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Toxicity, Biodegradation and Bioremediation of Triazophos (TAP): A Mini-Review.

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ABSTRACT

Triazophos (TAP) is a well-known Organophosphate pesticide (OP) widely used in agriculture because of its moderate toxicity and low persistence in the environment. While it is degrading in the environment, it also leaves specific intermediate and toxic residues. These intermediates are highly toxic to plants as well as animals. Hence, various physical and chemical techniques such as incineration and landfilling excavation are being used to detoxify these toxic residues. Due to the tedious procedures and high costs, these methods are not used effectively. However, Microbial degradation is considered to be a significant factor determining the fate of Triazophos for sustainable agriculture. In the present review, we focus on the efficacy and usefulness of integrating microorganisms for the detoxification of TAP in soil.

Keywords: Bioremediation; Microbial degradation; Sustainable Agriculture; Triazophos; Xenobiotics

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INTRODUCTION

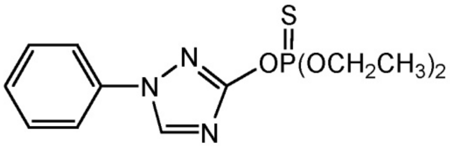
In current agricultural practices, the application of pesticides plays an inevitable role both in increasing agricultural production and in ensuring the supply of quality food. Among various pesticides that are being used worldwide, organophosphates (OPs) are a widely used group after the cessation of the use of organochlorine pesticides in 1970 [1]. Organophosphate compounds are the main components of herbicides, pesticides, and insecticides, and nerve gas as well [2]. The worldwide consumption of OPs had been risen from 5 to 6.8 million from 2011 to 2015 [3]. OPs are widely used all over the world because they are an effective alternative to persistent organochlorine pesticides and possess the ability to rapidly degrade under natural conditions such as sunlight, air, and soil [4]. Although OPs have an essential role in agriculture to solve the problem of feeding the world's ever-growing population but their recalcitrant nature to biodegradation makes them less efficient [5].
Among Organophosphates, Triazophos (TAP) is a broad spectrum and non-systemic pesticide in nature. TAP has been extensively sprayed to control pests on various crops like cotton, tea, maize, paddy, and vegetables [6]. TAP is accumulating in the environment because of its excessive and uncontrolled use. The high levels of triazophos residues have been found in a wide variety of foods according to several US and European food monitoring schemes causing threats to human health through food chains [7]. So, this review focuses on the degradation of TAP in the environment and the role of microorganisms in this process.

TRIAZOPHOS: AN ORGANOPHOSPHORUS PESTICIDE

Triazophos [0, 0-diethyl 0-1-phenyl-1H-1, 2, 4-triazol-3-yl phosphorothioate] has been put into agricultural use since the late 1970s on various crops such as cotton, maize, paddy and vegetables to control aphids, fruit borer, leafhopper, cutworms and many others (6). In China, it is one of the most extensively used insecticides for the control of lepidopteran pests in paddy rice fields [8,9]. Triazophos is a dark brown liquid at room temperature with a typical odor of phosphoric ester and possesses various chemical, physical and toxicological properties (Table No. 1). It has a freezing point ranges from 2-50C, and the boiling point cannot be determined due to its decomposition at temperatures above 1400C. It is slightly soluble in water (39mg/l) at 200C but is readily soluble in most organic solvents like toluene, methanol, 2-propanol, and ethyl acetate. It has acute oral LD50 value ranged between 171.3 to 224 mg/kg for rat and is non sensitizing to guinea pig. The phytoremediation of triazophos by Canna indica Linn. in a hydroponic system showed that the removal kinetic constant (K) of triazophos was 0.0229-0.0339 day-1 and the removal percentage of triazophos was 41-55% in the plant system [10]. The mean half-life of TAP in wheat plants (grain, stems, and leaves) is 5.22 days with a dissipation rate of 90% over 14 days, and the half-life in soil is 7.93 days with a dissipation rate of 90% over 21 days [11].

