE-ase) in target tissues. Chlorpyrifos, like other insecticide OPs are inhibitors of AChE-ase, an enzyme vital to the nervous systems of animals and humans. The transmission of impulses across certain nerve junctions involves the release of a neurotransmitter chemical, acetylcholine (ACh). The stimulant effect on ACh is rapidly cancelled by AChE-ase activity. The inhibiting effect of OPs on AChE-ase results in sustained high levels of ACh with consequent serious and widespread disruption of nervous activity [5, 6] [Fig 1].

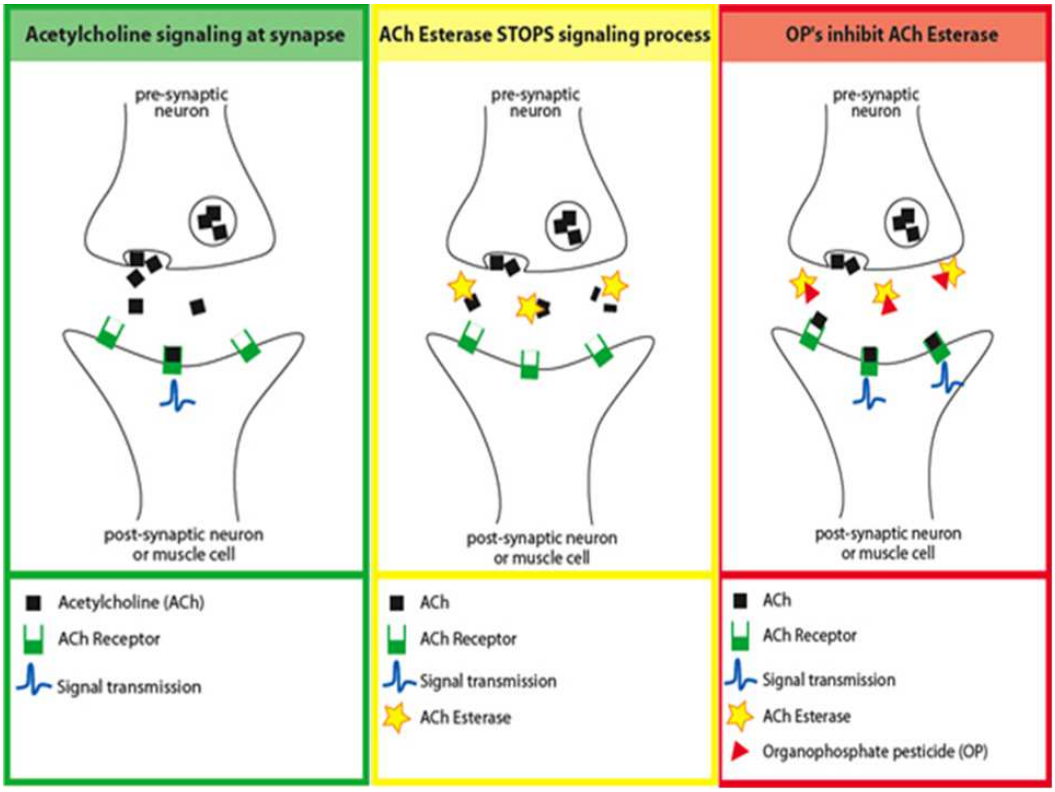


Fig. 1: Mechanism of action of Chlorpyrifos [10]

Besides AChE, there are numerous potential molecular targets for chlorpyrifos which have been identified in *in vitro* studies that includes cytotoxicity, effects on macromolecule synthesis (DNA, RNA,proteins), interactions with neurotransmitter receptors, interactions with signal transduction pathways and interactions with various enzymes. Symptoms of acute chlorpyrifos poisoning in humans occur when cholinesterase activity has been reduced by about 50% and includes headache, nausea, dizziness, muscle twitching, weakness, increased sweating and salivation [6].

Chlorpyrifos is also toxic to a variety of beneficial arthropods including bees, ladybird beetles and parasitic wasps. It kills fishes at concentrations as low as a few parts per trillion. Birds are also susceptible with effects ranging from reduced weight of nestlings, deformities and death. In plants, there have been reports of delayed seedling emergence, fruit deformities and abnormal cell division upon prolonged exposure to chlorpyrifos [10].

3. Analytical Methods for the Detection of Chlorpyrifos

The techniques for detection of chlorpyrifos are mainly based on three methods:

(i) Classical analytical techniques
(ii) Immunoassays
(iii) Biosensors

3.1 Classical Analytical Techniques for Detection of Chlorpyrifos

Classical analytical techniques for pesticide detection involve gas chromatography, high-performance liquid chromatography, gas chromatography coupled with mass spectrometry and liquid chromatography coupled with mass spectrometry. Chlorpyrifos in sample extracts like air, drinking water, river water, surface water, ground water, soil, sludge and pesticide formulations is typically determined using these techniques. The detection of chlorpyrifos and its intermediate TCP by GC

employs the use of selective detection systems such as flame photometric detection (FPD), nitrogen phosphorus thermionic detection (NPD), or electron capture detection (ECD). Other analytical techniques that have been used to detect chlorpyrifos include GC with atomic emission detection, GC with mass spectrometry, simultaneous analysis on two GC columns with both ECD and electrolytic conductivity detectors, and two-dimensional GC with simultaneous detection by ECD, NPD, and FPD. The chromatographic techniques used for pesticide analysis requires efficient isolation and concentration procedures. For air matrices, collection methods rely on the entrapment of chlorpyrifos onto a polymeric material, such as XAD or polyurethane foam, as the air is pulled through the sorbent. The analyte is subsequently recovered from the sorbent through solvent extraction. In the case of water, soil and waste, sample preparation is based on liquid/liquid extractions, solid phase extraction (SPE) or Soxhlet extractions [11]. Although chromatography based methods are sensitive and reliable, they require sophisticated equipment, skilled analysts and time consuming sample preparation steps.

3.2 Immunoassays for Detection of Chlorpyrifos

Immunoassay based detection methods have become indispensable analytical tools in a wide range of applications including environmental monitoring, clinical diagnosis and food safety. Immunological methods, which are suitable for both laboratory and field analysis, provide a unique opportunity to screen large numbers of samples quickly and effectively. Traditional immunoassays such as enzyme-linked immunosorbent assays (ELISAs) are invariably considered as the best for single analyte measurement. Several ELISAs were developed independently for the detection of pesticides [12, 13]. However, with the demand for multiplexing capability, shorter analysis time, smaller sample volume and higher sensitivity, a number of new techniques are being explored to perform immunoassays. An immuno-chromatographic assay (ICA) based on competitive antigen-coated format using colloidal gold as the label was developed for the detection of the chlorpyrifos. The ICA test strip consisted of a membrane with a detection zone, a sample pad and an absorbent pad. The membrane was separately coated with chlorpyrifos Hapten–ovalbumin (OVA) conjugate (test line) and anti-mouse IgG (control line). Based on the fact that the competition is between the migrating analyte in the sample and the analyte hapten immobilized on the test strip for the binding sites of the antibody–colloidal gold (Ab–CG) conjugate migrating on the test strip, this study suggests that the relative migration speed between the two migrating substances is a critically important factor for the sensitive detection by competitive ICA. The detection limit of the ICA for chlorpyrifos standard and chlorpyrifos spiked into agricultural samples were 10 and 50 ngmL−1, respectively. The assay time for the ICA test was less than 10 min, suitable for rapid on-site testing of chlorpyrifos [14] [Fig. 2].

