African Journal of Microbiology Research Vol. 5(3), pp. 177-183, 18 February, 2011
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2011 Academic Journals

Review

Eco-friendly role of biodegradation against agricultural pesticides hazards

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Accepted 23 December, 2010

This review aims to elaborate the potential applications of various biological agents in decontamination of agricultural soils, which have been polluted with continuous and higher doses of pesticides through process of biodegradation. Biodegradation is an eco friendly, cost effective, highly efficient approach and can be considered as a superior alternative to physical and chemical methods which are not only technically laborious and costly; also are not sufficient to completely degrade organic toxins. Development of experimental conditions in which all congruent biological agents are applied concurrently may be a promising strategy to enhance biodegradation and subsequently biodegradation. Much work remains to be done in carrying out field studies based on laboratory-scale results/experiments using plant-associated endophytic and rhizospheric bacteria to degrade a wide range of toxic organic compounds of concern in environmental soil before commercially viable systems.

Key words: Biodegradation, pesticides, fungi, bacteria, phytodegradation.

INTRODUCTION

Millions of tons of pesticides are applied annually in modern agriculture to increase the production through controlling harmful effects caused by the target organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops (Liu and Xiong, 2001). However, less than 5% of these products are estimated to reach the target organisms. The major environmental concern of used pesticides is their capacity to leach down to subsoil and contaminate the ground water (Kookana et al., 1998) or if immobile, they would persist on the top soil where it could accumulate to toxic level in the soil and become harmful to microorganisms, plants, animals and man (Amakiri, 1982). Pesticides have various characteristics that determine how they act once in soil. Excessive and persistent use of pesticides results in deterioration of the environment. The quality of soils, ground water, continental and coastal waters as well as the air, is compromised by pesticide contamination (Surekha et al., 2008). Globally, subsoil and groundwater pollution are the major consequences/outcomes environmental effects of pesticides application. Pesticides can reach water through surface runoff from treated plants and soil. Pesticide sprays usually directly hit non-target vegetation, or can drift or volatilize from the treated areas that contaminate air, soil, and non-target plants. Some pesticide drift occurs during every application, even from ground equipment (Johnson and Ware, 1991).

Pesticide exposure inflicts chronic and acute threats to human health. For example, long term low dose exposure to pesticide causes immune suppression, hormonal disruption, diminished intelligence, reproductive abnormalities and carcinema (Gupta, 2004). Amongst most of the important problems associated with pesticides

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application are their possible persistence in the environment and therefore, their possible incorporation into the food chain affects ecosystems and human beings (Liu and Xiong, 2001). Another problem is the conversion of pesticides into the obsolete form, which may even show more harmful effects than the former. When the pesticides are not used within the given time of their efficacy, they become obsolete. They are decomposed into other chemical components, which sometimes become even more toxic than the original pesticides. Most pesticides expire in two years after production, meaning they cannot be used unless they are tested and proved stable (Binod and Bhupendra, 2009). Therefore, these toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neuro-behavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure (Okon, 1985).

Bioremediation is the use of living organisms to minimize or eliminate the environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes. Bioremediation is a promising alternative to physico-chemical methods of remediation, because it is less expensive and can selectively achieve complete destruction of organic pollutants (Alexander, 1999). The use of microorganisms for the degradation and detoxification of numerous toxic xenobiotics, especially pesticides, proved to be an efficient tool to decontaminate the polluted sites in the prevailing environment (Mervat, 2009). Bioremediation methodology to treat xenobiotics such as pesticides in soil have gained considerable attention owing to its ecofriendliness and have been employed successfully in many countries (Enrica, 1994; Ritmann et al., 1988). Pesticides in soil and water can be biodegraded and is the primary mechanism of pesticide breakdown and detoxification in many soils (Surekha et al., 2008). Conventional approaches (e.g. landfilling, recycling, pyrolysis and incineration) for the remediation of contaminated sites are inefficient, costly and may lead to the formation of several toxic intermediates (Sayler, 1990). Thus, biological decontamination methods are preferable to conventional approaches because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates (Pieper, 2000; Furukawa, 2003). Aim of this review paper is to analyze the effect of various biological agents on biodegradation of various types of pesticides.

Different approaches for biodegradation

(a) Bacterial degradation.
(b) Fungal degradation.
(c) Phytodegradation.