TAP is generally regarded as safe for use on crops due to their relatively fast degradation rate, which varies as a function of microbial components, pH, temperature, hydrolysis, photolysis, and other factors and is active by contact, ingestion and vapor action but not systemically active. However, in the past several years, TAP has been used excessively, which has brought severe pollution in surface water, soil, food, and biota [12,13]. According to the International Union of Pure and Applied Chemistry (IUPAC), triazophos may harm the metabolism of non-target organisms as an acetyl cholinesterase inhibitor and neurotoxicant. The high levels of TAP residues in the environment, causing threats to human health through food chains [7]. The genotoxic effects of triazophos on male germ cells of Drosophila melanogaster showed that triazophos induced point mutations when assayed in the sex-linked recessive lethal test and induced a weak increase in the non-disjunction frequency [14]. It is highly toxic to most fishes, but less toxic to shellfishes. TAP has been utilized as a fungicide in intertidal aquaculture to protect farming shellfish such as Sinonovacula constricta and Tegillarca granosa from diseases, but due to the misuse of TAP, its pollution took place sporadically. This caused deaths of several other fishes. Hence, the evaluation of environmental safety for triazophos is thus of great concern [6].

Table 1. Chemical, physical and toxicological properties of Triazophos

S. No.	Name	Triazophos
1	Chemical structure	
2	CAS No.	24017-47-8
3	IUPAC name	O,O-diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate
4	Toxicity	Acute toxicity; LD50 Oral - rat - 57 mg/kg; LC50 Inhalation - rat - 4 hr - 280 mg/m3; LD50 Dermal - rat - 1.100 mg/kg;
5	Log Kow	3.34
6	Log Koc	4.50

MODE OF ACTION OF TRIAZOPHOS

TAP targets the central nervous system of insects, arachnids, and invertebrates, which have an acetylcholinesterase-based nerve response mechanism. In mammals, this mechanism involves the irreversible binding of organophosphate to receptor sites in acetylcholinesterase and inhibits the hydrolysis of acetylcholine. Acetylcholine chemically transmits sodium and potassium ion charge across the synapse that initiates nerve impulse. If acetylcholinesterase is neutralized, then acetylcholine maintains a charge potential on the postsynaptic side of the neural transmitting membrane forcing permanent nerve stimulation. In human beings, organophosphate poisoning causes general weakness, headache, excessive sweating, salivation, nausea, vomiting, diarrhea, abdominal cramps, and tremors. In very severe cases, respiratory failure may lead to death [1].

FATE OF ORGANOPHOSPHATE PESTICIDES IN THE ENVIRONMENT

Davisson et al in 2005 [15] studied that vapor pressure and hydrolysis rates can control the persistence of organophosphates released at large quantities into the environment. The environmental fate of OP compounds is primarily measured by persistence as a parent molecule. Although hydrolysis products are measured in some cases where they pose a toxicological hazard, for the most part, the rate at which a parent compound disappears is the typical measurement approach. However, as observed in many pesticide studies, OP compounds can be retarded in soil by adsorption or transported in water as a parent molecule. Chemical transport and fate are primarily governed by the fundamental physical properties of a particular compound. Its transition among the different physical states (i.e., gas, liquid, or solid) will depend on surrounding environmental conditions.

Triazophos could be easily up taken and degraded by plants [16]. This characteristic may be valuable when using phytoremediation to eliminate triazophos pollution from the environment. Phytoremediation technology, which utilizes plants to uptake, transform, stabilize, evaporate, and degrade pollutants [17], has proved to be a useful method to transport and transform pollutants from the environment [18,19].

Liang et al in 2011 [20] studied the adsorption and degradation of triazophos and its primary hydrolytic metabolite 1-phenyl-3-hydroxy-1, 2, 4-triazole (BZC) in paddy soil from Chaohu Lake, China. Adsorption tests demonstrated that the Freundlich equation could describe the adsorption of these compounds to soils. Triazophos displayed a higher affinity for adsorption than its primary metabolite BZC. Degradation of these compounds in non-sterile soil followed first-order exponential decay kinetics, and the half-life (t1/2) of these contaminants ranged from 8.40 to 44.34 d. Compared to the parent compound, BZC showed higher potential for leaching into groundwater.

