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Microbial Remediation of Organophosphates: A Step Forward Against Pesticidal Ecotoxicity

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ABSTRACT

Pesticides are the chemical compounds that act on pests and harmful pathogens in the agricultural field thereby enhancing the yield. Herbicides, pesticides, and insecticides mainly constitute to a diverse range of chemicals called organophosphates. There has been extensive use of organophosphates in the major fields namely agriculture, horticulture, pest control, plastic making, and other household applications. The level of toxicity in humans, plants, and animals varies by the exposure of organophosphates in both acute and chronic means and these inhibit the activity of acetylcholinesterase in insects, terrestrial and aquatic organisms leading to sever hepatic, nervous, reproductive, renal, and respiratory abnormalities. Due to the accumulation of organophosphates in the ecosystem, there is much disturbance in the essential factors for the growth of plants by inhibiting various enzymes, permeability, and diffusion. It is found to be toxic to other non-target organisms like fish leading to impairment of metabolism or even death. Pesticide residues remain in agricultural products and are transferred to the terrestrial and aquatic food chain causing major imbalance in the ecological cycles. The present work completely focuses on the various factors and possible mechanisms developed by microbes either by enzymatic or nano-based processes for the degradation of organophosphate pesticides, thereby, inhibiting the chances of biomagnification.

Keywords: *Pesticides; Organophosphates; Agriculture; Acetylcholinesterase; Biomagnification*

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INTRODUCTION

According to a statistical survey conducted during the span of 2003-2017, the utilization of pesticides has shown a spontaneous increase in Maharashtra and Uttar Pradesh, while a steep decrease was observed in the states of Bihar, Gujarat, Karnataka, and West Bengal. In India, crops subjected to the highest employment of pesticides mainly include cotton [66.70%] and paddy [48.62%] while the ones with the lowest employment of pesticides include maize [25.01%] and other species referring to the similar family. In Indian Agriculture, 76% of pesticides are used as insecticides while globally its reaches a staggering hike of 44%. In Asia, India is the second-largest for the production of pesticides and twelfth when compared globally. There are almost 2344 pesticides registered in India that are currently in use [1].

Bio-remediation of pesticides through the application of microbes is completely and usually referred to as the mineralization of its chemical derivatives. The effectiveness of microbial remediation depends upon the solubility, mobility, degradability, and bioavailability of the specific microbes in the areas of pesticidal exploitation [2]. In comparison to other modes of remediation processes, microbial remediation is an economically stabilized process which helps in eliminating the potent pesticides from the environment, as the applied microbes can be easily cultivated and efficiently maintained and entirely comprises of different mechanisms that are majorly enzyme-based. It was also noted that the degradation process can be enhanced by varying the conditions via isolating a potent strain in the laboratory, recombinant DNA technology [RDT] can prove to be a helpful strategy towards enhancing the properties of microbial bioremediation at genetic levels. One such example is *Bacillus subtilis* [WB800] which is a recombinant bacterium that disintegrates chlorothalonil production by chlorothalonil-hydrolytic dehalogenase enzyme operated by the CHD gene leading to the production of a water-soluble metabolite i.e. 4-hydroxytrichloroisophthalonitrile [3].

Globally organophosphates is the most pre-dominantly utilized pesticides comprising of a phosphate group as a structural framework where the terminal oxygen molecules are attached to a phosphate group by a double bond [3] [4]. The excessive use of pesticides and other chemical fertilizers in India began during the period of the green revolution, it was to enhance the production of crops as to fulfil the demands of the increasing population. In certain cases, organophosphates might lack target specificity which leads to long-lasting severe toxicological effects on both terrestrial and aquatic ecosystems. Although it had satisfactory results in terms of yield and growth of the plant, it also provided resistance towards pests invading them [5], primarily the weeds, insects, and fungal diseases, but in turn these pesticidal usage came with disastrous consequences as it accumulates in the ecosystem, not only affecting the terrestrial micro and macro fauna but also exerts adverse effects to the aquatic ecosystems. Organophosphates is widely used for pest control, horticulture, agriculture, industrial, vector control, and other diverse domestic purposes [6] [7] [8], although the results were fruitful, yet the chronic toxicity that persists in the environment has embellished very hazardous effects to the terrestrial and nautical ecosystems, in turn having direct and indirect negative aspects seen in humans. While studying the effects of the pesticides on the terrestrial biome, the most common effects seen are loss of soil fertility, soil acidification, nitrate leaching and increased weed species resistance [9]. A careful review, reveals that the organophosphates are found to be the main basis behind the contamination of vegetables, fruits, milk and other beneficial food products [10], and similar effects are seen in terrestrial and nautical bio-network when contaminated with organochlorines [a class of insecticide]. The level of toxicity in human, plants, and animals vary by the exposure of organophosphates in both acute and chronic cases and these inhibit the activity of acetylcholinesterase in insects, terrestrial and aquatic organisms leading to severe hepatic, nervous, reproductive and respiratory abnormalities [11] [12].

