

**Determining The Detoxification Potential of Some Soil Bacteria and Plants on Bioremediation of Deltamethrin, Fenvalerate and Permethrin Pesticides**

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**Abstract**

The aim of the study was upgrade the phytoremediation method for cleaning receiving environmet exposed to three kind of pesticides (Deltamethrin, Fenvalerate and Permethrin) for application of selected specific soil bacteria and agricultural plants. Efficient biodegradation of herbicides by bacteria depends of effectively uptake by the crops and expose aerobic biodegradation via detoxification enzymes of plants. These treatment techniques can take up degradation of toxic compounds into nontoxic compounds. The results of this study using *Microbacterium chocolatum*, *Ochrobactrum thiophenivorans*, *Sphingomonas melonis*, *Sphingomonas aquatilis* and *Bacillus subtilis* and crop phytoremediators in the experimental studies are presented. As a result of the experiments, It is understood that the using of recent techniques come close to decrease effects of pesticide pollution in artificially polluted agricultural lands.

**Keywords:** Phytoremediation, pesticide, biodegradation, enzyme

**Research article**

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## **INTRODUCTION**

The contamination of the environment by herbicides is a major problem (Mohamed et al., 2016). The enzymes that has a role in biotransformation are related with metabolizing enzymes. On the chemical structure of foreign compounds, their metabolic activities may also be used to identify the role of a livings, via their enzymes (Hodgson, 2010). Although Bioremediation methods of treatment of persistent organic pollutants are economically, effective, ecologically and also profitable for bioremediation of receiving environments, in addition, organochlorine herbicides have low productiveness as only a few strains of bacteria are adaptable to show the effective mineralization of these pesticides as a result of oxidative and non oxidative transitions (Khalid et al., 2017). Dehalogenation is principal mechanism for methydetoxification of various organochlorine pesticides, making them vulnerable to attack by other biodegradative (Shehu et al., 2019). Plants and biodegraders used for phytoremediation ativities may be suitable in case of pollution with organochlorine pesticides (Kurashvili et al., 2014).

Bioremediation of herbicides that comprises the potential of bacteria/fungi in the treatment of persistent organic compounds is related with low cost process. This process comprises the organic compound into inorganic compound. For the reason of properties of microorganisms, their biomass comparative to other organisms in the receiving environmet, and catalytic capabilities in their process (Paul et al., 2005), and their properties to purpose even in the deficiency of oxygen for chemical materials bioremediating bacteria, understanding their biochemistry, and also recent methods for their practical in the receiving environments have evolved in recent years Megharaj et al., 2011).

While herbicides exists in receiving environment, there is likely difference in biodegradation capabilities and microbial populations of receiving environments are composed of microbial communities instead of a single strain (Ramakrishnan et al., 2011)..

In habitat, biodegradation/bioremediation includes products within a well-coordinated bacterial community and transferring the substrates (Abraham et al., 2011). Most of the bacteria have the interaction properties, both physically and chemically, with substances preeminent to conclude biodegradation of target pollutants. Most of the microorganisms are pesticide degraders (Briceño et al., 2007).

When the biotransformed persistent organic pollutants are released into the soil or aquatic system, it is affected by by microorganisms (Diez, 2010). Microorganisms are known as the extracellular enzyme-producers. These properties are related with the production of enzymes that has an important role on persistents organic compounds. Most of the enzymes are taken in lignin degradation, such as oxidases, manganese peroxidase, lignin peroxidase and laccase. Various bacteria that biodegrade persistent organic pollutants have been isolated and the number of them is increasing day by day. These enzyme types involved in biodegradation are, glutathione S-transferases (GSTs), cytochrome P450 and esterases (Diez, 2010). These enzymes are very important for the biological properties of many herbicides (Riya et al., 2012). Certainly, many researches have proved that some pesticides are more toxic than other types which are first activated in vivo during metabolization by cytochrome P450 monooxygenas (Jokanović, 2001; Rinnofner et al., 2019). Enzymatic degradation of persistent organic pollutants is an advance remediation method for treatment of them from receiving habitat.

Enzymatic biodegradation of persistent organic pollutants can be more useful than other complex methods. Most of persistent organic pollutants are activated in situ by the

enzymes, and many herbicides activates by aiming enzymes with substantive physiological functions. (Scott et al., 2008; Rinnofner et al., 2019). Previous studies have shown that the enzyme can be produced at a large scale after immobilization into polyurethanes which may be considered in particular for incorporation into filtration devices (Guendouze et al., 2017).