The photocatalytic degradation of TAP in aqueous TiO2 suspension has been studied in a photoreactor operating with simulated solar radiation. The decrease in TAP concentration followed first-order kinetics with a half-life of 4.76±0.42 h at a TiO2 suspension concentration of 10 mg/L. Seventeen degradation products were identified using various techniques such as HPLC-UV, HPLC/MS/MS, GC/MS/MS, and IC, and by comparing retention times and spectra with commercially available authentic standards. Based on the observed transformation products, two routes were proposed, one based on the initial oxidative cleavage of P S bond to P O bond, and the other on initial cleavage of the ester P–O bonds. Photocatalysis holds promise for the solar treatment of pesticide-contaminated water [21].

Triazophos in canal water (pH 8.3) degraded with a half-life of approximately 25.44 days [22]. Triazophos degraded more rapidly in a photo-Fenton system to a series of degradation products and five major products, for example, o,o-diethyl phosphorothioic acid, monoethyl phophorothioic acid, phosphorothioic acid, 1-phenyl-3-hydroxy-1,2,4-triazole and phenyl semicarbazide were identified as their corresponding trimethylsilyl derivatives with gas chromatography and mass spectrometry [23].

EFFECT OF TRIAZOPHOS ON SOIL MICROORGANISMS AND THEIR ACTIVITIES

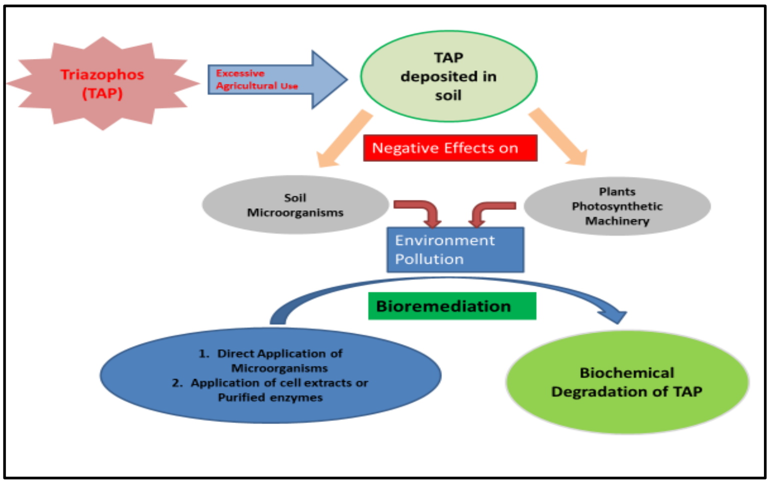
Pesticides, when used in the field to increase crop production besides combating insect pests, also affect the population and activity of beneficial microbial communities [24]. Microorganisms are involved in the recycling of essential plant nutrients, humus formation, soil structure stability, and all these processes are disturbed by the inefficient use of OPs such as TAP. Moreover, the excessive use of OPs has adverse effects on the microorganisms involved in ammonification, nitrification, denitrification.

Microbial communities of soil also interact with plant roots and soil constituents at the root-soil interface, and these interactions can be interrupted with the inefficient use of TAP and other OPs [25,26]. Hence, the addition of pesticides may disturb the equilibrium and, thus, the fertility of the soil (Fig. 1).

Further, Vig et al in 2008 [27] evaluated the effects of dimethoate, monocrotophos, endosulfan, and triazophos at recommended dosages in cotton fields in Punjab on non-target soil microorganisms. They reported that successive applications of the insecticides have short-lived adverse effects on the soil microorganisms. So, the inefficient and successive use of OPs have several adverse effects on the microbial community of the soil, which leads to poor soil quality and less fertility.

BIOREMEDIATION–A POTENTIAL DETOXIFICATION TECHNOLOGY

Figure 1. Effects of TAP on the environment and the Bioremediation Strategies for TAP degradation for sustainable agriculture.



The limitations of traditional methods of hazardous chemical disposal are overcome through bioremediation. This process brings about the actual destruction of various organic pollutants at a reduced cost. Microorganisms have many extraordinary characteristics such as their small size, ubiquitous distribution, high specificity, surface area, rapid growth rate, and unrivaled enzymatic and nutritional versatility, which make them one of the most critical recycling agents of pesticides in nature. Biodegradation of xenobiotic compounds is a metabolic process that involves the complete breakdown of an organic compound into its inorganic forms; the process is referred to as mineralization. In the context of environmental sciences, it is defined as the use of biological agents to eliminate hazardous substances from the environment [28].