ORGANOPHOSPHATES: TYPES & EXPLOITATION

Organophosphates [OP] is a critical problem in many agricultural factors, especially in rural regions of the developing world, which kills almost 200,000 people every year. Among the health effects caused due to pesticidal exposure, neurological dysfunction is the one widely documented. A wide range of peripheral and central neurological symptoms have been reported due to the acute exposure of organophosphates [13]. More neurological symptoms have been recorded by many farmworkers, pesticide factory workers, and greenhouse workers.

Types of organophosphates include- parathion, chlorpyrifos, phosmet, azamethiphos, malathion, diazinon, fenitrothion, azinphos-methyl, dichlorvos, tetrachlorvinphos, methyl parathion, and terburfos. Most of them are highly toxic organophosphates that has been widely used in the agricultural sector, due to this, most of the contaminants is accumulated in soil and rivers which ultimately lead to the toxication of non-target organisms. The direct exposure to organophosphates could be through inhalation, direct contact, or even ingestion. Approximately, 3million people worldwide are exposed to organophosphates and around 300,000 mortalities are observed annually. Chronic exposure could lead to adverse effects such as lack of coordination, speech loss, impairment of memory and can also cause nausea, vomiting, and weakness. Based on the type of organophosphates, there can be a possibility of development of cancer. Concerning the reports presented by the International Agency for Research on Cancer, parathion, diazinon, malathion, and tetrachlorvinphos are classified as possible carcinogens.

ECOTOXICITY OF ORGANOPHOSPHATES ON NON-TARGET ORGANISMS

Most organophosphorus pesticides are liquids and have different vapor pressures at room temperature. All organophosphorus substances are subjected to degradation by hydrolysis. Organophosphates are frequently used due to their impact on wide range of pests. The various approaches like nano-filtration and adsorption are common physical methods used for the remediation of organophosphates, but these methods are not sufficiently effective to remove organophosphate pesticide [14]. Common members of the organophosphate family are Methyl parathion, malathion, dimethoate, phosphamidon, phorate, fenitrothion, and monocrotophos. Methyl parathion is one that is structurally very similar to ethyl parathion. Degradation of methyl parathion by ozonation in aqueous solution was studied under constant ozone dosage and variable pH conditions. In the process of degradation of methyl parathion, it was observed that ozonation is more effective at the alkaline reaction of the medium than other conditions. The degree of methyl parathion conversion was achieved a maximum of 98% at pH 9 in comparison to pH 7 and 3 respectively [15]. The microbial community present in the ecosystem has the potential towards remediating the harmful toxic chemicals in form of pesticides persisting in the environment. These pesticides persist in the environment due to partial degradation by the physicochemical factors.

Ecotoxicity of Organophosphates on Plants:

Organophosphate pesticides have clearly shown a wide range of effects on photosynthesis [16], plant mineral nutrition [17], carbon metabolism, chlorophyll synthesis, and oxidative stress [18]. Organophosphates inhibits the biosynthesis of catalase, peroxidase and **δ**-aminolevulinic acids [ALA] which are the main component of chlorophyll biosynthesis pathway by causing iron deficiencies in plants [19], it affects ALA production by competing with a major producer of ALA. Recent studies on soya bean, show that the organophosphate active site leads to deprivation of the glutamate content by competing with glycine during photorespiration [20].