The biodegradation includes enzymes converging on particular intermediates form a funnel topology, the novel response prevails in the exterior part of the network, and finally, the sustainable pathway between pesticides and the centre of the metabolism can be arrived in view of all the required enzymes in a given organism (Trigo and Valencia, 2009). For biodegradation of herbicides, three enzyme systems are most useful. These are; esterases, hydrolases, the mixed function oxidases and glutathione S-transferases (Li et al., 2007).

Biodegradation methods is related with the metabolic potential of bacteria/fungi to detoxify the herbicides, which is dependent on bioavailability (Ramakrishnan et al., 2011). In the metabolism of pesticide step, the properties of herbicides are transformed via oxidation process and turned to generally produce a less toxic product than the original form. Other step includes conjugation of a herbicide to an amino acid, which reduces the toxic effects according to the original form. The last step comprises changeover of second step metabolites into secondary non-toxic conjugates. In these steps, microorganisms are changeover producing extra cellular enzymes (Ortiz-Hernández et al., 2013; Van Eerd et al., 2003).

The main purpose of our study is the understanding bioremediation and phytoremediation potential of targeted three kind of pesticides for remediate polluted receiving environment, with application of plants and microorganisms.

## **MATERIAL AND METHOD**

### **Pesticides, plants and agricultural soil used in the study**

Pollutants investigated in this study were Deltamethrin (IUPAC NAME: (S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate), Fenvalerate (IUPAC NAME: cyano-(3-phenoxyphenyl)methyl] 2-(4-chlorophenyl)-3-methylbutanoate and Permethrin (IUPAC NAME: [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate). Maize, Alfalfa and soybean used for testing.

Crops after application on pesticides (0.01, 0.1 or 1 mM) with five days were cleaned with pure water, sprouts and roots separated in 0.05 M phosphate solution at pH 7.4. Separates were tightened through muslin cloth and they centrifuged at 1000 g for 30 minute for acquire the supernatant activities of enzymes. The artificial land has been polluted with Deltamethrin, Fenvalerate and Permethrin for model study step. The soil type used in the study was fertile, alkaline and humid. Maximum soil particle was about 2 mm. The experimental studies have been continued in 250 ml of Erlenmayer flasks. Each air dried sample equals approximately 100 g. About 100 ppm of pesticide applied in soil samples for pollution. At the starting phase of experimental studies, the suspension of microorganism inoculated in the studied polluted agricultural soil about 20%. The samples have put in the thermostat at room temperature over five days. The grains of crops planted in divided samples of agricultural soil (20 grains for maize, soybean and alfalfa in every sample). Experimental studies contains two type of control with and without water.

### **Isolation and identification of bacteria**

In bacteria isolation process, agricultural soil samples taken from agricultural fields of Mediterranean, East Anatolian and Marmara region of Turkey transferred in sterile glass containers (Zelles et al., 1991). 10 g of agricultural soil was diluted to  $10^{-4}$  in 0.8% sodium chlorate isotonic solution. In a laminar flow sterile cabin, 0.1 ml of this sample was inoculated into plate count agar (PCA) which was sterilized in an autoclave before. After this phase, the petri dishes were put in a incubator at 28°C; about five days while the growing phase of the bacteria finished. For identification of bacteria, Phire Hot Start II DNA Polymerase was used. Polymerase Chain Reaction (PCR) bands of various lengths (1000–3000 bp) were gained through 16S ribosomal general primers. 16S rRNA forward primers used were as “AGA GTT TGA TCC TGG CTC AG” while 16S rRNA reverse were as “ACG GCT ACC TTG TTA CGA CTT”

### **Determination of pesticides**

The model experiments carried on with 40 days. The bacteria in The Munzur University Department of Environmental Engineering Collection of Microorganisms Cultures were used for determining the biodegradation properties on herbicides. The capability of bacteria to degrade organic pesticides was revealed by growing phase on PCA media at 28°C. For the screening modified PCA media, containing Deltamethrin, Fenvalerate and Permethrin was used. As inoculant, vegetative culture grown up to the exponential phase of growing media used. The PCA media were inoculated with 20% of the microbial suspension.