Xenobiotic compounds are those to which microorganisms have not been exposed; therefore, they may be recalcitrant to bioremediation or not completely degraded. Biodegradability represents the susceptibility of substances to be altered by microbial processes. The alteration may occur by intra or extracellular enzymatic attack that is essential for the growth of the microorganisms. The attacked substances are utilized as a source of carbon, energy, nitrogen, or other nutrients or as a final electron acceptor. Co-metabolic reactions occur when an enzymatic attack is not necessarily beneficial to the microorganism's i.e., a physiologically useful primary substrate induces production of enzymes that fortuitously alter the molecular structure of another compound. Bioremediation is a combination of several processes like bioaccumulation, biodegradation, biostimulation, and bioaugmentation; all involve livings organisms in a different way of using them. One technique is biostimulation that involves the stimulation of microorganisms by adding the nutrients and other chemicals required for the metabolism of microorganisms that encourage the degradation of contaminants of native microorganisms at the contaminated site. Biostimulation is considered as the most preferred and cheapest techniques to decontaminate the environment. If the contaminated site has no native microorganisms, then another way is to introduce some microorganisms that can degrade these contaminants, which is known as bioaugmentation. In this technique, genetically modified microorganisms can also be used. Another way, is to isolate and purify the enzymes from the microorganisms and introduce into the contaminated area either land or water, to decontaminate it from the contaminants. Decontamination by the organisms depends on three factors, the type of microorganisms, the type of contaminant, and the geological and chemical conditions of the contaminated site [29,30].

There are two main approaches proposed for the bioremediation with microorganisms. In the first case, microorganisms can be applied directly to degrade pollutants and wastes in a reactor or in situ. In the second case, cell extracts or purified enzymes preparations of microbial origin could be used for decontamination purposes (Fig. 1) [31].

Direct application of microbes in contaminated sites for the remediation of toxic chemicals is the cheapest way of remediating the toxic compounds when conditions are favorable for the growth of microorganisms. However, if the conditions are unfavorable, their growth would be prolonged, and sometimes they die. In these conditions, the addition of nutrients plays a vital role in the bioremediation process, which enhances the growth of microbes and, ultimately, the rate of biodegradation [32].

MICROBIAL DEGRADATION OF TRIAZOPHOS

Naturally occurring triazophos-degrading microorganisms may be relatively rare in pristine environments and non-exposed agricultural soils. Therefore, isolation of bacterial strain through enrichment technique has been extensively used for the study of biodegradation of pesticides.

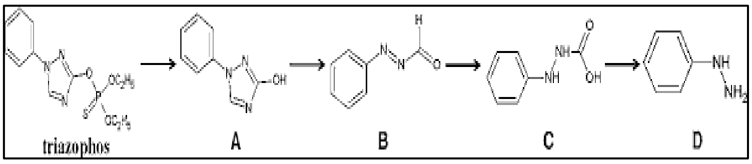
The use of microorganisms for bioremediation requires an understanding of all physiological, microbiological, ecological, biochemical, and molecular aspects involved in pollutant transformation [33, 34]. Optimizing process parameters based on an understanding of the effects of abiotic factors on the capacity of microorganisms is essential when designing bioremediation strategies.

A strain designated as GS-1, capable of degrading triazophos efficiently, was isolated by Guo et al in 2009 [35] from the sludge in an organophosphate pesticide wastewater treatment plant. Strain GS-1 was identified preliminarily as Diaphorobacter sp. based on its physiological and biochemical characters and the result of the 16S rDNA homologue sequence analysis. Strain GS-1 could grow with triazophos as its sole carbon source, and degrade 100 mg/L triazophos to non-detectable level within 12 h. The metabolite, 1-phenyl-3-hydroxy-1,2,4-triazole, produced during triazophos biodegradation, was finally transformed within 36 h. The optimal initial pH value and temperature for triazophos degradation by GS-1 was 7.0 and 30°C, respectively. Strain GS-1 could also degrade some other organophosphorus-pesticides such as fenitrothion, phoxim, chlorpyrifos, and methyl-parathion. Strain GS-1 also showed chemotaxis to triazophos, fenitrothion, and phoxim, indicating the close relationship between degrading and chemotaxis characteristics. This study demonstrated that Diaphorobacter sp. has excellent potential in the bioremediation of organophosphorus-pesticides contaminated sites.