They also reduce the availability of amino acids and metal ions associated with PS-I and PS-II to transfer photons [light energy] in the electron transport chain system. Foliar spray of glyphosate and its metabolites decreases the net stomatal conductance and carbon exchange in plants thus reducing the CO2 assimilation capacity [17] [21]. Exposure to an organophosphate pesticide, glyphosate, also lowers the levels of 3-phosphoglyceric acid [PGA] and ribulose-1,5-bisphosphate [RuBP] which affects the activity of ribulose 1,5-bisphosphate carboxylase oxygenase [RuBisCO] in plants. The reactive oxygen species are also part of the normal metabolism of the cell, but their deleterious effect materializes, when the balance between the ROS [Reactive Oxygen Species] produced and the ROS eliminated is disturbed. Upon damage to this balance, the accumulation of ROS can affect cell growth and survival [22].

The organophosphates have also been found to affect non-target plants that causes inhibition of chlorophyll synthesis as well as protein and carbohydrate synthesis. The profuse use of organophosphates in agriculture and other areas has caused severe damage to the environment. Dichlorvos have been detected in high amounts in the freshwater bodies of China [23] and Korea[24]. It has also been reported that In India, organophosphates [glyphosate] reduces the soluble sugar content in *Pisum sativum*along with alterations in potassium and sodium concentrations present in tissues [25].The most commonly used organophosphates that have harmful effects on non-target photosynthetic organisms include glyphosate, phorate, chlorpyrifos, dimethoate, imidacloprid, chlorsulfuron, meta-sulfomethyl, dichlorvos, trichlorfon, etc.

Ecotoxicity of Organophosphates on Terrestrial and Aquatic Life:

Excessive use of organophosphate pesticides affects non-target crops and non-target animal species found in various aquatic and terrestrial ecosystems [26]. United States Environmental Protection Agency [USEPA, 2013] classifies most organophosphate pesticides in the toxicity class I to IV for inhalation and oral exposure. It is responsible for causing irritation, cancer, vomiting, nausea and dermatitis in humans, mild to moderate toxicity was observed in amphibians and fish. Organophosphate pesticides are used extensively throughout the world and these harmful chemicals, especially in developing countries, are a real general health problem. The component of lethality is the containment of acetylcholinesterase, which determines an aggregation of the neurotransmitter acetylcholine and the stimulation of acetylcholine receptors [27]. Patients who receive prompt treatment usually recover from acute toxicity but may suffer from neurological sequelae. The standardized and widely accepted plant bioassay is lemna bioassay for determination aquatic ecotoxicity.

Since, some of the organophosphate pesticides are highly persistent in the environment, inducing sublethal or lethal effects in both aquatic and terrestrial organisms. Therefore, it is of concern to monitor human organophosphate pesticides and food residues to verify population exposure size.

MODE OF ACTION OF ORGANOPHOSPHATES

Organophosphates being a widely manoeuvred class of synthetically synthesised acute toxic compounds that are released into the environment in the form of pesticides which are predominantly constituted of thiophosphoric acid and esters of phosphoric acid [28]. These compounds have a simple structure and are comparatively less toxic than organochlorinated pesticides.Studies have shown that the mode of action of these organophosphate pesticides are selectively toxic and have diverse effects depending on the species they affect. These pesticides affect insects irrespective of it being a target or non-target species more than mammals when recorded on a wider scale. The reason behind these organophosphates being highly hazardous to insects than mammals is because they are more biodegradable compared to other pesticides released to the environment, the toxicity mostly occurs due to intense exposition rather than accumulation. Studies on the mode of action of organophosphates forms to be a key element for the development of better bio-safe pesticides in modern days [29].

Most of these organophosphates pesticides undergo various types of mechanisms based on their use and structural configurations. Despite these various inducing factors and several mechanisms of toxifications, the primary method of toxification that occurs in non-target organisms is the inhibition of acetylcholinesterase [AChE] enzyme that disrupts the activity of the central and peripheral nervous system due to the obstructed propagation of nerve signalling caused by the accumulation of acetylcholine in the nerve endings on the neurons [30]. The catalytic activity of acetylcholinesterase is interrupted by the organophosphates producing a phosphinyl abduct due to the spontaneous process of phosphorylation making it temporarily inactive, this inactive acetylcholinesterase enzyme can be readily reactivated by certain nucleophilic reactivators primarily oximes, nonetheless, the organophosphates that are attached by covalent bonds undergo the process of dealkylation making the AChE enzyme permanently resistant to the reactivation (Fig.1) [31].