For determine amount of Deltamethrin, Fenvalerate and Permethrin residues in the soil, 10 g of soil sample were combined with anhydrous sodium sulfate and then these samples were extracted with a proper solvent using EPA 3550C (EPA, 2007) ultrasonic extraction method. For analytical determination of pesticides, the extracted samples were taken into a 250 ml beaker, and surrogate spiking solutions and matrix spiking (1.0 ml of each) solutions were added to the samples. The soil samples were scanned twice for 30 min with 50 ml of the extraction solvent mixture (1:1 Hexane and acetone for GC-ECD analyses pure) in ultrasonic bath. The extract supernatants were filtered through Whatman Grade No. 41 Quantitative Filter Paper, Ashless, Whatman 1441-047/ 28477-974 using a Buchner funnel. For eliminate unwanted interaction of organic matters such as PAHs, PCBs, etc., an alumina-silicic acid column used. These chemicals were heated at 450°C in baker for 6 hours and then let cooled down to the room temperature in a desiccator. Separation column was forged by 3 g silicic acid that contents of 3% ultra pure water, 2 g neutral alumina that consist of 6% ultra pure water, and 2 g Na<sub>2</sub>SO<sub>4</sub> according to the Ref. given by Jantunen et al., (2000). After this process ended, column was pre-washed with 20 ml of dichloromethane solvent. Sample was evaporated to 2 ml and spilled to the column. At least, dichloromethane was added to resolve the herbicides according to Cindoruk (2011). Aliquot part of the samples were placed into a concentrator tube and evaporated to 1 ml in a warm bath using a gentle stream of clean dry nitrogen, for analyzing procedure. Dichloromethane was used for washing up step. After that, the final extract (approximately 1 ml) was analyzed for the Deltamethrin, Fenvalerate and Permethrin residues using the method described in EPA 8081B (EPA, 2007). Retention time for tested pesticides are: Deltamethrin: 7.1 minute; Fenvalerate; 7.5 minutes and Permethrin; 7.0 minutes. The quantification of all pesticides was carried out using a Perkin Elmer Clarus 500 gas chromatograph with Electron Capture Detector.

**Determining protein content**

For determining the protein content of the samples, Bradford (1974) method was chosen. Phenoloxidase activity was identified spectrophotometrically at 420 nm, according to the pyrocatechol oxidation (Lanzarini et al., 1972), while Peroxidase activity was identified at 470 nm, in conformity with the rate of H<sub>2</sub>O<sub>2</sub> addicted oxidation of guaiacol. Activity of monooxygenase was identified polarographically, by oxygen depletion rate at NADP-H addicted oxidation of N, N-dimethylaniline (Khatisashvili et al., 1995), while Glutathione S-transferase activity identified spectrophotometrically at 340 nm with the line of Schroder and Rennenberg, 1992). Definitive activities adjusted as mmole dissipated oxygen in min per mg protein and mmole 1-chloro-2,4-dinitrobenzene (CDNB) in min per mg protein respectively.

**Statistical analysis**

All of the results obtained from experimental studies were evaluated with SPSS (SPSS Inc, Chicago, IL, USA). The values are the averages of the results of three replicates of each experiment with a standard error (SE). To compare the Enzyme activity and removal efficiencies, the datas were analyzed by analysis of variance (ANOVA).

**RESULTS AND DISCUSSION**

**Determination of pesticide metabolism**

Studies for determination methods of metabolism of pesticides in used crops accodingly of used herbicides on the process of detoxification enzymes (cytochrome P450, glutathione stransferase and peroxidase) were fulfilled. In *Zea mays*, nearly all examined enzymes are influenced for modification of Deltamethrin and Fenvalerate, merely the activation of glutathione S-transferase occure for Permethrin (Figure 1-3).