Bacterial degradation

Complete biodegradation of a pesticide involves the oxidation of parent compound to form carbon dioxide and water. This process provides both carbon and energy for the growth and reproduction of microbes. Each degradation step is catalyzed by specific enzyme produced by a degrading cell or enzyme found external to the cell. Degradation of pesticide by either external or internal enzyme will stop at any step if an appropriate enzyme is not present. Absence of an appropriate enzyme is one of the common reason for persistence of any pesticide. If an appropriate microorganism is absent in soil or if biodegrading microbial population has been reduced due to toxicity of pesticide in that case a specific microorganism can be added or introduced in soil to enhance the activity of the existing population (Singh, 2008).

The use of bacteria for the degradation and detoxification of numerous toxic chemicals such as pesticides is an effective tool to decontaminate the polluted sites. Isolation of indigenous bacteria capable of metabolizing pesticides provides environmentally friendly means of *in situ* detoxification (Mervat, 2009). Degradation by microbes depends not only on the presence of degradative enzymes, but also on a wide range of environmental parameters. Temperature, pH, water potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factor for pesticide degrading microorganisms, which requires further exploration in relation to total microbial population and their biochemical activities (Singh, 2008). Some pesticides are readily degraded by microorganisms; others have proven to be recalcitrant (Aislabie and Lloyd-Jones, 1995; Richins et al., 1997; Mulchandani et al., 1999). Many pesticide degrading genes harboring in soil bacteria have been reported on plasmids (Chung and Jong, 1998; Laemmli et al., 2000). These genes encoding for enzymes capable of degradation have been studied well, these plasmids are known as catabolic plasmids; the organism, containing them have the ability to degrade certain compounds. Many catabolic plasmids have been found in species of *Pseudomonas, Alcaligenes, Actinobacter, Flavobacterium, Klebsiella, Moraxella* and *Arthrobacter* (Sayler et al., 1990). A diverse group of bacteria, including members of the genera *Alcaligenes*, *Flavobacterium, Pseudomonas* and *Rhodococcus*, metabolize pesticides (Aislabie and Lloyd-Jones, 1995; Richins et al., 1997; Mulchandani et al., 1999). Actinomycetes have considerable potential for the biotransformation and biodegradation of pesticides. Members of this group of Gram-positive bacteria have been found to degrade pesticides with widely different chemical structures, including organochlorines, s-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, and sulfonylureas. A limited number of these xenobiotic pesticides can be

mineralized by single isolates, but often consortia of bacteria are required for complete degradation. Co metabolism of pesticides is frequently observed within this group of bacteria. When compared with pesticide degradation by Gram-negative bacteria, much less information about molecular mechanisms involved in biotransformations of pesticides by actinomycetes is available. Progress in this area has been seriously hampered by a lack of suitable molecular genetic tools.

Overcoming this constraint would enable a better exploitation of biodegradation and biotransformation mechanisms for applications such as bioremediation and to construct transgenic herbicide-resistant crops (De Schrijver and DeMot, 1999). Contaminated environments have resulted through time in the evolution of autochthonous microbial populations; therefore, these sites are the most appropriate ecological niches for the isolation of strains which are also able to degrade such compounds (Oshiro et al., 1996; Ortiz-Hernández et al., 2001; Horne et al., 2002).

Methomyl belongs to a class of compounds known as oxime carbamates, and it is widely used for the control of insects and nematode pests by inhibiting the enzyme acetyl cholinesterase, which hydrolyzes the neurotransmitter acetylcholine. The IUPAC name of methomyl is S-methyl N- (methylcarbamoyloxy) Thioacetimidate. The World Health Organization (WHO), Environment Protection Agency (EPA) and European Chemical Classification (ECC) classify methomyl as a very toxic and hazardous pesticide (Clive, 2003), and since the sorption affinity of soils for this pollutant is rather low, it can easily cause contamination of both ground and surface water resources (Strathmann and Stone, 2001). Microorganisms are thought to play an important role in the removal and detoxification of these toxicants from the environment. Many bacteria that are able to degrade carbamate pesticides have been isolated from soil around the world (Desaint et al., 2000). It has been reported that *Stenotrophomonas maltophilia* is able to degrade many xenobiotic compounds (Lee et al., 2002) and to detoxify high molecular weight polycyclic aromatic hydrocarbons (PAHs) (Juhasz et al., 2000). Therefore, it has a great potential for soil decontamination (bioremediation). *S. maltophilia* M1 strain possesses a strong ability for methomyl degradation. This strain contains two plasmids; one of them (PMb) was believed to be responsible for the degradation of methomyl carrying the degrading gene. This plasmid could be transferred to other bacterial strains to enhance their ability to degrade methomyl pesticide. These two plasmids PMa and PMb were used to transform *Escherichia coli* DH5α strain. Only PMb plasmid (5 kb) was successfully transferred to this strain that was allowed to grow on methomyl while PMa plasmid was not detected and may lack the gene responsible for the degradation process. Transformed strain (*E. coli* DH5α strain M2) had the ability to hydrolyze methomyl (100 ppm) in M9 media and maintained its activity after repeated subculture. However, efficiency of transformed strain M2 was less as compared to M1 strain. This may be attributed to the fact that the strain M2 acquired the ability for methomyl degradation after transformation with PMb plasmid, which encodes the degradative gene (s) from M1 strain but rate of degradation was slower than M1, which may be due to the slower rate of replication of the transferring plasmid to the host DH5α strain M2 than the original host (Mervat, 2009).

