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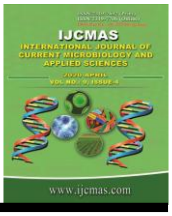

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Microbial Bioremediation of Pesticide Residues: A Review

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ABSTRACT

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Plant protection using synthetic pesticides has become one of the essential components of modern agriculture. Although need based judicious application of pesticides have made a significant impact on increasing crop productivity in agricultural sector by combating heavy losses due to pest infestation but there are lots of serious associated bottlenecks with pesticides. The major issues related to pesticide application are environmental pollution, health hazards, pest resurgence, secondary pest outbreak etc., as a very less per cent of the applied pesticides reaches to the target organisms and rest remains as residues. Moreover, most of these pesticides becomes persistent pollutant of the environment because of their relative stable nature and many a times, the extreme toxicity results in pesticidal poisoning in living beings which is considered as a matter of great concern. Considering the ill effects of pesticides, many physical and chemical efforts have been attempted to lower down the possible effects of pesticide residues mostly in agricultural field however, those approaches are highly expensive and not eco-friendly. Of late, the bioremediation approach mostly by exploring pesticide degrading microorganisms has emerged as an eco-friendly, effective and economical alternative to address the concerned issues. Major groups of microbes having pesticide residue degrading properties are bacteria, fungi and actinomycetes. Under favourable conditions, most of these microbes utilize pesticides as a sole source of carbon whereas some synthesize various metabolic enzymes to degrade and detoxify many harmful pesticides. Nevertheless, some difficulties of those microbes in respect of specificity, spectrum of activity, environmental sensitivity, registration, lack of formulated products etc. are need to be addressed. Exploration of modern biotechnological tools for selecting appropriate potential strains of microbes and their further improvement through genetic engineering may pave the way for the wide applicability in true sense.

Introduction

India is one of the seven largest countries in the world having an arable land area of 159.7 million hectares. The economy of India is solely based on agriculture and allied sectors and approximately 70 per cent of the population depends on it. India's population is expected to reach 1.6 billion by 2050, surpassing China in 2025 and in that situation, feeding the whole population will be a great challenge for the country (Wolf *et al.*, 2011).

Moreover due to the pest infestation, approximately 45 per cent of the total food production is reduced or lost annually (Sharma *et al.*, 2016). To conquer the loss of infestation due to various pests and diseases as well as to enhance the productivity of crops throughout the world, quantum jumps in the use of synthetic pesticides have now become a part of modern agriculture. Pesticides are organic chemicals purposefully intended for increasing agricultural yield through minimizing the losses of agricultural products caused by crop pests and to control the insect vectors for prevention of the outbreak of human and animal epidemics. In India, the use of pesticides began in 1948 (just after the world war-II) when DDT was imported for the control of malaria and benzene hexachloride (BHC) for locust control (Buyuksonmez *et al.,* 1999). However, pesticide production was started in the country just after the setting up of manufacturing plants of DDT and BHC in 1952. The first report of use of pesticides in agricultural field was started with the advent of Green Revolution which certainly increased the total food production in the country and the globe as well. Of late, 75 per cent of the total pesticides produced in the world are only being used in agricultural practices and over 500 compounds are registered and used worldwide as pesticides or their metabolites (Parte *et al.,* 2017).