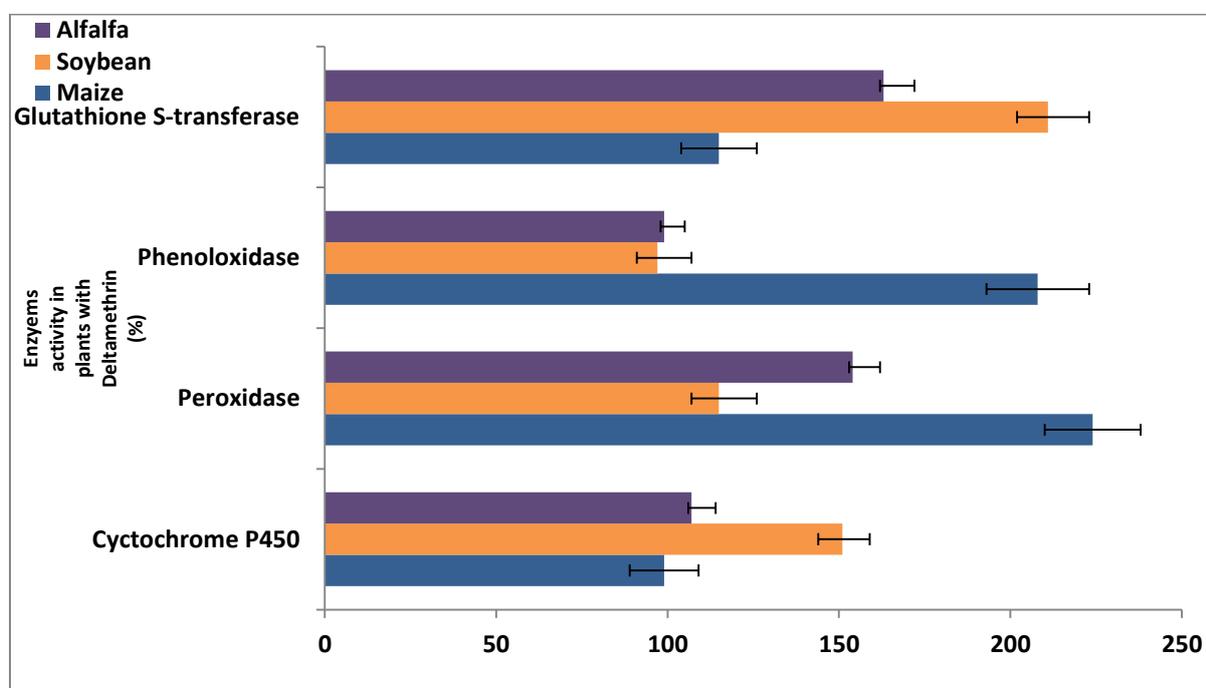


Figure 1: Causation of oxidative enzymes in roots of two weeks old plantings after developing on solution 0.1 mM of Deltamethrin during ten days. The enzymatic activation in control differencies are take into account as full.