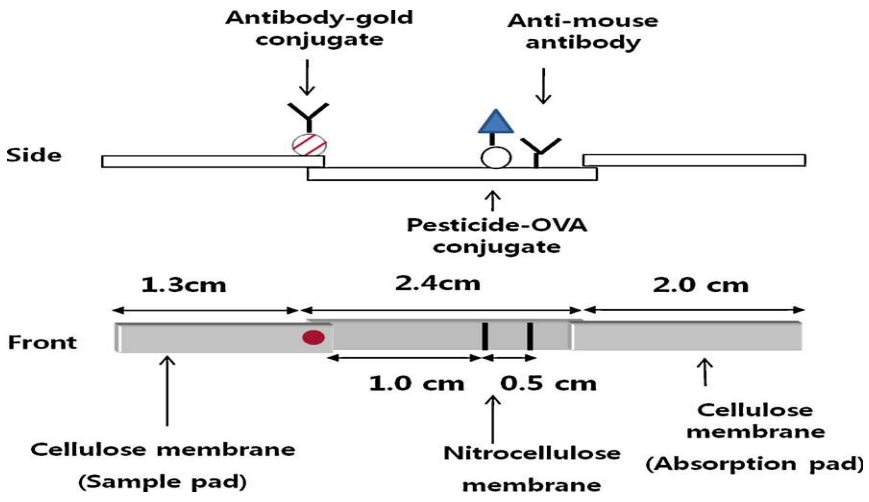


Fig. 2: Schematic diagram of the lateral flow ICA test strip [14]

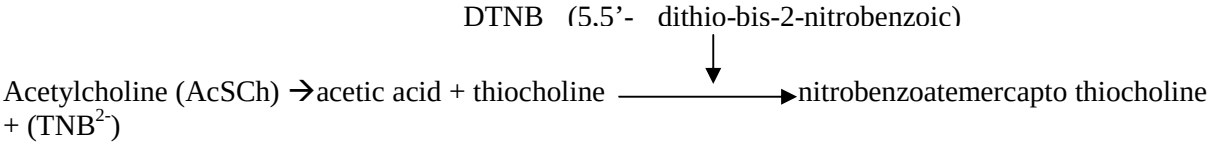
3.3 Biosensors Based Detection of Chlorpyrifos

In case of some environmental samples, where nothing is known about the nature of possible contaminants, universal and reliable analytical methods are required. These methods enable one to analyze in a single run and within the shortest possible time as many pesticides as possible from samples of unknown origin [7]. It is then an advantage to use methods that permit the determination of pesticides residues of different chemical classes in the same extract. In this context, various biosensors to detect the organophosphorus compounds have been developed [15].

A biosensor is a miniaturized device integrating a biological sensing element (antibody, enzyme, receptor, cell, etc) on intimate contact with an appropriate transducer (optic, electrochemical, piezoelectric, etc) for conversion of the recognition success to a primary signal (optical or electrical) that can be amplified and subsequently processed eventually to take automatic remediation actions.

The detection principle of biosensors is usually based on the inhibition by organophosphorus compounds of AChE-ase which is an enzyme with narrow specificity for acetylcholine and related compounds in nerve tissue [16].

The determination of enzyme activity is based on two coupled reactions [15].



The product 5-thio-2-nitrobenzoate (TNB^{2-}) is yellow with an absorption maximum at 410 nm and can be detected optically [17].

A biosensor based on quartz crystal microbalance for detection of chlorpyrifos was reported in which AChE-ase was immobilized on multilayer films assembled by poly diallyl dimethyl ammonium chloride (PDDA) and ι-carrageenan (IC) on silver-coated crystal electrode surfaces. The lowest concentration of chlorpyrifos that could be detected was 0.1 μg l^{-1} [18].

Fibre-optic biosensors to detect the organophosphorus compounds have been developed based on the inhibition of AChE-ase by organophosphorus compounds [19, 20, 21, 22]. It has many advantages compared with the other sensor types due to their capability of remote and multiple sensing [23]. It is not interfered with an electric field and is easy to miniaturize, which can lead the development of a very small, light and flexible sensor [16]. A fibre optic biosensors consisting of an AChE-ase immobilized Langmuir-Blodgett film has been developed to detect chlorpyrifos in contaminated water. The sensing scheme was based on the decrease of yellow product, *o*-nitrophenol, from a colorless substrate, *o*-nitrophenyl acetate, due to the inhibition by organophosphorus compounds on AChE-ase. Absorbance change of the product as the output of enzyme reaction was detected and the light was guided through the optical fibres. This biosensor could successfully detect the organophosphorus compounds upto 2 ppm in the response time of about 10 min [24]. In another report, a fibre-optic biosensor consisting of AChE-ase and bromothymol blue (BTB) doped sol–gel film was employed to detect organophosphate pesticide chlorpyrifos. The main advantage of this optical biosensor is the use of a single sol–gel film with immobilized AChE-ase and BTB. In the presence of a constant AChE-ase, a color change of the BTB and the measured reflected signal at wavelength 622 nm could be related to the pesticide concentration in the sample solutions. The detection limit for chlorpyrifos was 0.04 mg/L [25]. Gaberlein et al. (26) had deviced potentiometric biosensors based on immobilized whole cell and cytoplasmic membrane fractions of *Flavobacterium* sp.on the surface of a glass pH electrode. The protons released by hydrolysis of chlorpyrifos as a sole carbon source by the enzyme organophosphatase hydrolase was detected by the pH electrode which gives a signal that can be measured [26].

Screen printed carbon electrodes modified with dialdehydes, glutaraldehyde and terephthaldicarboxaldehyde and then polyethylenimine had also been utilized for production of pesticide biosensors based on acetylcholinesterase. The detection limit of the biosensors produced by non-covalent immobilization of AChE-ase onto polyethyleneimine modified carbon electrodes was found to be about 10^{-10} M for the organophosphate pesticide [27].