Despite this mechanism showing negative effects on the nervous system, it is also found that organophosphates show inhibition in the activity of certain sex hormones like testosterone, converting it to estradiol or sometimes even hydroxyl group substituted testosterone, this mechanism of organophosphates is enhanced with the presence of other pesticides prominently triazine herbicides which cause major molecular toxicities [32] [33](Fig.2).

BIOREMEDIATION OF ORGANOPHOSPHATES USING MICROBES:

In the modern days, organophosphates take its place as the largest family of pesticides used for pest control basically for better crop yield in the agricultural fields, but, due to its adverse effects, toxic nature and high persistence certain compounds under the class of organophosphates especially malathion, parathion and methyl parathion have been listed and register as extremely hazardous by the World Health Organisation [WHO], as they pose negative impacts on the nervous, reproduction, endocrine, excretion, respiratory and cardiovascular system, thereby creating a wide space for research interest and development of effective bioremediation techniques [34].

The degradation of these pesticides in the environment is primarily determined by the biotic and abiotic factors, out of which the action of indigenous microorganisms especially bacteria and fungus such as *Aspergillus, Pseudomonas, Arthrobacter*and other soil bacteria play a frontline role in the remediation of organophosphates [35] (Table 1). Organophosphates are exploited as sources for carbon, nitrogen and phosphorus by agricultural soil microorganisms and converting it into safer organic compounds [36] (Fig. 3). The toxicity of organophosphates is spontaneously reduced by microorganisms as these pesticides belonging majorly to the organic class of ethers are highly vulnerable to the process of enzyme hydrolysis since these compounds have the presence of bonds such as P=O and P=S. The most significant step involved in the process of detoxification is the active hydrolysis of the P-O-alkyl and P-O-aryl bonds [37]. Organophosphates hydrolase is one significant enzyme that is released by the microbes which bind to the central metal atom cleaving the P=O and P=S bonds [38]. Studies on reaction kinetics have proven to show that the process of microbial remediation is comparatively faster than the chemical degradation and significantly 10 times faster than photolysis [36]. Microbial bioremediation is carried out worldwide in modern days most prominently on the most widely used organophosphates namely Malathion [39], Parathion and Chlorpyrifos [38].

Mechanism of microbial bioremediation occurs within two major pathways that are enzymatic degradation and nanoparticle-based degradation [40].

Enzymatic degradation (Enzyme hydrolysis):

Enzyme hydrolysis being highly capable in hydrolysing a variant class of pesticides has gained immense interest worldwide. Enzymes such as esterase, phosphotriesterase, paraoxonase, parathion hydrolase and diisopropylfluorophosphataseshow excellent actiontowards the degradation of organophosphates, specifically the enzymes that show hydrolases activity are the primary enzymes behind the degradation activity [41]. For example, the bacterial strain *Pseudomonas diminuta* is known to produce an enzyme called as parathion hydrolase which hydrolysis the organophosphate parathion which decreases the toxicity of the molecule to 100 folds lesser [40].

Among this diverse range of enzymes, organophosphorus hydrolases [OPH] is one of the enzymes that alone acts against a wide range of organophosphates irrespective of its molecular characteristics, but the OPH molecule suffers highly restrictive stability or poor stability during non-ideal physical conditions and this enzyme cannot be recovered or reused [42].

Nanoparticle based degradation:

Organophosphates have a great tendency of prevailing in the environment for a long period of timethat causes direct or indirect negative effects on the ecosystems [43]. Due to this high resistance power of organophosphates and loss of stability of hydrolases enzymes the introduction to nano-particle based degradation showed a very fruitful effect on the bioremediation of organophosphates. The basic mechanism of nano-particle degradation comprises of the encapsulation of the microbial degradation enzyme over the synthesized metal nanoparticles. For example, the Atrazine-dechlorinating enzyme [AtzA] produced by the cells of *Escherichia coli* are encapsulated on the silica nanoparticles which showed enhanced degradation effect and a very high level of stability [44]. This system was found to work with excellent stability under the temperatures ranging for 23°C to 45°C and also under the wide p^H changes of the soil.