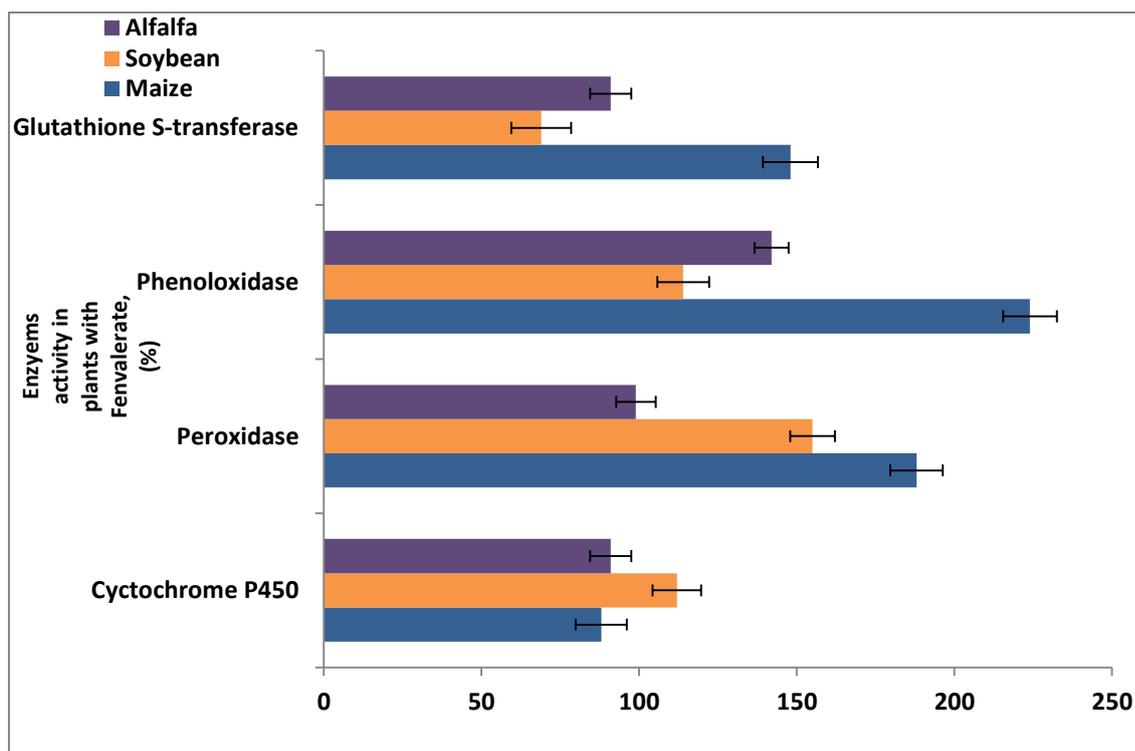


Figure 2: Causation of oxidative enzymes in roots of two weeks old plantings after developing on solution 0.1 mM of Fenvalerate during ten days. The enzymatic activation in control differences are take into account as full.

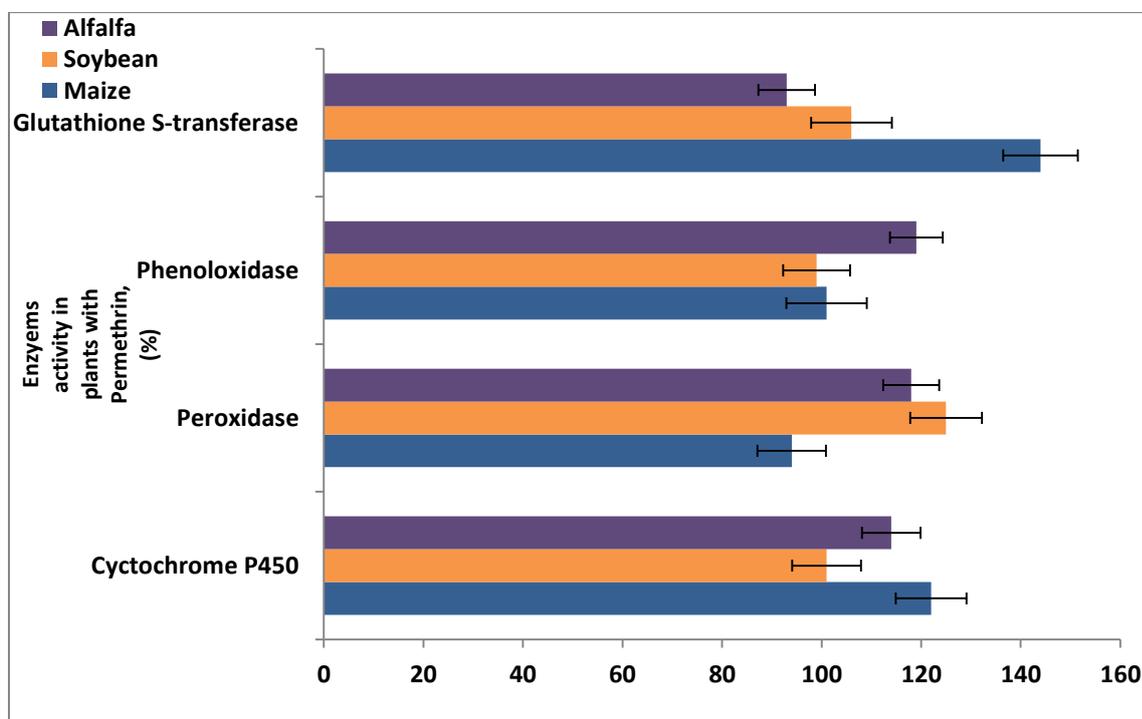


Figure 3: Causation of oxidative enzymes in roots of two weeks old plantings after developing on solution 0.1 mM of Permethrin during ten days. The enzymatic activation in control differences are take into account as full.