Another detection system based on bacterial phosphotriesterase (PTE) for the degradation of organophosphate (OP) insecticides in water had also been developed. PTE was immobilised on an activated agarose gel via covalent coupling. The efficiency of insecticide degradation was controlled using a highly sensitive biosensor allowing the detection of OP concentration as low as 0.004 $\mu g\ l^{-1}$ [28].

A novel portable immunochromatographic electrochemical biosensor (IEB) had been developed for simple, rapid, and sensitive biomonitoring of trichloropyridinol (TCP), a metabolite biomarker. Under optimal conditions, the IEB had demonstrated a wide linear range (0.1–100 ng ml^{-1}) with a detection limit as low as 0.1 ng ml^{-1} TCP [29].

In another report, a novel, low potential and highly sensitive acetylcholinesterase (AChE) biosensor based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotube composite gel thiocholine sensor have been developed. Chlorpyrifos could be determined in the range of 10^{-8}–10^{-6}M with a detection limit of 4 nM [30].

A novel amperometric immunosensor based on multiwalled carbon-nanotubes-thionine-chitosan (MWCNTs-THI-CHIT) nanocomposite film was developed by Sun et al. (31) for the detection of chlorpyrifos residue. The nanocomposite film was dropped onto a glassy carbon electrode. The anti-chlorpyrifos monoclonal antibody was covalently immobilized on the surface of MWCNTs-THI-CHIT/GCE using the cross-linking agent glutaraldehyde (GA). The immunosensor exhibited detection limit of 0.046 ng ml^{-1}, high reproducibility, stability and good selectivity and regeneration [31].

A disposable amperometric immunosensor for sensitive detection of chlorpyrifos-methyl (CM) has been developed by the modification of the screen-printed carbon electrode by doping of bovine serum albumin conjugated chlorpyrifos-methyl antigen (BSA-Ag) and the platinum colloid into silica sol-gel [32].

AChE-ase biosensor based on chitosan/prussian blue/ multiwall carbon nanotubes/ hollow gold nanosphere nanocomposite film developed by Zhai et al. (33) showed a wide range, low detection limit, good reproducibility and high stability [33]. Various biosensors developed for detection of cholrpyrifos has been shown in Table 1.

Table 1: Different type of Biosensors developed for the detection of Chlorpyrifos

Type of Biosensor	**Design**	**Detection Characteristics**	**References**
Fibre optic biosensor	Acetylcholinesterase (AChe) immobilized on Langmuir Blodgett film	Detects chlorpyrifos in contaminated water	[15]
	Acetylcholinesterase (AChe) and Bromothymol blue (BTB) doped on sol-gel film	Detection limit- 0.04 mg/L	[25]

Potentiometric biosensor	Whole cell and cytoplasmic membrane fractions of Flavobacterium sp. immobilized on the surface of a glass pH electrode	Detects the release of protons on hydrolysis of chlorpyrifos by enzyme Organophosphatase hydrolase	[26]
Modified screen printed carbon electrode based biosensor	Acetylcholinesterase is immobilized onto polyethyleneimine modified carbon electrode.	Detection Limit: 10^{-10} M for organophosphate pesticide dichlorvos	[27]
Phosphotriesterase (PTE) based biosensor	Phosphotriesterase (PTE) is immobilized on activated agarose gel via covalent coupling.	Detects OP concentration as low as 0.004 μg/L	[28]
Acetylcholinesterase (AChE) biosensor based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwall carbon nanotube composite film	AChE was immobilized in sol–gel matrix	Detection limit: 4 nM.	[30]
Amperometric immunosensor based on muti-walled carbon-nanotubes-thionine-chitosan (MWCNTs-THI-CHIT) nanocomposite film	The anti-chlorpyrifos monoclonal antibody was covalently immobilized on the surface of MWCNTs-THI-CHIT/GCE using the crosslinking agent glutaradehyde (GA).	Detection limit : 0.046 ng/ml	[31]
Modified screen-printed carbon electrode based amperometric immunosensor	Doping of bovine serum albumin conjugated chlorpyrifos-methyl antigen (BSA-Ag) and the platinum colloid into silica sol-gel.	Detects chlorpyrifos-methyl (CM)	[32]
Acetylcholinesterase biosensor based on chitosan/prussian blue/ multiwall carbon nanotubes/ hollow gold nanosphere nanocomposite film	Acetylcholinesterase immobilized on chitosan/prussian blue/ multiwall carbon nanotubes/ hollow gold nanosphere nanocomposite film	Wide range , low detection limit	[33]

4. Degradation Pathway of Chlorpyrifos

Like most OP pesticides, chlorpyrifos is oxidized to its oxon form, chlorpyrifos-oxon, which is generally regarded as the principal toxic metabolite and is responsible for inhibition of cholinesterases. Chlorpyrifos-oxon is either enzymatically or spontaneously hydrolyzed to form the diethylphosphate and 3,5,6-trichloro-2- pyridinol (TCP). In addition to the formation of chlorpyrifos-oxon, chlorpyrifos is oxidized via cytochrome(s) P-450 to an unstable intermediate that spontaneously hydrolyses to form diethylthiophosphate and TCP. The major intermediate hydrolysis product of chlorpyrifos are 3,5,6-trichloro-2-pyridinol (TCP) and Diethylthiophosphoric acid (DETP) [Fig. 3].