A triazophos degrading strain; Klebsiella sp. E6 was isolated by Wang et al in 2005 [7] using enrichment technology from soil that had been exposed long term to triazophos. The strain grew well at a pH of 7.0–8.0 with a broad temperature profile ranging from 32 to 370C. It could keep good growth on methanol as a carbon source and triazophos as an additional carbon source or nitrogen source. The experiment on the degradation activities of strain E6 showed that it utilized triazophos more effectively when triazophos was supplied as the sole nitrogen source, as opposed to additional carbon source. The intermediates of triazophos metabolism indicated that degradation occurred through a hydrolysis mechanism, one of the products of which, 1-phenyl-3-hydroxy-1,2,4-triazole, was also mineralized by strain E6.

A bacterial strain, Diaphorobacter sp. TPD-1, capable of using triazophos and its intermediate, 1-phenyl-3-hydroxy-1,2,4-triazole (PHT), as its sole carbon sources for growth, was isolated by Yang et al in 2011 [11] from a triazophos contaminated soil in China. This strain could completely degrade 50 mg/l triazophos and PHT to non-detectable levels in 24 and 56 h, respectively. During PHT degradation, three metabolites were detected and identified based on tandem mass spectrometry (MS/MS) analysis. Using this information, a biochemical degradation pathway of triazophos by Diaphorobacter sp. TPD-1 was proposed. The first step involved in the degradation of triazophos is the hydrolysis of the P–O ester bond of triazophos to form PHT and o,odiethyl phosphorothioic acid, then the triazole ring of PHT is subsequently cleaved to form (E)-1-formyl-2-phenyldiazene. Subsequently, (E)-1-formyl-2-phenyldiazene is transformed into 2-phenylhydrazinecarboxylic acid by adding one molecular of H2O. Finally, the carboxyl group of 2-phenylhydrazinecarboxylic acid is decarboxylated to form phenylhydrazine (Fig.2).

Figure 2: The proposed degradation pathway of triazophos by *Diaphorobacter* sp. TPD-1. A: PHT, B: (E)-1-formyl-2-phenyldiazene, C: 2-phenylhydrazinecarboxylic acid, D: phenylhydrazine. [11].



Further, the use of enzymes for the degradation of pesticides can be developed as a technology for bioremediations, where growth conditions are not unfavorable for microorganisms [36]. Organophosphorus hydrolases (OPH) play essential roles in the decontamination of organophosphate pesticides, and extensive study has been done on enzymological aspects of degradation of OPs [37]. Bacterial enzymes, such as OPH, MPH, and OpdA, are responsible for the preliminary hydrolysis of organophosphates, and OPH has been the most widely studied for its hydrolytic activity on organophosphates [38,39].

TAP degradation is a complex enzymatic process. The responsible enzymes are usually intracellular and constitutively produced. However, the mechanism of enzymatic degradation is still not precise. So, there is a high need for research to understand the enzymatic process of TAP degradation [40,41].

CONCLUSION

The use of OPs such as TAP, has bestowed many benefits on the farming community, such as the enhancement in agricultural production, soil productivity, and product quality, which is reflected in economic benefits. However, this is only the fruitful side of this synthetic chemical; its extensive utilization causes a deleterious effect on the ecosystem. For this reason, it is necessary to generate strategies for the remediation of polluted sites. Uses of microbes for the detoxification of TAP proved to be a vital technology from an economical and environmental point of view. Presently, the use of indigenous or genetically modified microorganisms to degrade or remove OPs such as TAP has emerged as a powerful technology for in situ bioremediation. The integration of microbial cells with organophosphates can be proved as the most efficient method to control several insect pests, and this will lead us to sustainable agriculture.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest.

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