Though pesticides plays pivotal role in augmenting food production as well as socio-economic upliftment of farming community, its large scale and indiscriminate use has created a highly unstable ecosystem by developing insecticide resistance in insects, pest resurgence, elimination of parasites, predators and pollinators etc. Moreover, due to the unplanned application of pesticides, only 10 per cent actually penetrate the target organism and the remaining are deposited as residues on non-target areas such as soil and water causing serious environmental pollution. The unwanted side effects of pesticide residues to humans and other life forms in terms of carcinogenicity, mutagenicity, reproductive disorder, neurological and various other health problems have already been reported by Forget, 1993 and Abhilash and Singh, 2009. Many of the pesticides which were reported to be lethal to both nature and humans were subsequently banned, although there is every possibility that the residues accumulated in the soil or water may enter again at any trophic level of the food chain (Parte *et al.*, 2017). Thus, looking towards the environmental concerns and health hazards resulting due to the continuous use of these noxious pesticides, concerted efforts have already been made to lower down the possible effects of pesticide residues mostly in agricultural field through many physical (photo degradation and high temperature incineration) and chemical (by using powerful transient chemicals) methods (Torres-Duarte *et al.*, 2009). However, both physical and chemical approaches are highly expensive and not eco-friendly in nature and hence the bioremediation approach mostly done by exploring pesticide degrading microorganisms has emerged as an ecofriendly, effective and economical alternative to address the concerned issues (Aislabie and Lloyd-jones, 1995; Ortiz-Hernández *et al.*, 2003 and Singh and Thakur, 2006). Different microorganisms, their enzymes and genes responsible for bioremediation of pesticide residues, challenges and future thrust areas related to the subject have critically reviewed in this paper.

Factors related to pesticide residues degradation

The amount of pesticides initially laid down after application on any surface is termed as deposit and those deposits after a lapse of time is referred to as pesticide residues (Prasad, 2014). The pesticide residues are subjected to many biotic and abiotic factors after getting deposited into soil or water. Out of the abiotic factors, temperature, humidity, pH, water content, organic matter content, viscosity and climate plays an important role in the degradation of residues. High temperature along with high humidity climates, high organic matter and alkaline pH of the soil generally resulted in a rapid degradation of pesticide residues. Amount of pesticide applied and the molecular structure of the pesticide also depends on the degradability of its residues. There are reports that the degradation of 2,4,5-T is fourteen fold higher as compared to 2,4-D due to the addition of one chlorine atom at the 5th carbon (Ye *et al.*, 2018). However, the degradation of pesticide residues principally depends on the diversity and action of microorganisms.

Potentiality of microorganisms in pesticide residue remediation

The ability of the soil to reduce the concentration of any contaminants naturally is known as “natural attenuation”. The microorganisms occurred in the soil plays an important role in assisting chemical reactions which break down the molecular structures of the contaminants to less toxic molecules. On the other hand, the exploration of such naturally occurring microorganisms by the intervention of humans to break down or degrade toxic chemical compounds accumulated in the environment is known as “Bioremediation”. Bioremediation of pesticide residues through microorganisms has been recognized since time immemorial.

Rapid rate of reproduction, high surface area volume ratio and high catalytic power make microorganisms a potential source for bioremediation of pesticide residues. Some species of microbes are even reported to synthesis pesticide degrading enzymes either naturally or through random mutation whereas in some species synthesis of degrading enzymes are induced in the presence of a particular pesticide (Tewari, 2012). Out of all the microorganisms, certain species of bacteria, especially actinomycetes and cyanobacteria, algae and fungi presented in Table 1 are reported to have the pesticide degrading ability.

The three phases of pesticide residue degradation by microorganisms

The first step of degradation of any contaminants is primarily carried out by fungi in which they biotransform pesticides and other xenobiotics to certain nontoxic products intermediate by changing the original molecular structure. The nontoxic intermediates are then susceptible to further degradation by bacteria (Diez, 2010). Both the fungi and bacteria produces extracellular enzymes such as esterases, glutathione S-transferases (GSTs) and cytochrome P450 which induced breakdown of the pesticide molecules through hydrolysis, oxidation, addition of amino group or hydroxyl group, dehalogenation, reduction of a nitro group to an amino group, replacement of a sulfur with an oxygen molecule, metabolism of side chains, ring cleavage etc. (Bass and Field, 2011).