Various biochemical methods showed that some of the soil bacteria have the ability to utilize aldicarb (a carbamate insecticide has been known to have the potential to cause adverse effects on human health by inhibition of acetyl cholinesterase activity in the neuromuscular junction (Burgess et al., 1994; Lifshitz et al., 1997) through hydrolysis and degradation by bacterial enzyme-esterase. *S. maltophilia* found to be the most degradative effect on aldicarb (Turan et al., 2008). Nine different bacterial strains have been identified for biodegradation of aldicarb *S. maltophilia, Bacillus species Alcaligenes denitrificans*, Gram (+) *bacillus, Bacillus subtilis, Enterobacter gergoviae, Flavimonas oryzihabitans, Flavimonas species.* Aldicarb can be hydrolyzed enzymeticaly by esterase and amidase, however in case of its degradation by *S. maltophilia* the enzyme involved was found to be esterase by determining the cytoplasmic enzyme activity of *S. maltophila* (Turan et al., 2008).

Cometabolism means addition of an easily metabolized organic matter such as glucose increases biodegradation of recalcitrant compounds that are usually not used as carbon and energy sources degradations by microorganisms (Prescot et al., 2002) use of glucose as co-substrate increases the rate of biodegradation (Swaminthan and Subrahmanyam, 2002) as found in case of degradation of dicofol by isolated tea rhizosphere microflora. In this case out of 13 *Pseudomonas sp*, chemically adapted to dicofol as they were collected from a soil that received repeated application of pesticide (dicofol), two strains P9 and P13 were tolerant to high dicofol concentration and degradative potential of these strains raised up to 70 and 32% in presence of glucose whereas it was only 38 and 14% in its absence respectively. This process is finding a great venue for diverse application in biodegradation management (Soumik, 2009).

Fungal degradation

Fungi, from natural sources can be screened out as an effective tool for biodegradation of toxic organic chemicals. *Phanerochaete* and related fungi that have the ability to attack wood possess a powerful extracellular enzyme that, acts on a broad array of organic

compounds. The enzyme is a peroxidase that, with H_2O_2 produced by fungus, catalyzes a reaction that cleaves a surprising number of compounds. Culture of *Phanerochaete chrysosporium,* the most widely studied of these fungi for its biodegradative capacity, can degrade a number of Polycyclic aromatic hydrocarbons (PAHs) including pyrene, anthracene, di- and tribenzoic acids, several Polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, DDT (Dichlorodiphenyltrichloroethane), lindane, and chlordane (Alexander, 1999). Bioremediation technologies using this fungus thus have considerable promise, especially for compounds not acted on readily, if at all, by bacteria. The transformations by the fungus are slow, and a test of the biodegradation of DDT in soil pans failed to show an effect of *Phanerochaete sordid* in promoting bioremediation (Safferman et al., 1995). However, the addition of large inocula of this fungus resulted in an enhanced degradation of PCP as well as three- and four- but not five and six rings PAHs (Lamar et al., 1994). Another study performed by Glaser and Lamar (1995) showed a stimulation in the degradation of PCP by two species of *Phanerochaete.*