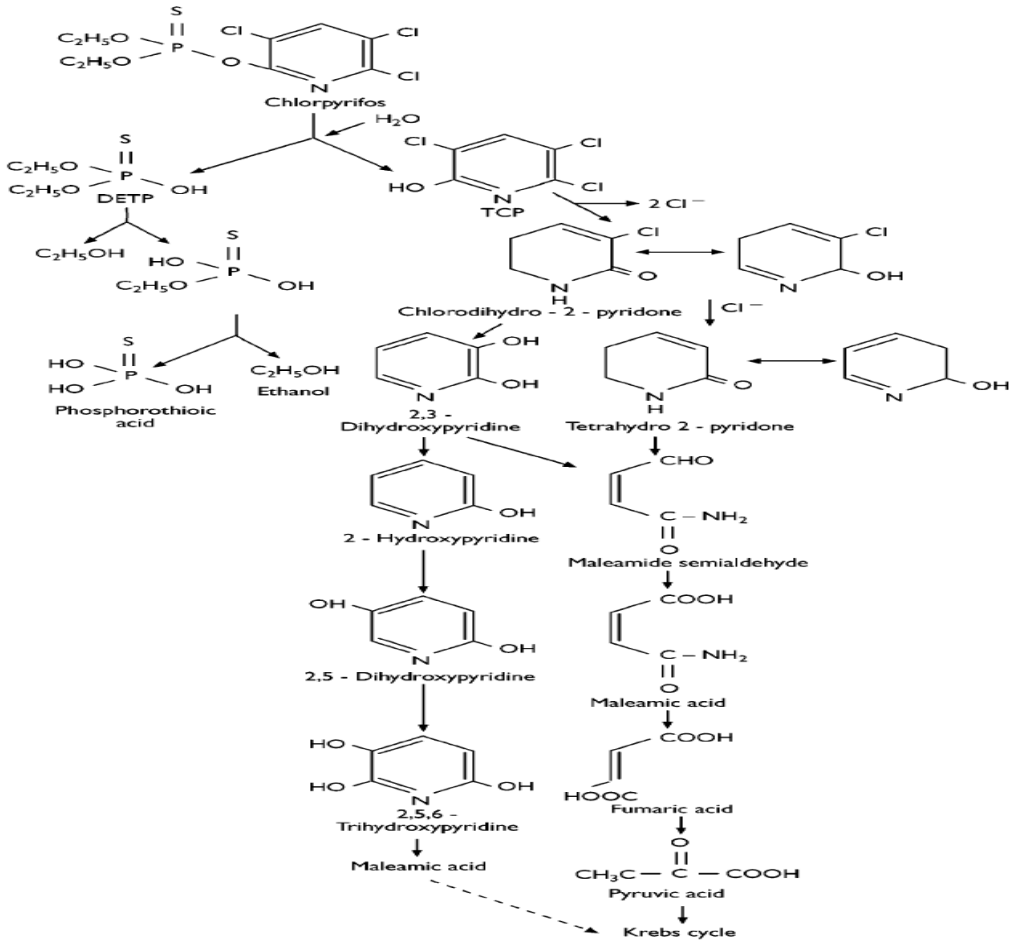


Fig. 3: Proposed degradation pathway of Chlorpyrifos [35]

Out of them, TCP has greater water solubility than chlorpyrifos and causes the widespread contamination in soils and in the aquatic environment. TCP is not only persistent towards degradation by microorganisms but also limits the biodegradation of chlorpyrifos owing to its antimicrobial activities [34]. But some organisms have been reported to completely degrade the chlorpyrifos into CO_2 and organic matter [35]. Following application to crops, chlorpyrifos binds to soil and plants. Though it degrades rapidly in the environment, residual levels of chlorpyrifos can last for long periods of time. Human exposure pathways from agricultural applications include dermal, oral, or inhalation. While in the environment, chlorpyrifos and its metabolites are susceptible to photodegradation, with a half-life of approximately 3 days, in the presence of hydroxyl radicals in the atmosphere the half-life is lowered to about 6 h. Upon entering surface water, chlorpyrifos degradation is associated with abiotic hydrolysis or photosensitized oxidation. In soil, photodegradation plays a role in hydrolysis, dechlorination and oxidation of chlorpyrifos. However, in indoor environment, chlorpyrifos can persist for several months because of the relative lack of sunlight, water, and/or soil microorganisms that contribute to its rapid degradation in the outdoor environment [36].

4.1 Biodegradation of Chlorpyrifos

Microorganisms have considerable capacity for the metabolism of many pesticides. Although they are capable of catalyzing similar metabolic reactions as in mammals and plants, they possess the unique ability to completely mineralize many aliphatic, aromatic and heterocyclic compounds. There are two major types of microbial degradation of organic chemicals. The first, termed catabolism is a type of degradation in which the organic chemical or a portion thereof is completely degraded and the energy or nutrient gained contributes to cell growth. The second, incidental metabolism or co-metabolism, involves the partial degradation of an organic chemical with no net benefit to the organism, the

compound being merely caught up in some metabolic pathway during the normal metabolic activities of the microorganisms. The most important microbial role in the chlorpyrifos degradation pathway is the metabolism and mineralization of 3, 5, 6-trichloro-2-pyrinidinol (TCP) and 3, 5, 6-trichloro-2-methoxypyridine (TMP) metabolites which are toxic intermediates in chlorpyrifos degradation. Although chlorpyrifos has been used as an agricultural household pesticide, few attempts to isolate chlorpyrifos degrading microorganisms have been successful. The major obstacle is the intermediate TCP accumulation which is toxic and has antimicrobial activity which prevents the degradation of chlorpyrifos in soil [37]. However, some microorganisms have been reported which degrades chlorpyrifos completely. Important chlorpyrifos degrading microorganisms have been summarized in Table 2.

Table 2: Chlorpyrifos degrading Micro-organisms

Microorganism	**References**
Bacteria	
Micrococcus sp.	[39]
Flavobacterium sp.	[40]
Enterobacter strain B-14	[54]
Alcaligenes faecalis DSP3	[44]
Stenotrophomonas YC-1	[45]
Klebsiella sp.	[46]
Sphingomonas sp. Dsp-2	[47]
Paracoccus sp. strain TRP	[50]
Pseudomonas putida MAS-1	[51]
Bacillus pumillus strain C2A1	[52]
Pseudomonas sp. (Ch1D)	[54]
Bacillus licheniformis ZHU-1	[55]
Ralstonia sp. strain T6	[56]
Cupriavidus sp. DT-1	[59]
Lactobacillus bulgaris	[69]
Streptococcus thermophilus	[69]
Serratia sp.	[70]
Agrobacterium sp.	[72]

Enterobacter sp.	[72]
Fungi	
Phanerochaete chrysosporium	[61]
Verticillium sp. DSP	[64]
Acremonium sp. Strain GFRC-1	[65]
Cladosporium cladosporoides strain Hu-01	[66]
Ganoderma sp. JAS4	[67]
Trichoderma sp.	[68]
Trichosporon sp.	[70]
Aspergillus niger	[71]
Trichoderma viridae	[71]

4.1.1 Bacteria Involved in Chlorpyrifos Degradation

Munnecke et al. (38) reported the ability of parathion hydrolase, an organophosphorus ester-hydrolyzing enzyme isolated from a mixed microbial culture, to hydrolyze chlorpyrifos [38]. However, the first ever documented organophosphorus biodegrading Opd gene was identified in *Pseudomonas diminuta* which was found to be plasmid based which was responsible for chlorpyrifos degradation has also been reported from *Micrococcus* sp. [39]. Mallick et al (40) reported the rapid degradation of chlorpyrifos when added to a mineral salt medium or applied to the soil, as a sole carbon source by the *Flavobacterium* sp. studied the degradation of chlorpyrifos in poultry and cow-derived effluents and reported that chlorpyrifos was degraded by aerobic microbial processes in animal derived lagoon effluents [40]. Further analysis of the microbial community involved in the degradation process by denaturing gradient gel electrophoresis (DGGE) of PCR amplified16S RNA genes showed that a single band became dominant in effluents during chlorpyrifos degradation, thereby indicating the role of a single aerobic-bacterial population in the degradation of chlorpyrifos [41].