Among the variant class of nanoparticles, silica nanoparticles are the most prominentlyused in the nano-particle degradation process. Some of the examples include OPH enzyme derived from *Lavibacterium* species show positive effectiveness towards the pesticides such as malathion and paraoxon when encapsulated in the mesoporous silica-gel nanoparticles [45]. Although most of the enzymes are extracted from bacterial strains, certain fungal species namely *Coriolopis polyzona* produce enzymes called as

Laccases which are a class of copper-containing hydrolases enzyme that show excellent action in the degradation of Bisphenol responsible for severe hormonal disturbances when encapsulated in silica-gel nano-particles [46]. Laccase produced by another fungal species called as *Trametes versicolor* is used for the degradation of 2,4-dinitrophenol and its actions are found to be optimal even in high temperatures of around 40°C and high p^H fluctuations [47].

Figure 1: Mechanism of Acetylcholinesterase inhibition by organophosphate pesticides

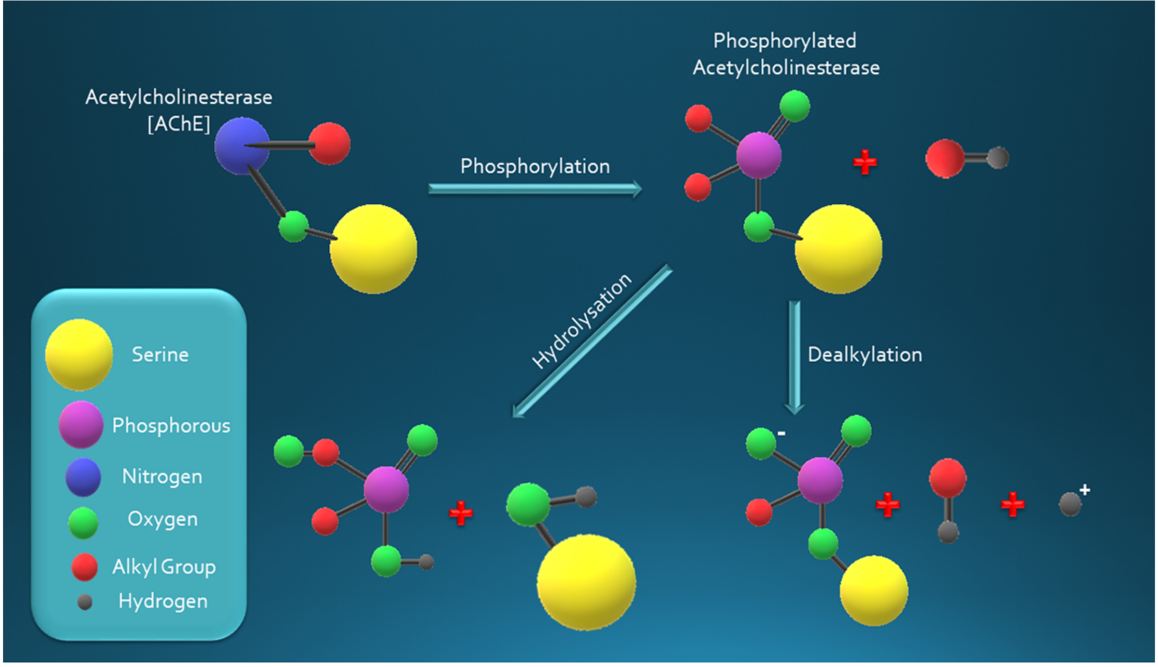

Figure 2: Effect of Organophosphates incorporated with Triazines on Testosterone hormone