Most of the white-rot fungi secrete various extra cellular enzymes that degrade lignin. Among them, peroxidase or ligninolytic enzymes, predominantly lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. Presence of nonspecific enzyme system, enable fungi to degrade natural complex aromatic polymers of lignin as well as complex aromatic polymers that share structure with lignin, such as pesticides, PAHs, PCBs and dyes (Cameron et al., 2000). In most researches *Phanerochaete chrysosporiume* are utilized as a model for studying the biodegradation of a wide variety of pollutants present in both liquid and soil cultures. *P. chrysosporium* is used as a model for studying the degradation of DDT, 2,3,7,8 tetrachlorodibenzo-p-dioxin, lindane and, benzo pyrene. *P. chrysosporium* oxidizes these organopollutants to carbon-dioxide using its lignin-degrading enzyme system under nitrogen deficient conditions (Bumpus et al., 1985). *P. chrysosporium* degraded polychlorinated biphenyl mixtures of Aroclors 1242, 1254 and 1260 by 60.9, 30.5 and 17.6%, respectively. In defined media under high and low N conditions in a defined medium degradative potential of *P. chrysosporium* rose for mixtures of Aroclors 1242 and 1254 (Yadav et al., 1995). Fungal isolates *Trametes* sp., *Polyporus* sp., *Nigroporus* sp, F33 (un identified) and U11 (un identified) were able to biodegrade different organic compounds. These isolates having peroxidases, proved to be having ability to degrade 2, 8-DCDD (2,8-dichlorodibenzo-p-dioxin) and DDT in liquid culture medium with different kinetics. In contrast, degradation of DDT by all these tested isolates was less as compared to 2, 8-DCDD. Of these five isolates, F33 exhibited the greatest ability to degrade 2, 8-DCDD during 30 day incubation (Premjet et al., 2009). Fungal technology appears promising for biodegradation of recalcitrant contaminants (Glaser and Lamar, 1995). Fungi do not generally metabolize contaminants; degradation occurs extracellular by enzymes excreted by the fungi. Much research remains to be done to identify the fungal strains most capable of degrading specific contaminants.

Phytodegradation

Phytoremediation is defined as the use of green plants and their associated microorganisms, soil amendments and agronomic techniques to remove content or render harmless environmental contaminants. Plants can either accumulate and metabolise organic pollutants (phytodegradation) or stimulate appropriate rhizospheric microorganisms (phytostimulation). Both approaches have been explored to remediate soils contaminated with pesticides. Phytoremediation is an eco-friendly, cost effective, easy to be employed approach for remediation of contaminated soil and sub-soil water using plants. It is the use of unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contaminant storage/degradation. Plant-based soil remediation systems can be viewed as biological, solar-driven, pump -and-treat systems with an extensive, self extending uptake network (the root system) that enhances the under-ground ecosystem for subsequent productive use (Cobbet, 2000). Contamination of soil and water with pesticides may be recovered partially by using filter strips and buffer zones of non-target plants (Borner, 1994). Such technologies are valuable and economically cheaper, exploiting the inherent abilities of plants to reduce pesticide runoff and their metabolic capacity to accumulate and transform toxicants (Dobson et al., 1997). Detoxification potential of higher plants, analogous to “green livers,” may be effectively used as a base line to develop treatment technologies to replenish the contaminated environments (Hall et al., 2001; McCutcheon and Schnoor, 2003). In nature, plants for example, a hydrophyte *Typha latifolia* metabolize organic pollutants (Ops), remove malathion and dimethoate pollutants from soil. Phytoremediation along with conservation biology may be employed to enhance the recovery of natural ecosystems from local or more widespread anthropogenic changes (Dobson et al., 1997). Accumulating evidences suggest the possible use of plant-based remediation systems (Shimp et al., 1993; Bicki et al., 1994; Cunningham et al., 1996; Davis et al., 2002; Scheper and Tsao, 2003; Tsao, 2003).

Generally speaking, plant-based remediation systems are designed on the basis of known plant capabilities to stabilize or remediate contaminated soil and water. Because plants use significant quantities of water, plume control and groundwater management are benefits that should be considered in plant-based remediation. Several characteristics of plants, such as local adaptation, metabolism, uptake, and tolerance, are important factors in designing plant-based treatment systems. Plants may enhance transport of volatile compounds from the soil

into the atmosphere (Davis et al., 2002; 2003). Pesticides which enter plants may be transformed into relatively less toxic forms that may further be degraded or incorporated into plant biomass such as lignin (Hall et al., 2001; McCutcheon and Schnoor, 2003).

Plants create a favorable micro environment around their root-zone that facilitates the contaminant degradation. Degradation of toxic organic compounds in environmental soil by plant-associated bacteria involve endophytic and rhizospheric bacteria. Endophytic bacteria are usually non-pathogenic bacteria occuring naturally in the internal tissues of plants which may promote plant growth, be beneficial to the plant host by producing a wide range of natural products, and contribute biodegradation of soil pollutants as well (Bacon and White, 2000; Sessitsch et al., 2002). Almost all 300,000 plant species identified, have at least one species of endophytes (Strobel et al., 2004). The major endophytic bacterial species isolated from plants, include *Acetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Herbaspirillum* and *Pseudomonas,* (Lodewyckx et al., 2002).