Enhanced degradation of chlorpyrifos by an *Enterobacter* strain B-14 was shown to utilize chlorpyrifos as a sole source of carbon and phosphorus and hydrolyzed chlorpyrifos to diethyl thiophosphoric acid (DETP) and 3, 5, 6-trichloro-2- pyrinidol. DETP was utilised for growth and energy. Further studies revealed that the strain possessed a novel phosphotriesterase enzyme system, as the gene coding for this enzyme had a different sequence from the widely studied organophosphate degradative gene (opd) [42].

Ajaz et al. (43) found that three isolates *viz*., *Klebsiella* sp., *Pseudomonas putida* and *Aeromonas* sp., offered resistance upto 2 mg ml^{-1}, 4 mg ml^{-1} and 8 mg ml^{-1} of chlorpyrifos respectively [43]. Yang et al. (44) also isolated *Alcaligens faecalis* DSP3, which was capable of degrading both chlorpyrifos and TCP [44].

Yang et al. (45) isolated a strain *Stenotrophomonas* YC-1 which degraded 100 mg ml^{-1} of chlorpyrifos to DETP and its intermediate TCP within 24 hour. DETP was utilized as a source of carbon and phosphorus, but it did not degrade TCP. They were successful in cloning the opd gene from this strain and used it for bioremediation of contaminated soil [45].

Klebsiella sp. isolated from activated sludge sample [46] and *Sphingomonas* sp. Dsp-2 [47] has also been reported to degrade chlorpyrifos. Rani *et al.* had isolated four morphologically distinguishable bacterial colonies on mineral salts agar enriched with chlorpyrifos pesticide. The four isolates were identified as *Providencia stuartii, Serratia marcescens, Klebsiella oxytoca* and *Bacillus subtilis* respectively. The growth experiments showed that *P. stuartii* strain MS09 is able to grow in the presence of high concentrations of chlorpyrifos and utilized it as carbon source [48].

Lakshmi et al. (49) utilized *P. fluorescence, Brucella melitensis, B. subtilis, B. cereus, Klebsiella* sp., *S. marcescens* and *P. aeroginosa f*or degradation of chlorpyrifos. They showed 75-87% of degradation of chlorpyrifos [49].

Xu et al. (50) isolated *Paracoccus* sp. strain TRP from activated sludge with the ability to completely biodegrade chlorpyrifos and its metabolite 3,5,6-trichloro-2 pyridinol [50].

A chlorpyrifos degrading bacterium *Pseudomonas putida* MAS-1 from the cotton grown soil of NIAB, Faisalabad, Pakistan have been isolated. The plasmid genes (conferring chlorpyrifos degrading ability) from *P. Putida* MAS-1 was transferred to *E. coli* DH5α competent recipient cells [51].

Anwar et al. (52) have isolated *Bacillus pumillus* strain C2A1 which is able to degrade chlorpyrifos and its first hydrolysis metabolite 3, 5, 6-trichloro-2-pyridinol (TCP). Chlorpyrifos was utilized by the strain as sole carbon source as well as it was co-metabolized in the presence of glucose, yeast extract and nutrient broth. Maximum pesticide degradation was observed at pH 8.5. The strain C2A1 showed 90% degradation of TCP (300 mg ml^{-1}) within 8 days of incubation [52].

Four CP degrading lactic acid bacteria that degraded chlorpyrifos upto 83 % by day 3 and completely by day 9 have been isolated. These four strains also degraded coumaphos, diazinon, parathion and methyl parathion [53].

Singh et al. (54) isolated *Pseudomonas* sp. (Ch1D) from agricultural soil by enrichment culture technique in the presence of chlorpyrifos, with the capability to produce biosurfactant (rhamnolipids) and to degrade chlorpyrifos (0.01 g l^{-1}) [54].

Zhu et al. (55) isolated *Bacillus licheniformis* ZHU-1 capable of utilizing chlorpyrifos as the sole carbon source. The addition of this bacteria to soil treated with chlorpyrifos resulted in a higher degradation rate than non inoculated soils. The degradation rate of chlorpyrifos (100 mg kg^{-1}) could reach 99% or above after 14 days [55].

A *Ralstonia* sp. strain T6 which was able to metabolize 100 mg ml^{-1} TCP within 12h and 700 mg ml^{-1} TCP in 80h was also reported. A green metabolite 3,6-dihydroxypyridine-2,5-dione was detected as one of the end product [56].

Liu et al. (2012) reported the ability of *Bacillus cereus* to degrade chlorpyrifos under different culture conditions [57]. Harishankar et al. (2013) analysed the efficiency of five model intestinal bacteria (*Lactobacillus lactis, L. fermentum, L. plantarum, E. coli and Enterococcus faecalis*) in the degradation of the chlorpyrifos. It was found that *Lactobacillus fermentum* degraded 70% CP with 3,5,6-trichloro-2-pyridinol (TCP) detected as the end product, *L. lactis* degraded upto 61% CP with chlorpyrifos oxon detected as the end product whereas *E. coli* degraded a lesser concentration (16%) to chlorpyrifos oxon and diethylphosphate [58].

A novel chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol degrading bacterial strain *Cupriavidus* sp. DT-1 was isolated and characterized by Lu et al.(2013) . It was suggested that chlorpyrifos was hydrolysed to TCP, successively dechlorinated to 2-pyridinol and then underwent pyridine ring cleavage and further degradation. The mpd gene encoding for the enzyme responsible for hydrolysis of chlorpyrifos to TCP was cloned and expressed in *E. coli* BL21. The strain exhibited the degradation rate of chlorpyrifos and TCP of 100% and 94.3% respectively [59].

4.1.2 Fungi Involved in Chlorpyrifos Degradation

Besides bacteria, many fungal isolates have also been reported which can degrade chlorpyrifos. Lal and Lal (60) observed some degree of degradation by the yeast *Saccharomyces cerevisiae*. Only half of the initial chlorpyrifos was recovered 12 h after the cultures were inoculated with 1-10 ppm [60]. Bumpus et al. (61) reported the ability of *Phanerochaete chrysosporium* to degrade 27.5 % of chlorpyrifos during the 18-day incubation in nitrogen-limited stationary cultures. During the biodegradation process, the chlorinated pyridinyl ring of chlorpyrifos underwent ring cleavage and was hydrolysed to carbon dioxide [61]**.** Strains of *Aspergillus flavus* and *Aspergillus niger* isolated from agricultural soil exposed to chlorpyrifos has been reported to biomineralise chlorpyrifos in liquid culture medium [62]. Chlorpyrifos-degrading fungi, such as *Phanerochaete chrysosporium* and *Aspergillus terreus* have also been reported [63]. A fungal strain of *Verticillium* sp.DSP which significantly shortened the half life of chlorphyrifos by 37% in inoculated soil was isolated by Hua et al. [64].