Detoxification of non-polar molecules of Deltamethrin and Fenvalerate, the ineptive subsequent and oxidation junction is essential. Differently from Deltamethrin and Fenvalerate, Permethrin includes polar hydroxyl group and Permethrin can be exposed to manage conjugation with glutathione. Comparable outcomes acquired for another crops or plants, but causation influences like them are less stated. At the outcome of the study, different induction degree of enzymes take part in conjugation and oxidation of studied pesticides action on crops occurred. When each detoxification enzyme catalyze conjugation or oxidation response, it is understood that the transformation of studied herbicides in crops develops via various ways. Deltamethrin exposed to first oxidation by peroxidase, monooxygenase, phenoxidase and subsequent conjugation by glutathion S-transferase and this situation is mainly due to footpath of this pesticides modification. Additionally, these pollutants may be coupled by glutathione S-transferase directly. Fenvalerate exposed to oxidation by monooxygenase, peroxidase or phenoxidase and subsequent conjugation by glutathione S-transferase primerly. The basic tools of metabolized Permethrin exposed squarely conjugation by glutathion S-transferase and other tools are conjugated after primary oxidation by monooxygenase and peroxidase.

### **Biomass studies**

*Microbacterium chocolatum*, *Ochrobactrum thiophenivorans*, *Sphingomonas melonis*, *Sphingomonas aquatilis* and *Bacillus subtilis* identified and used for monitoring on solid nutritive area with Deltamethrin, Fenvalerate and Permethrin. According to the results, after cultivation on Deltamethrin contaminated area, *Microbacterium chocolatum* reveals best growth with glucose. In media with Fenvalerate, *Ochrobactrum thiophenivorans* reveals best growth with glucose and at least, *Sphingomonas melonis*, *Sphingomonas aquatilis* and *Bacillus subtilis* exposes best grown are selected with madium with glucose. After refinement with Permethrin, *Bacillus subtilis* unveils best growth with glucose. The affect of Deltamethrin, Fenvalerate and Permethrin pesticides on biomass accretion by selected bacteria were determined. According to the experimental results, *Microbacterium chocolatum* Deltamethrin, *Ochrobactrum thiophenivorans* in Lindane and *Bacillus subtilis* in Permethrin accumulate biomass higher than on Czapek's agar without glucose.

### **Bioremediation studies**

The detoxification capacity of bacteria that related with biomass accretion properties at submerge culture for pesticides containing areas determined. Pesticide residual analyses results gained from GC-ECD, in incubation medium after planting, high pesticide degradation efficiencies of the bacteria have been determinated. These bacteria can biodegrade pollutants from agricultural area with removal efficiency of nearly 80% from suggested concentration for farmers and these results are also can be helpful highly-developed phytoremediation methods. Similar studies for expand of new advance of phytoremediation methods for remediate agricultural fields caused by these type of pesticides have been also investigated. For example, the microbial consortia (Mixture of bacteria and/or fungi) and crops like *soybean*, *maize* and *alfalfa* studied. Phytoremediation of agricultural soils contaminated by Deltamethrin is very effective with using of *Microbacterium chocolatum* concertia with soybean. Deltamethrin content in agricultural soils decreased by 76%. For phytoremediation of agricultural soils contaminated by Permethrin shows best removal performance using of *Bacillus subtilis* with maize as 66%. In experiments with Fenvalerate, the content of pesticide residuals decreased by bioremediation capacity of *Ochrobactrum thiophenivorans* and maize by 67% (Figure 4).