The unique status of the rhizosphere as a treatment-zone has great potential for remediation (Anderson et al., 1993; Cunningham et al., 1996; Davis et al., 1998; 2003). The enhanced rate of biodegradation in the rhizosphere may be due to co metabolism and/or the larger microbial populations stimulated root exudates, root turnover, and improved soil moisture, oxygen, and nutrient conditions. Plant roots also sorb pesticides onto their surfaces, and dead roots add organic matter to soil, which can enhance the sorption of pesticides onto soil organic matter where microbial transformation may occur (Karthikeyan et al., 2004). Several laboratory studies were conducted in order to assess the impact of the rhizosphere on pesticide degradation (Nair et al., 1993; Anderson et al., 1994; Hoagland et al., 1994; Shann and Boyle, 1994; Zablotowicz et al., 1994; Anderson and Coats, 1995; Perkovich et al., 1995; Kruger et al., 1997a, 1997b; Zablotowicz et al., 1997).

In general, the rhizospheric soil has a potential to support degradation of various agrochemicals, mostly because of enhanced microbial activity. However, the extent to which the rhizosphere effect is species specific is poorly understood. Some plants such as leguminous plants obviously support large populations of certain microbes. These may have selective capacity to degrade various pesticides. Nevertheless different plant species have been identified that release aromatic compounds either as root exudates or through root turnover. These plants may selectively stimulate microbial populations which degrade aromatic compounds like PCBs, PAHs, and pesticides like DDT (Leigh et al., 2002).

An alternative to the use of plant-associated bacteria to degrade toxic organic compounds in soil is the use of recombinant DNA technology to generate transgenic plants expressing bacterial enzymes exhibiting improved tolerance in plants and catabolic activity against toxic organic compounds in soil (Kawahigashi et al., 2009), organic explosives (Aken, 2009), TCE (James and Strand, 2009) and PCBs (Sylvestre et la., 2009), using bacterial genes encoding pollutant degrading enzymes have been used in the detoxification of the target organic contaminant. Villacieros et al. (2005) reported that the modified rhizospheric bacteria colonized roots as effectively than the wild type rhizospheric bacteria and rhizospheric bacteria could degrade PCBs more efficiently than the wild type rhizospheric bacteria, indicating considerable potential for the manipulation of the rhizosphere as a useful strategy for bioremediation. This phyto-technology using *Amaranthus caudate*, *Lactuca sativa*, *Nasturtium officinale* and *Phaseolus vulgaris* could detoxify and degrade malathion and dimethoate during cultivation periods (Fahd and Ahmed, 2009).

Evidence showed that transgenic plants could secrete organophosphorus hydrolase (OPH) into the growth medium. The transgenic plants were resistant to methyl parathion (Mep), as evidenced by a toxicity test showing that the transgenic plants produced greater shoot and root biomass than did the wild-type plants. Furthermore, at 0.02% (v/v) Mep, the transgenic plants degraded more than 99% of Mep after 14 days of growth. Transgenic plants expressing an OPH gene may provide a new strategy for decontaminating organo phosphorous pollutants (Wang et al., 2008).

Though phytodegradation is still a relatively new area of green research, there are many research groups engaged in studying the underlying science necessary for a wide range of applications for plant-based remediation system against organic contaminants (Newman and Reynolds, 2004).

Much work remains to be done in carrying out field studies based on laboratory-scale results/experiments using plant-associated endophytic and rhizospheric bacteria to degrade a wide range of toxic organic compounds of concern in environmental soil before commercially viable systems. Using biotechnology tools, bacterial strains can be engineered expressing specific enzymes to degrade toxic organic substances in an effective way. Additionally bacteria (rhizospheric and/or endophytic) can be engineered, through gene transfer to degrade toxic organic pollutants present in prevailing environment. However, genetic engineering of endophytic as well as rhizospheric bacteria along with transgenic plants is seems to be promising approach for remediation of contaminated environmental sites (McGuinness and Dowling, 2009).

CONCLUSION

Decontamination of polluted areas is the need of hour. The conventional means (physio chemical methods) of degradation of toxic recalcitrant chemicals are not only expensive, labour intensive less efficient but may harm

the natural microenvironment of soil as well. Biodegradation is becoming a method of choice for the remediation of polluted site. To mitigate hazardous chemicals from environment collaborative use of biological agents (bacteria, fungi plants), along with innovative molecular techniques must be conducive, to the effects of each other, can be applied over a larger area of land/water. Further studies should be conducted to investigate the mechanisms by which the plants, microorganisms and their enzymes can assimilate these compounds. Knowledge of these enzymatic processes, especially concepts related to pesticides mechanism of action, resistance, selectivity, tolerance and environmental fate, has advanced our understanding of pesticide science and of plant and microbial biochemistry and physiology.

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