Kulshreshtha and Kumari (65) first reported the isolation of *Acremonium* sp. strain GFRC-1 from agricultural soils in India using enrichment culture technique, which utilized chlorpyrifos as a source of carbon and nitrogen and had highest chlorpyrifos degradation capacity (83.9%) when cultivated in the nutrient medium with full nutrients [65].

Chen et al. (66) isolated and characterized a new fungal strain *Cladosporium cladosporoides* strain Hu-01 which has high chlorpyrifos degradation activity. It utilized 50 mg ml^{-1} of chlorpyrifos as the sole carbon source and was able to tolerate upto 500 mg ml^{-1} of chlorpyrifos concentration. The optimum conditions for degradation were found to be 26.8°C and pH 6.5. It completely metabolized chlorpyrifos under these conditions and no accumulation of 3, 5, 6-trichloro-2-pyridinol was observed. It was suggested that the isolate harbours the metabolic pathway for complete detoxification of chlorpyrifos and its hydrolysis product TCP [66].

Silambarasan and Abraham (67) recently reported the isolation of a new fungal strain *Ganoderma* sp. JAS4 from agricultural soil which exhibited high efficiency in degrading chlorpyrifos and its major degradation product 3,5,6 –trichloro-2-pyridinol (TCP) [67].

4.1.3 Chlorpyrifos Degradation by Coculture of Micro Organisms

Although many chlorpyrifos degrading microorganisms have been isolated but there are very few reports of microorganisms which can degrade chlorpyrifos and its toxic intermediate product TCP completely. Therefore, some attempts have been made in which by co-culturing the two or more microorganisms, complete degradation of chlorpyrifos can be achieved. Microorganisms which degrade chlorpyrifos to TCP are co-cultured with microorganisms which degrade TCP which results into complete degradation.

Ivashina (68) studied chlorpyrifos degradation by several microbial cultures maintained in liquid media containing 10 ppm chlorpyrifos. Dissipation was more rapid in a sucrose supplemented media containing *Trichoderma* sp. and glucose supplemented media containing *Bacillus* sp. [68].

Two lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was also reported which displayed 72-83% loss in chlorpyrifos after 96 h [69]. Gangming et al. (70) reported co-culture

of *Serratia* sp. and *Trichosporon* sp. for degradation of chlorphyrifos. *Serratia* sp. could transform Chlorphrifos to TCP and *Trichosporon* sp. further mineralized TCP. The fungus could degrade 50 mg ml^{-1} Chlorphyrifos within seven days. Co-culture completely mineralized 50 mg ml^{-1} Chlorphyrifos within 18 h at 37°C [70].

Agrobacterium and *Enterobacter* sp. exhibited growth linked biodegradation of chlorpyrifos. Such efficient bacterial strains can be used successfully for the removal of chlorpyrifos from the contaminated sites [71].

5. Conclusions

Along with other organophosphorus pesticides, the use of chlorpyrifos has raised a number of concerns regarding human health. Extensive research is going on for the development of sensitive detection methods and efficient degradation strategies for chlorpyrifos. This review comprises substantial information regarding the degradation and detection of chlorpyrifos that can be useful for the researchers involved in these studies. Previously, chlorpyrifos was thought to be immune to biodegradation but now a large number of microorganisms have been isolated and characterized that can degrade this compound. Further studies are required for the complete mineralisation of chlorpyrifos as some of the intermediate products of its degradation like TCP is more persistent towards degradation by microorganisms and has antimicrobial activity. More research on Co-culturing of two or more microorganisms, that can completely degrade both chlorpyrifos and TCP, can be beneficial in this regard.

References

[1] M. Gavrilescu, Fate of pesticides in the environment and its bioremediation, *Eng Life Sci.*, 5(6) (2005), 497-526.

[2] T.R. Fukuto, Mechanism of action of organophosphorus and carbamate insecticides, *Environ. Health Perspect.*, 87(1990), 245-254.

[3] B.K. Singh, Organophosphorus–degrading bacteria: Ecology and industrial applications, *Nature Rev Microbiol.*, 7(2008), 156-164.

[4] S.D.K. Singh, Utilization of chlorpyrifos by *Aspergillus niger* and *A. flavus* as carbon and phosphorus source, *17th World Congress of Soil Science*, 14-21 August (2001), Bangkok, Thailand, (2002).

[5] R.K. Bhagobaty, S.R. Joshi and A. Malick, Microbial degradation of organophosphorous pesticide: Chlorpyrifos (Mini-Review), *Inter J Microbiol.*, 4(1) (2006) 1-12.

[6] D.L. Eaton, R.B. Daroff, L. Autrup and J. Bridges, Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment, *Crit Rev Toxicol.*, 38(2) (2008), 1-125.

[7] T.A. Albains and D.G. Hela, Multi-residue pesticide analysis in environmental water samples using solid-phase extraction discs and gas chromatography with flame thermionic and mass-selective detection, *J Chromatogr.*, 707(2) (1995), 283-292.

[8] K.D. Racke, K.P. Steele, Y.R.N. Dick and A.E. Wa, Factors effecting the hydrolytic degradation of chlorpyrifos in soil, *J Agric Food Chem.*, 44(1996), 1582-1592.

[9] B.K. Singh, A. Walker, J.A.W. Morgan and D.J. Wright, Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacterium, *Appl Environ Microbiol.*, 69(2003), 5198-5206.

[10] U.S. EPA, Office of Prevention, Pesticides and Toxic Substances, Review of chlorpyrifos poisoning data, *Memorandum*, 14 January (1997).

[11] J.L. Armstrong, R.A. Fenske, M.G. Yost, M. Tchong-French and J. Yu, Comparison of polyurethane foam and XAD-2 sampling matrices to measure airborne organophosphorus pesticides and their oxygen analogs in an agricultural community, *Chemosphere*, 92(4) (2013), 451-457.

[12] A.C. Young, S.H. Lee, Y.E. Park, T.Y. Lee, B.D. Hammock, K.C. Ahn and J.K. Lee, Development of an ELISA for the organophosphorus insecticide chlorpyrifos, *Bull Korean Chem Soc*., 23(3) (2002), 481-487.

[13] P.Y. Chen, B. Ning, N. Liu, Y. Feng, Z. Iu, X. Liu and Z.X. Gao, A rapid and sensitive fluroimmunoassay based on quantum dot for the detection of chlorpyrifos residue in drinking water, *J. of Environ. Science and Health*, 45(Part B) (2010), 508-515.

[14] A.K. Young, E.H. Lee, K.O. Kim and Y.T. Lee, Competitive immunechromato graphic assay for the detection of the organophosphorus pesticide chlorpyrifos, *Anal Chim Acta*., 693(2011), 106-113.