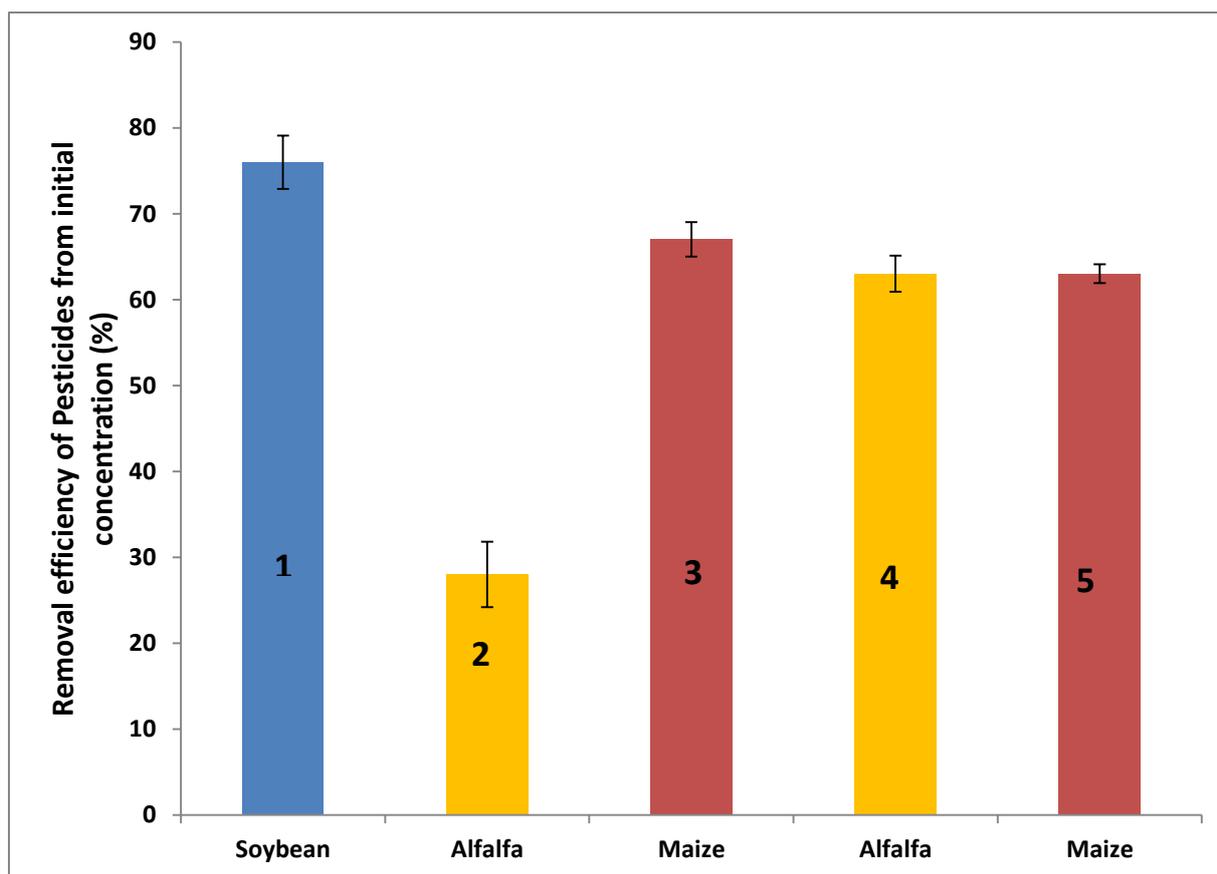


Figure 4: The results of model experiments using bacterial strains and crops for bioremediate agricultural soils contaminated with herbicides. Column 1: Pollutant: Deltamethrin; Bioremediation agent: *Microbacterium chocolatum*; plant: soybean. Column 2: Pollutant: Fenvalerate; Bioremediation agent: *Ochrobactrum thiophenivorans*; Plant: Alfalfa. Column 3: Pollutant: Fenvalerate; Bioremediation agent: *Ochrobactrum thiophenivorans*; Plant: Maize. Column 4: Pollutant: Permethrin; Bioremediation agent: *Bacillus subtilis*; Plant: Alfalfa. Column 5: Pollutant: Permethrin; Bioremediation agent: *Bacillus subtilis*; Plant: Maize.

Thus it can be understood that model studies reveals new technological perspective discovered in recent years is successful for phytoremediation of agricultural lands contaminated by these kind of pesticides. Microorganisms biodegrade persistent organic pollutants and interact with the environment. This issue is important for successful implementation of the technology for in situ remediation (Hussain et al., 2009).

Herbicide detoxification was first identified by microorganisms that genetically changed and the genes encoding these hydrolases were *Escherichia coli*, *P. Pseudoalcaligene*, *Yarrowia lipolytica*, *Streptomyces lividans* and *Pichia pastoris* (Wang et al., 2012). Different method for bioremediate herbicides is phytoremediation. This method is economically and environmentally friendly (Eapen et al., 2007). In what way, the limitation with crops is that they lack the catabolic pathways for full biodegradation of herbicides. The properties of agricultural crops to biodegrade pesticides encourage by crops via effective genes that are embody in the biodegradation of these toxic pollutants (Singh et al., 2011).

Recent years studies identify that nearly most of the bacteria present in soil and aquatic environment are not cheerfully culturable and consequently not adaptable for biotechnological studies (Zhou et al., 2010). According to result of some recent studies about biodegradation and phytoremediation of organochlorine pesticides (Lindane, DDT and PCP) on the growth parameters of different crops, the using of these crops for bioremediate soil was not adequate because of low solubility and bioavailability.