Degradation of pesticide residues in soil generally undergoes three processes. The parent compounds forms some intermediate molecules which are more water soluble through oxidation, reduction or hydrolysis in the first phase. In the second phase of metabolism, the intermediate molecules conjugated with a sugar or amino acid which makes the final molecule more water soluble and comparatively less toxic. In the final phase, Phase II metabolites are transformed in to a final secondary conjugates with relatively non-toxic molecules. In each of these process, there is an involvement of certain intracellular or extra cellular enzymes like oxidoreductases (mixed function oxidase, cytochrome p450, monooxygenases, dioxygenases etc.), transferases (Glutathione S-transferases) and hydrolases (hydrolase, esterase, dehalogenases etc.) which were reported to be synthesized by both the bacteria and fungi (Jauregui *et al.*, 2003, Van Eerd *et al.*, 2003, Singh and Singh, 2005, Joosten *et al.*, 2007, and Pizzul *et al.*, 2009). Lists of different microbial enzymes responsible for the degradation of pesticide residues as reported byScott *et al.*, (2008); Ortiz-Hernandez *et al.*, (2013) and Sharma *et al.*, (2016) are presented in Table 2.

Microbial genes involved in pesticide residue degradation

Unlike normal environmental conditions, the genes of the microorganisms expressed differently when they are exposed to any stressed situations. Of late, the adoption of microorganisms to altered environment as well as mechanism to degrade contaminants from the soil has already been studied through recent biotechnological tool like genomics, metagenomics, proteomics and bioinformatics (Arora and Bae, 2014).

Expression of genes or a particular protein responsible for the degradation of pesticides through genome sequencing or recombinant DNA technology have also been reported (Widada *et al.*, 2002). The gene responsible for degrading pesticide residues mostly occurs either on chromosomes or plasmids and transposons. Li *et al.*, (2007) identified "opd" gene, a gene having 996 nucleotides responsible for degrading organophosphate pesticides and its residues.

Similarly, "mpd" gene having the ability to degrade methyl parathion has been identified and recorded in *Pseudaminobacter* sp., *Achrobacter* sp., *Brucella* sp., *Ochrobactrum* sp. and *Pleisomonas* sp. (Zhongli *et al.*, 2001). Some genes responsible for degrading pesticides and its residues as reported by Singh and Walker (2006) and Ortiz-Hernandez *et al.*, (2013) are listed in Table 3.

Examples of pesticide residues degraded through microorganisms

Endosulfan biodegradation

Endosulfan is an organochlorine insecticide currently banned in almost all the countries. Tow bacterium viz., *Mycobacterium tuberculosis* and *Arthrobacter* sp. are reported to degrade the residues of endosulfan in soil. After deposition in the soil, the endosulfan undergoes metabolism to form endosulfan sulphate and endosulfan diol as primary metabolites.

Mycobacterium tuberculosis synthesized ESD enzyme which degrades beta endosulfan to monoaldehyde and hydroxyether and transforms alpha endosulfan to endosulfan sulphate as more toxic compounds. However, monooxygenase enzyme encoded by *ese* gene in *Arthrobacter* sp. KW oxidize endosulfan sulphate to endosulfan monoalcohol as relatively less toxic compound to the environment (Weir *et al.*, 2006).

Carbamate biodegradation

Synthesis of carbofuran hydrolase encoded by "mcd" genes resulted in hydrolysis of the methyl carbamate linkage which leads to the degradation of carbamate pesticides. Tomasek and Karns (1989) first described the gene in *Achromobacter* sp. Later, the enzyme as well as gene was also reported from an array of bacteria *viz*., *Pseudomonas*, *Sphingomonas*, *Arthrobacter*, *Mesorhizobium*, *Ralstonia*, *Rhodococcus*, *Ochrobactrum*, *Spingobium*, *Bosea*, *Microbacterium* and *Bacillus* (Desaint *et al.*, 2000). Of late, *Aspergillus niger* has also been reported to degrade carbamate pesticides and its residues (Qing *et al.*, 2006).