[15] J.W. Choi, Y.K. Kim, I.H. Lee and J. Min, Optical organophosphorus biosensor consisting of acetylcholinesterase/viologen hetero Langmuir-Blodgertt film, *Biosens Bioelectron*., 16(2001), 937-943.

[16] M.E. Leon-Gonzalez and A. Townshend, Flow-injection determination of paraoxon by inhibition of immobilized acetylcholinesterase, *Anal Chim Acta*., 236(1990), 267-272.

[17] G.L. Ellman, K.D. Courtney, V.J.R. Andres and R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem Pharmacol*., 7(1961), 88-95.

[18] OSHA, Method 62, In: Manual of methods, Organic methods evaluation branch, OSHA analytical laboratory, Occupational safety and health administration, Salt lake city, UT, (1986).

[19] J. Sherma and R. Slobodien, Determination of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol in tap water and bananas by quantitative TLC on pre adsorbent silica gel, *J Liq Chromatogr*., 7(14) (1984), 2735-2742.

[20] B.B. Gerhart and H.J. Cortes, Determination of chlorpyrifos in water by large-volume direct aqueous injection capillary gas chromatograph, *J Chromatog*., 503(2) (1990), 377-384.

[21] S. Lacorte, C. Molina and D. Barcelo, Screening of organophosphorus pesticides in environmental matrices by various gas chromatographic techniques, *Anal Chim Acta*., 281(1) (1990), 71-84.

[22] G.C. Mattern, J.B. Louis and J.D. Rosen, Multipesticide determination in surface water by gas chromatography/chemical ionization/mass spectrometry/ion trap detection, *J Assoc Anal Chem Int*., 74(6) (1991), 982-986.

[23] K. Helrich, Method 981.03, Liquid chromatography method, Chlorpyrifos in pesticide formulations, In: K. Helrich (Ed.), Official Methods of Analysis of the Association of Official Analytical Chemists (15th ed.), Arlington, VA: Association of Analytical Chemists, Inc., 1999.

[24] J.W. Choi, J. Mm and W.H. Lee, Signal analysis of fiber-optic biosensor for the detection of organophosphorus compounds in the contaminated water, *Korean J Chem Eng*., 14(2) (1997), 101-108.

[25] B. Kuswandi, H.J. Nuriman and W. Verboom, Optical sensing systems for microfluidic devices: A review, *Anal Chim Acta*, 601(2007), 141-155.

[26] S. Gaberlein, M. Knoll, F. Spener and C. Zaborosch, Disposable potentiometric enzyme senor for direct determination of organophosphorus insecticides, *Analyst.*, 125(2007), 2274-2279.

[27] A. Vakurov, C.E. Simpson, C.L. Dalya and T.D. Gibson, Acetylecholinesterase-based biosensor electrodes for organophosphate pesticide detection II: Immobilization and stabilization of acetylecholinesterase, *Biosen Bioelectron*, 20(2004), 2324-2329.

[28] G. Istamboulie, R. Durbiano, D. Fournier, J.L. Marty and T. Noguer, Biosensor-controlled degradation of chlorpyrifos and chlorfenvinfos using a phophotriesterase based detoxification column, *Chemosphere*, 78(2000), 1-6.

[29] L. Wang, D. Lu, J. Wang and D. Du, A novel immunochromatographic electrochemical biosensor for highly sensitive and selective detection of trichloropyridinol: A biomarker of exposure to chlorpyrifos, *Biosen Bioelectron*, 26(2011), 2835-2840.

[30] L. Zamfir, L. Rotariu and C. Bala, A novel, sensitive, reusable and low potential acetylcholinesterase biosensor for chlorpyrifos based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotubes gel, *Biosen Bioelectron*, 26(2011), 3692-3695.

[31] X. Sun, Y. Cao, Z. Gong and X. Wang, An amperometric immunosensor based on multi-walled carbon nanotubes-thionine-chitosan nanocomposite film for chlorpyrifos detection, *Sensor*, 12(2012), 17247-17261.

[32] W. Wei, X. Zong, X. Wang and L. Yin, A disposable amperometric immunosensor for chlorpyrifos-methyl based on immunogen/platinum doped silica sol-gel film modified screen-printed carbon electrode, *Food Chem*., 135(3) (2012), 888-892.

[33] C. Zhai, X. Sun, W. Zhao and Z. Gong, Acetylcholinesterase biosensor based on chitosan/prussian blue/multiwall carbon nanotubes/hollow gold nanospheres nanocomposite film by one-step electrode position, *Biosen Bioelectron*., 42(2013), 124-30.

[34] D. Yang, H. Lauridsen, K. Buels, L.H. Chi, J. La Du, D. Bruun, J.R. Olson, R.L. Tanguay and P. Lein, Chlorpyrifos-oxon disrupts zebrafish axonal growth and motor behavior, *Toxicol Sci*., 121(1) (2011), 146-59.

[35] B.K. Singh and A. Walker, Microbial degradation of organophosphorous compounds, *FEMS Microbiol Rev*., 30(3) (2006), 428-471.

[36] P.S. Chauhan, N. George, S. Sondhi, N. Puri and N. Gupta, An overview of purification strategies for microbial mannanases, *Int J Pharm Biosci*., 5(2014), 176-192.

[37] P.S. Chauhan, N. Puri, P. Sharma and N. Gupta, Mannanases: Microbial sources, production, properties and potential biotechnological applications, *Appl Microbiol Biotechnol*., 93(2012), 1817-1830.

[38] D.M. Munnecke and D.P.H. Hsieh, Development of microbial systems for the disposal of concentrated pesticide suspensions, *Med Facul Landbouw Rijksuniv Gent.*, 40(1975), 1237-1247.

[39] A. Guha, B. Kumari, T.C. Bora and M.K. Roy, Possible involvement of plasmids in degradation of malathion and chlorpyrifos by *Micrococcus* sp., *Folia Microbiol.*, 42(6) (1997), 574-576.

[40] B.K. Mallick, A. Banerji, N.A. Shakil and N.N. Sethunatahn, Bacterial degradation of chlorpyrifos in pure cultures and in soil, *Bull Environ Contam Toxicol.*, 62(1999), 48-54.

[41] X. Huang, L.S. Lee and C. Nakatsu, Impact of animal waste lagoon effluents on chlorpyrifos degradation in soils, *Environ Toxicol Chem.*, 19(12) (2000), 2864-2870.

[42] B.K. Singh, A. Walker, J.A.W. Morgan and D.J. Wright, Biodegradation of chlorpyrifos by enterobacter strain B-14 and its use in bioremediation of contaminated soils, *Appl Environ Microbiol.*, 70(80) (2004), 4855-4863.