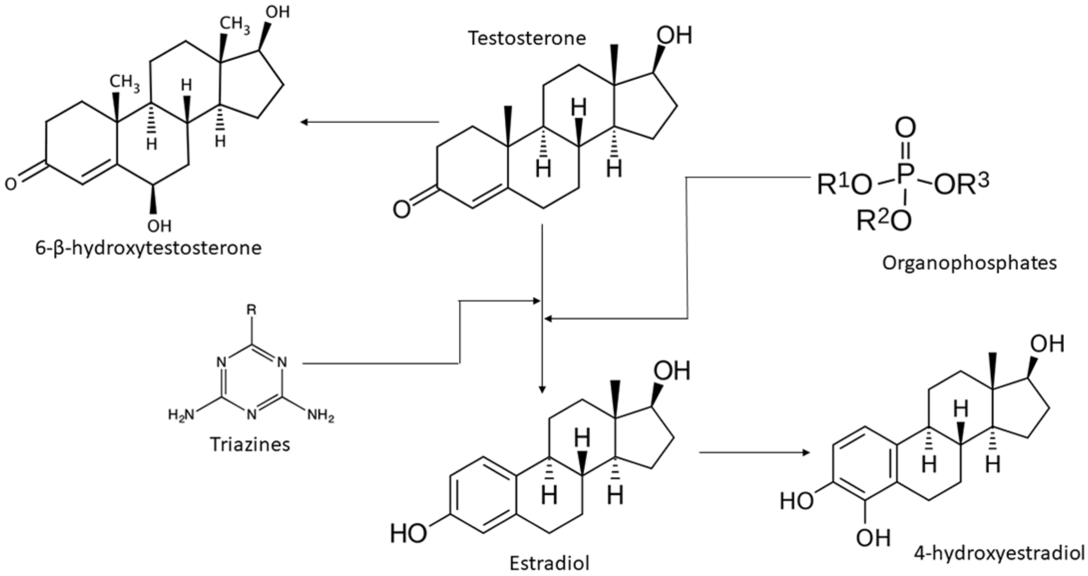

Figure 3: Degradation Mechanism of Organophosphates for various biotic and abiotic factors

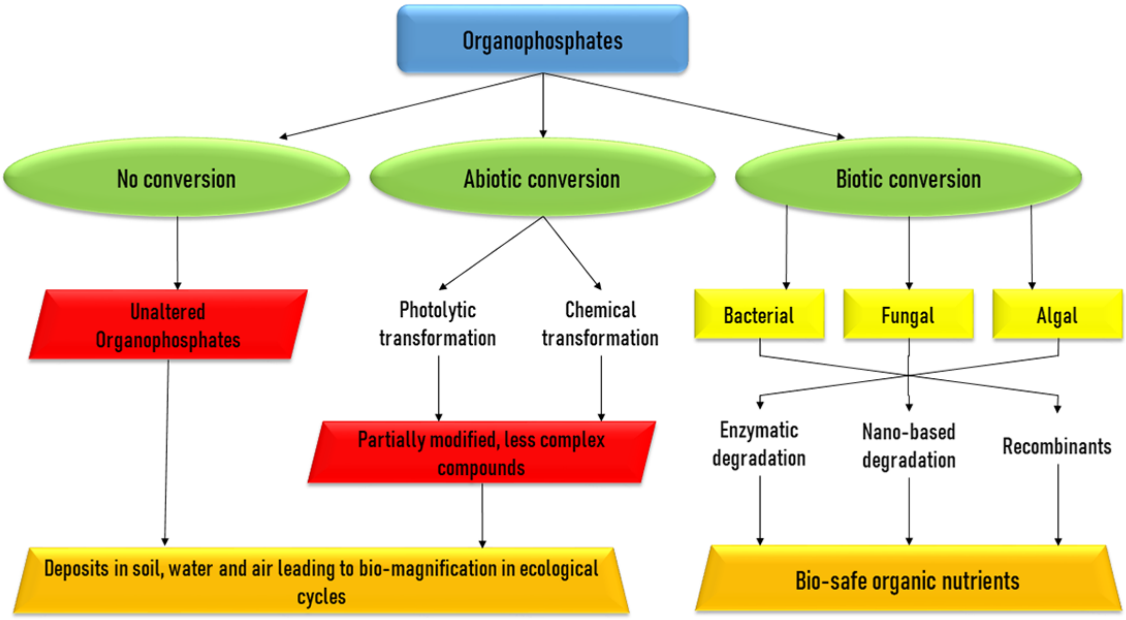

Table 1: List of bacterial bioremediators working against the degradation of particular organophosphate pesticides.