Organophosphorous biodegradation

The work on organophosphorous pesticide residue degradation started in 1973 when Sethunathan and Yoshida isolated *Flavobacterium* sp. to degrade diazinon and parathion. This soil bacterium synthesizes organophosphate hydrolase or phosphotriesterase enzymes which are the prime requisite for organophosphate degradation. These enzymes hydrolyze phosphoester bonds, such as P–O and the hydrolysis mechanism involves a water molecule at the phosphorus center (Ortiz-Hernández *et al.,* 2003). Some of the bacteria responsible for degrading organophosphorous componds are *Flavobacterium* sp., *Plesimonas* sp. strain M6, *Pseudomonas moteilli* etc. (Ortiz-Hernandez *et al.,* 2013).

Neonecotinoid biodegradation

Like organophosphorus compounds, the neonicotinoids are also degraded by many bacterial species such as *Stenotrophomonas maltophilia* CGMCC 1.1788, *Pseudomonas sp.* 1G, *Leifsonia* sp. and *Rhodotorula mucilaginosa* strain IM-2 etc. (Dai *et al.*, 2007 and Pandey *et al.*, 2009)

Table.1 Microorganisms used in the degradation of different pesticide and their residues

Pesticides	Microorganisms	References
DDT	*Escherichia coli* *Enterobacter aerogenes* *E. cloacae* *Klebsiella pneumonia* *Pseudomonas putida* *Bacillus circulans* *Hydrogenomonas* sp. *Pseudomonas aeruginosa* *Micrococcus* sp. *Bacillus pumilus* *Flavobacterium* sp.	Ortega *et al.*, 2011 Fang *et al.*, 2010 Kamanavalli and Ninnekar, 2005 Pesce and Wunderlin, 2004 Patil *et al.*, 1970 Wedemeyer, 1967
Endosulfan	*Pseudomonas aeruginosa* *Bacillus circulans* *Flavobacterium* sp.	Bhalerao and Puranik, 2007
Lindane	*Bosea thiooxidans* *Sphingomonas paucimobilis* *Streptomyces* sp. *Pleurotus ostreatus*	Benimeli *et al.*, 2008 Rigas *et al.*, 2005
Aldrin and Endrin	*Trichoderma viridae* *Pseudomonas* sp. *Micrococcus* sp. *Bacillus* sp. *Arthrobacter* sp.	Patil *et al.*, 1970
Toxaphene	*Bjerkandera* sp.	Patil *et al.*, 1970
Dieldrin	*Pseudomonas aeruginosa*	Matsumura *et al.*, 1968
PCP	*Arthrobacter* sp. *Flavobacterium* sp.	Crawford and Mohn, 1985 Stanlake and Finn, 1982

Heptachlor	*Phanerochaete chrysosporium* *Phlebia* sp.	Xiao *et al.*, 2010 Arisoy and Kolankaya, 1998
Cypermethrin	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *P. Stutzeri* *Bacillus subtilis* *Enterobacter asuburiae*	Pankaj *et al.*, 2016
Lambda-cyhalothrin	*Klebsiella* sp. *Pseudomonas oleovorans*	Chen *et al.*, 2015
Carbofuran	*Pseudomonas* sp. *Flavobacterium* sp. *Achromobacterium* sp. *Sphingomonas* sp. *Arthrobacter* sp.	Head *et al.*, 1992
Chlorpyriphos	*Sphingomonas* sp. *Enterobacter* sp.	Li *et al.*, 2007 Singh *et al.*, 2004
Dimetoate and Malathion	*Pseudomonas frederiksbergensis*	Al-Qurainy and Megeed, 2009
Methyl parathion	*Sphingobium* sp.	Yuanfan *et al.*, 2010
Diazinon	*Serratia liquefaciens* *S. marcescens* *Pseudomonas* sp.	Cycon *et al.*, 2009
Prophenofos	*Pseudomonas putida* *Burkholderia gladioli*	Malghani *et al.*, 2009
Atrazine and Alachlor	*Arthrobacter* sp. *Clavibacter* sp. *Nocardia* sp. *Rhodococcus* sp. *Nocardioides* sp. *Streptomyces* sp.	Behki *et al.*, 1993
2,4-D	*Ralstonia eutropha*	Chung and Ka, 1998
Propiconazole	*Pseudomonas putida*	Sarkar *et al.*, 2009
Carbendazin	*Pseudomonas aeruginosa*	Tian and Chen, 2012
Pentachloronitrobenzene	*Rhizoctonia solani* *Botrytis* sp. *Aspergillus* sp. *Penicillium* sp. *Fusarium* sp. *Sclerotinia* sp. *Tilletia caries*	Pesce and Wunderlin, 2004 Spain and Nishino, 1987
Iprodione	*Pseudomonas aeruginosa*	Bending and Rodríguez, 2007