[43] M. Ajaz, N. Jabeen, S. Akhtar and S.A. Rasool, Chlorpyrifos resistant bacteria from Pakistani soil: Isolation, identification, resistance profile and growth kinetics, *Pak J Bot.*, 37(2) (2005), 381-388.

[44] L. Yang, Y.H. Zhao, B.X. Zhang and C.H. Yang, Isolation and characterization of a chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol degrading bacterium, *FEMS Microbiol Lett.*, 251(2005), 67-73.

[45] C. Yang, N. Liu, X. Guo and C. Qiao, Cloning of mpd gene from a chlorpyrifos degrading bacterium and use of this strain in bioremediation of contaminated soil, *FEMS Microbiol Lett.*, 265(2006), 118-125.

[46] I. Ghanem, M. Orfi and M. Shamma, Biodegradation of chlorpyrofos by *Klebsiella* sp. isolated from an activated sludge sample of waste water treatment plant in Damascus, *Folia Microbiol. (Praha)*, 52(4) (2007), 423-427.

[47] X. Li, H. Jian and L. Shunpeng, Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2 and cloning of the mpdgene, *Res Microbiol*., 158(2007), 143-149.

[48] M.S. Rani, V.K. Lakshmi, D.P. Suvarnalatha, R.J. Madhuri, S. Aruna, K. Jyothi, G. Arasimha and K. Venkateswarlu, Isolation and characterization of a chlorpyrifos-degrading bacterium from agricultural soil and its growth response, *Afri J Microbiol Res*., 2(2008), 026-031.

[49] C.V. Lakshmi, M. Kumar and S. Khanna, Biotransformation of chlorpyrifos and bioremediation of contaminated soil, *Int Biodeterio Biodegrad.*, 62(2) (2008), 204-209.

[50] G. Xu, W. Zheng, Y. Li, S. Wang, J. Jhang and Y. Yan, Biodegradation of chlorpyrifos and 3,5,6- trichloro-2-pyridinot by a newly isolated *Paracoccus* sp. strain TRP, *Int Biodeterio Biodegrad*., 62(2008), 51-56.

[51] M. Ajaz, N. Jabeen, T.A. Ali and S.A. Rasool, Split role of plasmid genes in the degradation of chlorpyrifos by indigenously isolated *Pseudomonas putida* Mas-1, *Pak J Bot*., 41(2009), 2055-2060.

[52] S. Anwar, F. Liaquat, Q.M. Khan, Z.M. Khalid and S. Iqbal, Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1, *J Haz Mater*., 168(2009), 400-405.

[53] K. Cho, R.K. Math, S.M.A. Islam and W.J. Lim, Biodegradation of chlorpyrifos by lactic acid bacteria during kimchi fermentation, *J Agr Food Chem.*, 57(2009), 1882-1889.

[54] P.B. Singh, S. Sharma, H.S. Saini and B.S. Chadha, Biosurfactant production by *Pseudomonas* sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos, *Lett Appl Microbiol*., 49(3) (2009), 378-383.

[55] J. Zhu, Y. Zhao and J. Qi, Isolation and application of a chlorpyrifos-degrading *Bacillus licheniformis* ZHU, *Afri J Microbiol Res*., 4(22) (2010), 2410-2413.

[56] J. Li, J. Liu, W. Shen and X. Zhao, Isolation and characterization of 3,5,6-trichloro-2-pyridinol-degrading *Ralstonia* sp. strain T6, *Bioresour Technol.*, 101(19) (2010), 7479-7483.

[57] Z. Liu, X. Chen, Y. Shi and Z.C. Su, Bacterial degradation of chlorpyrifos by *Bacillus cereus*, *Adv Mate Res.*, 356-360(2012), 676-680.

[58] M.K. Harishankar, C. Sasikala and M. Ramya, Efficiency of the intestinal bacteria in the degradation of the toxic pesticide, chlorpyrifos, *Biotech*., 3(2) (2013), 137-142.

[59] P. Lu, Q. Li, H. Liu, Z. Feng, X. Yan, Q. Hong and S. Li, Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by *Cupriavidus* sp. DT-1, *Bioresour Technol.*, 127(2013), 337-42.

[60] S. Lal and R. Lal, Bioaccumulation, metabolism and effects of DDT, fenitrothion and chlorpyrifoson *Saccharomyces cerevisae*, *Arch Environ Contam Toxicol*., 16(6) (1987), 753-757.

[61] J.A. Bumpus, S.N. Kakkar and R.D. Coleman, Fungal degradation of organophosphorus insecticides, *Appl Biochem Biotechnol.*, 39/40(1993), 715-726.

[62] I. Mukherjee and M. Gopal, Degradation of chlorpyrifos by two soil fungi *Aspergillus niger* and *Trichoderma viride*, *Toxicol Environ Chem*., 57(1-4) (1996), 145-151.

[63] A.S. Nair and T. Pradeep, Extraction of chlorpyrifos and malathion from water by northwest coalition for alternatives to pesticides NCAP, *2000 Insecticide Factsheets*, (2007).

[64] F. Hua, Y.Q. Xiang, Y.J. Hao, X.Q. Chu, X.D. Pan, J.Q. Yu and Y.L. Yu, Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi, *Int Biodeteri Biodegrad*., 61(4) (2008), 294-303.

[65] G. Kulshrestha and A. Kumari, Fungal degradation of chlorpyrifos by *Acremonium* sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil, *Biol Fertil Soils*, 47(2011), 219-225.

[66] S. Chen, C. Liu, C. Peng, H. Liu and M. Hu, Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* Hu-01, *PLoS ONE*, 7(10) (2012), e 47205.

[67] S. Silambarasan and J. Abraham, Efficacy of *Ganoderma* sp. JAS4 in bioremediation of chlorpyrifos and its hydrolyzing metabolite TCP from agricultural soil, *J Basic Microbiol*., 54 (1) (2013) 44-55.

[68] S.A. Ivashina, Interaction of Dursban with soil micro-organisms, *Agrokhimia*, 8(1986), 75-76.

[69] N. Shaker, S. Abo-Donia, Y.A. El-Shaheed and Ismail, Effect of lactic acid bacteria and heat treatments on pesticides contaminated milk, *Egypt J Diary Sci*., 16(1988), 309-317.

[70] X. Gangming, L. Yingying, Z. Wei and P. Xiang, Mineralization of chlorpyrifos by co-culture of *Serratia* and *Trichosporon* spp., *Biotechnol Lett.*, 29(2007), 1469-1473.

[71] Z. Chishti and M. Arshad, Growth linked biodegradation of chlorpyrifos by *Agrobacterium* and *Enterobacter* spp., *Int J Agric Biol.*, 15(2013), 19-26.