Organophosphates Pesticides	Bacterial Bioremediator	References
Parathion	*Stenotrophomonas* spp.	[56]
	Serratia marcescens	[57]
Malathion	*Bacillusspp.*	[58]
	Enterobacter aerogenes	[59]
Monocrotophos	*Arthrobacter atrocyaneus*	[36]
Chlorpyrifos	*Streptomyces olivochromogenes*	[60]
	Sphingobacteriumspp.	[61]
	Pseudomonas pseudoalcaligenes	[62]
Coumaphos	*Serratia marcescens*	[63]
Diazinon	*Lactobacillus brevis*	[64]
Dimethoate	*Paracoccus* spp.	[65]
Ethoprophos	*Flavobacterium* spp.	[36]
Fenamiphos	*Acinetobacter rhizosphaerae*	[66]
	Microbacterium esteraromaticum	[36]
Acephate	*Exiguobacterium* spp.	[67]
	Pseudomonas aerugtnosa	[68]
Phorate	*Ralstonia eutropha*	[69]
	Enterobacter cloacae	
Tetrachlorvinphos	*Vibrio metschnikovi*	[70]
Cadusafos	*Pseudomonas putida*	[71]
Fenitrothion	*Burkholderia* spp.	[36]
Profenofos	*Bacillus subtilis*	[72]
Quinalphos	*Ochrobactrum* spp.	[73]
Triazophos	*Stenotrophomonas* spp.	[74]

FATE OF ORGANOPHOSPHATES

Increase in toxicity due to organophosphates pose a major threat to the environment and a primary cause for soil pollution. Due to these prospects in toxicity studies bioremediation process due to microbes in undoubtedly the best and safest approach towards the degradation of organophosphates. Despite these microbes working over the degradation of organophosphates, studies on future prospects of introducing genetically modified microbes and recombinants into the agriculture fields for optimizing higher rates of microbial degradation [48]. Certain other techniques that have been worked into for enhancing the bioremediation methods include directed evolution [49]and molecular engineering of metabolic pathways [50] [51].

Advancements and increased studies in environmental microbiology and geochemistry play a vital role in the peaking up the progression of applied microbial bioremediation. Future improvement in management campaignsand knowledge awareness regarding biohazards caused by organophosphates can cause a spontaneous decrease in the use of organophosphate pesticides [52].Bioaugmentation is also one the best techniques that can be applied for higher rates of bioremediation, it involves the introduction of cultured microbes into the soil for pesticidal degradation such that the microbial count reaches to several billions in per gram of soil [53]. Recent studies have proven that introducing nutrients such as vitamins and sources of carbon and oxygen cause exhaustion in the microbes resulting in the increase of enzymatic activities of microbes in turn playing a vital role for the microbial bioremediation of organophosphates, this process is termed as bio-stimulation. Compounds known for enhancing the microbial remediation and stimulating the microbes for enzymatic hydrolysis of organophosphates include $NaNO_3$, KNO_3, $MgNH_4PO_4$ and NH_3NO_3 that show excellent properties as bio-stimulants [54].

In recent years, nano-biotechnology has been used widely in enhancing the properties of microbes for well-versed enhancements in the bioremediation processes. One of the crucial steps involved in the fusion of nano-biotechnology and microbial remediation includes the understanding of the relationship between the microbes, type of organophosphates and the nano-materials, this is necessary because certain nano-materials prove to be as bio-stimulants for the microbes while some are toxic. The main perspective behind this analysis include the introduction of principles behind the nanomaterials assisted microbial remediation and their interaction with the ecological matrices [55].

CONCLUSION

In the rapidly increasing global population and spontaneous modern urbanization, there is also a simultaneous peaking in the production of pest-free agricultural crops to fulfil the human edible needs. Organophosphates being the most lethal and the highly opted pesticide that has been exploited worldwide, has now become a concern due to its hazardous toxic effects caused by the accumulation of the chemical substances in the agricultural soil, effectuating direct damage to crops and beneficial agricultural organisms, in-turn initiating toxic damage towards humans and other mammals by the consumption of such treated crops. In such scenarios, the need for remediation of these organophosphates has become the most crucial parts of modern agriculture, gaining immense interest among researchers for the development of easy, beneficial and safe biological ways for the degradation of these pesticides. Microbial remediation has been found to be the most effective way towards the degradation of these pesticides, for which, recent developments such as nano-based technologies, enzymatic activation and genetically engineered microbes prove to enhance the effectiveness of the microbial degradation.

Although, these techniques have been brought into practice in many parts of the world, much more studies and investigations regarding the microbial degradation of these organophosphates have to be attained to achieve an expected peak in future for a complete bio-safe degradation and non- hazardous consumption of organic non-pesticidal variants of crops.

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CONFLICT OF INTEREST

We the authors of this paper declare no potential conflict of interest.

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