Table.2 Microbial enzymes reported for degradation of pesticide residues

Enzyme	**Microorganism responsible for synthesis**	**Pesticide residues**
Oxidoreductases (Gox)	*Pseudomonas* sp. LBr *Agrobacterium* sp. strain T10	Glyphosate
Monooxygenases:		
ESd	*Mycobacterium* sp.	Endosulphan
Ese	*Arthrobacter* sp.	Endosulphan, Aldrin, Malation and DDT
Cyp76B1	*Helianthus tuberosus*	Linuron, Chlortoluron and Isoproturon
P450	*Pseudomonas putida*	Hexachlorobenzene and Pentachlorobenzene
Dioxygenases		
TOD	*Pseudomonas putida*	Trifluralin
E3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester
Phosphotriesterases: OPH/OpdA	*Agrobacterium radiobacter* *Pseudomonas diminuta* *Flavobacterium* sp.	Insecticides phosphotriester
Haloalkane Dehalogenases:		
LinB	*Sphingobium* sp. *Shingomonas* sp.	Hexachlorocyclohexane (β and δ isomers)
AtzA	*Pseudomonas* sp.	chloro-s-trazina
TrzN	*Nocardioides* sp.	chloro-s-trazina
LinA	*Sphingobium sp.* *Shingomonas sp.*	Hexachlorocyclohexane (γ isomers)
TfdA	*Ralstonia eutropha*	2,4 - D and pyridyl-oxyacetic
DMO	*Pseudomonas maltophilia*	Dicamba

Table.3 Microbial genes responsible for the degradation of pesticide residues

Gene	**Source**
Bacterial gene	
opdA	*Agrobacterium radiobacter*
Opd	*Pseudomonas diminuta*
adpB	*Nocardia* sp.
Phn	*Bacillus cereus*
ophB	*Burkholderia* sp. JBA3
Imh	*Arthrobacter* sp. scl-2
Mpd	*Ochrobactrum* sp. *Yw28* and *Rhizobium radiobacter*
opdE	*Enterobacter* sp.
Fungal genes	
A-opd	*Aspergillus niger*
P-opd	*Penicillium lilacinum*

Synthetic chemicals has significantly increased the farmer's economy by saving food loss and also saved the millions of lives by managing pests of public health importance. However, due to the injudicious, indiscriminate and unplanned application of pesticides has certainly affected the ecosystem and its allied services. In many cases, very less per cent of applied pesticides could actually reach the targeted organisms and rest remain deposited as residues in various surfaces causing serious problems to the environment.

Being an eco-friendly and cost effective tool, microorganisms can be explored extensively to address the issue. However, lack of pesticide residue specific microorganisms, less adaptation of microorganisms to the changing environment and lack of formulated pesticide degrading formulated marketable products stands as key bottlenecks of the subject. Selection of appropriate potential strains and their further improvement through genetic engineering to development of a formulated pesticide degrading microbial consortia along with standard method of application may pave the way for the wide applicability of such microbes.

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