For handle with this negativeness, Kurashvili et al. (2014) found a new bioremediation system with high detoxification potential for efficiency reducing the effects of organochlorine pesticides from receiving environment. According to their study, this system based on microorganisms that degrade initial degradation of pesticides with the action of enzymes. Cavka and Jönsson, (2014) found that *S. cerevisiae* strain exhibited the best capability to grow in high concentrations of lignocellulosic media, which indicates that it has better resistance to inhibitors according to other types of fungi. In their study, they found, *P. Pastoris* and *S. cerevisiae* reduced mannose and glucose while the other bacteria or fungi were more varied from a metabolical perspective.

As a results of the experiments, *maize*, *alfalfa* and *soybean* are qualified with causation of detoxification enzymes like cytochrome P450 containing monooxygenase, peroxidase, glutathione S-transferase and phenoloxidas. It is indicated that the transformation of Deltamethrin, Fenvalerate and Permethrin in plants mainly due to pathways of direct oxidation, or oxidation and subsequent conjugation, or conjugation. According to the experimental results, *alfalfa*, *maize* and *soybean* as crops remediators and *Sphingomonas melonis*, *Sphingomonas aquatilis* and *Bacillus subtilis* as biodegrading tools for phytoremediation method aimed to remediate receiving environments contaminated with these type of pesticides have been chosen. As a results of experimental models, the discovered treatment will be alternatively and prevalently used for remediate the receiving environments such as agricultural fields contaminated with these pesticides. The toxicity of parathion-ethyl, diazinon, fenitrothion and biodegradation products of them generated through enzyme hydrolysis was investigated. Each pesticides were confirmed by chromatographic and spectrometric analysis and determined to be in accordance with catalytic mechanisms (Elias et al., 2007), Rodriguez et al., (2010) studied paraoxon-ethyl concentrations up to 800  $\mu\text{M}$  for incubating planarians while concentrations ranging from 13  $\mu\text{M}$  up to 57  $\mu\text{M}$  for recently used pesticides. *D. japonica* for concentrations up to 500  $\mu\text{M}$  was also described and it was found that dichlorvos was 100 times more toxic than chlorpyrifos suggesting that toxicity is strongly dependent on the pesticide (Hagstrom et al., 2015).

### CONCLUSION

The results show that *maize*, *alfalfa* and *soybean* indicate detoxification induction of enzymes used in the study. The transformation of the examined herbicides in crops generally occur through direct oxidation, direct conjugation, or through oxidation and subsequent conjugation pathways. The results indicate that *maize*, *alfalfa* and *soybean* can be used as plant phytoremediators; some soil bacteria as herbicide biodegradation agents, for decontamination of contamination of an environment caused by herbicides. Thus, according to the results of the model experiments, subsequent biotechnological method is effective in remediating soil from pollution of herbicides. Bacteria also have a similar degradative process of herbicide, and unlike plants they genetically rapidly adapt to chemical substances. The degradation and detoxification potential of microorganisms are being used for contamination of receiving environments caused by a wide variety of pesticides. Additionally, phytoremediation, known as a process where microorganisms and plants jointly detoxify and degrade contaminants resulting in eradication of contaminants.

Bioremediation process for detoxification of herbicides from the contaminated environment has now emerged as the best option. Today, various bioremediation approaches are available to address the problem of decontaminating the environmental compartments from these hitherto essential toxicants at least needed for vector control despite the persistent and recalcitrant nature of several pesticides as well as their associated health hazards. Pest control over the last years had a significant change due to biotechnology techniques and there is a continual exploration of interesting new perspectives.

However, such perspectives continue to be reliant on more advances in the microbial of whole-plants. An understanding of the biodegradation mechanism, enzyme expression implicated in herbicide metabolism may solve problems. Finally, our study will help in promoting an appreciation of the environmentally unharmed and commercial applications of herbicides. With high budget projects, changes in the molecular structure of pesticides may be monitored. In addition to this, the differences that occur in the genes of the microorganisms that are involved in the decomposition process may also be observed. Since agricultural areas include microorganisms which decompose pesticides, studies may be conducted on different pesticides with microorganisms obtained from different agricultural soils.

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