

Interactions of antioxidant defense mechanisms developed by plants and microorganisms against pesticides



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ABSTRACT

In the developing world and increasing human population, great importance is given to food security and crop cultivation in climate change. Therefore, different strategies are needed to maintain and revive the crop resources required for agriculture. Pesticides in soil solution can either be adsorbed by soil colloids or degraded by microorganisms. The use of pesticides against diseases and pests that reduce the yield of plants continues to increase day by day. However, it also threatens the life of living things. Over time, many insects and weeds resistant to pesticides have emerged, and it has been seen that the dose of pesticides should be increased. This situation harms both the environment and the economy. Pesticides stay in the soil for many years, preventing microorganism activities and causing oxidative stress induction. Besides, it is known that pesticides inhibit enzyme activities by participating in some enzymatic events. In this review, we discussed the antioxidant defense mechanisms that develop in microorganisms due to the effect of pesticides on microorganisms and provide biodegradation. When exposed to pesticide toxicity in microorganisms that provide biodegradation, specific biological systems characterized by enzymatic reactions become active. In cases where the enzyme defense of microorganisms is insufficient, biotransformation is carried out by artificially supplementing the environment with enzymes; It contains a series of enzyme-catalyzed reactions, and in this way, the negative factors that the plant is exposed to can be eliminated or mitigated. Microbial enzymes provide biodegradation. In addition, to prevent the biodegradation effect; It is essential to examine this subject to improve the understanding and practical use of enzyme use, enzyme extraction methods and future enzyme-assisted bioremediation processes for their development and evaluation.

Keywords:

Pesticide, microorganisms, plant, antioxidant defense mechanisms

1. Introduction

Especially pesticides, which are chemical wastes, are the main causes of soil pollution. XX. Since the beginning of the century, the developing industry and technology have brought intensive agricultural practices to obtain more products from the unit area with

the increasing world population. Accordingly, plants and herbal products have been inevitable to use pesticides or pesticides to be protected from the effects of pests, disease factors, and weeds and obtain quality and abundant products[1, 2]. Pesticides are synthetic organic compounds used to destroy

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undesirable organisms in products. All kinds of drugs and preparations used for plant protection and the substances used in their manufacture are included in this group. In addition to the benefits of pesticides, it has been determined that they harm the ecosystem and human health due to their long-term use, so some rules limit the use of chemicals for agricultural purposes. The importance of pesticide residues was first understood in 1948 by discovering organic chlorine pesticide residues in the human body. While some pesticides do not cause harm in terms of toxicology, it has been determined that some of them are carcinogenic and affect the nervous system. The most important source of pesticide residues in food. For this reason, the "Codex Committee for Pesticide Residues" was established by FAO and WHO in 1960. As a result of the work of this committee, definitions on the subject were made, and the maximum residue values allowed in foods were determined based on scientific research data [3, 4].

Pesticides are applied to agricultural fields; it passes into the air, water and soil, and from there to other living things living in these environments and transforming. The actions of a pesticide in the environment are affected by factors such as its chemical structure, physical properties, formulation type, application method, climate and agricultural conditions. Pesticides are used worldwide to protect agricultural products from pests, diseases and weeds and obtain quality products. As a result of this use, some problems arise regarding human, animal and environmental health [5, 6].

Pesticides can be classified according to their target organism, chemical structure and physical state. Pesticides include microbial and biochemical pesticides. The term pesticide includes all chemicals that can be listed as algicide (alg killer), avicide (bird killer), bactericide (bacteria killer), fungicide (fungus killer), herbicide (weed killer), insecticide (insect killer), ovicide (egg killer), larvicide (larva killer), adulticide (adult killer), acaricide (Acar killer), molluscicide (slug and snail killer), nematicide (nematode

killer), rodenticide (rodents killer) and viricide (viral killer)(Fig 1) [7].

Soil, which forms the basis of life for humans, animals, plants and microorganisms, produces carbohydrates, proteins and fats necessary for animals and humans through the assimilation of plants. On the other hand, microorganisms break down the dead plant, animal and human mass in the soil and ensure the recirculation of the main nutrients to the ecosphere. Agricultural pesticides are applied directly to the soil surface and inside, on the plant or the seedlings in seed spraying. Most of the pesticide thrown on the plant falls into the soil. If the applied pesticides are permanent, significant drawbacks arise. Soil acts as an effective buffer and filter against pesticides, keeping harmful substances in physicochemical and biological ways[8-11].

Pesticides that pass into the soil are subject to photochemical degradation by the effect of sunlight and biologically degraded by the effect of plants, soil microorganisms and other organisms; They are adsorbed (adhesion on the surface), desorbed (separation from the surface) or chemically degraded by soil solids (clay and organic matter). Pesticides that have passed into the soil are carried to the soil surface by capillary water and mixed into the air. The structure of the soil, clay type and amount, organic matter content, iron and aluminium oxide content, pH and the dominant microorganism species in the soil are the factors affecting all these events. With the retention of the pesticide in the soil, its movement and biological uptake are inhibited. It either loses its toxic properties or becomes more toxic by degradation in various ways. It is important to know and examine all these events, as it is not desired that the pesticide itself or its toxic transformation products contaminate non-target places or organisms[12].

Soil microorganisms regulate the physical and chemical structure of the soil. With their killing, the balance in the soil is disturbed. Pesticides can break down into harmless forms due to soil microorganism activities, and in some cases, prevent the beneficial activities of microorganisms for nature. For example, pesticides cause the death of

earthworms, which play an important role in increasing soil fertility. Pesticides can directly or indirectly affect a microbial population by altering its metabolic and physiological activity. Plants, animals and other microorganisms affected by the pesticide can cause different situations to arise with the deterioration of their balance. According to the common view of researchers working on pesticide microorganism interactions in field

conditions, due to the accumulation of fungicides, insecticides, and herbicides in the soil, little change occurs in the microbial environment. However, larger changes can occur when pesticides are applied in large quantities and frequently. Microbial activities such as carbon dioxide production, oxygen consumption, nitrification, growth rate, and legume nodulation are indicators used to measure pesticide response[13, 14].

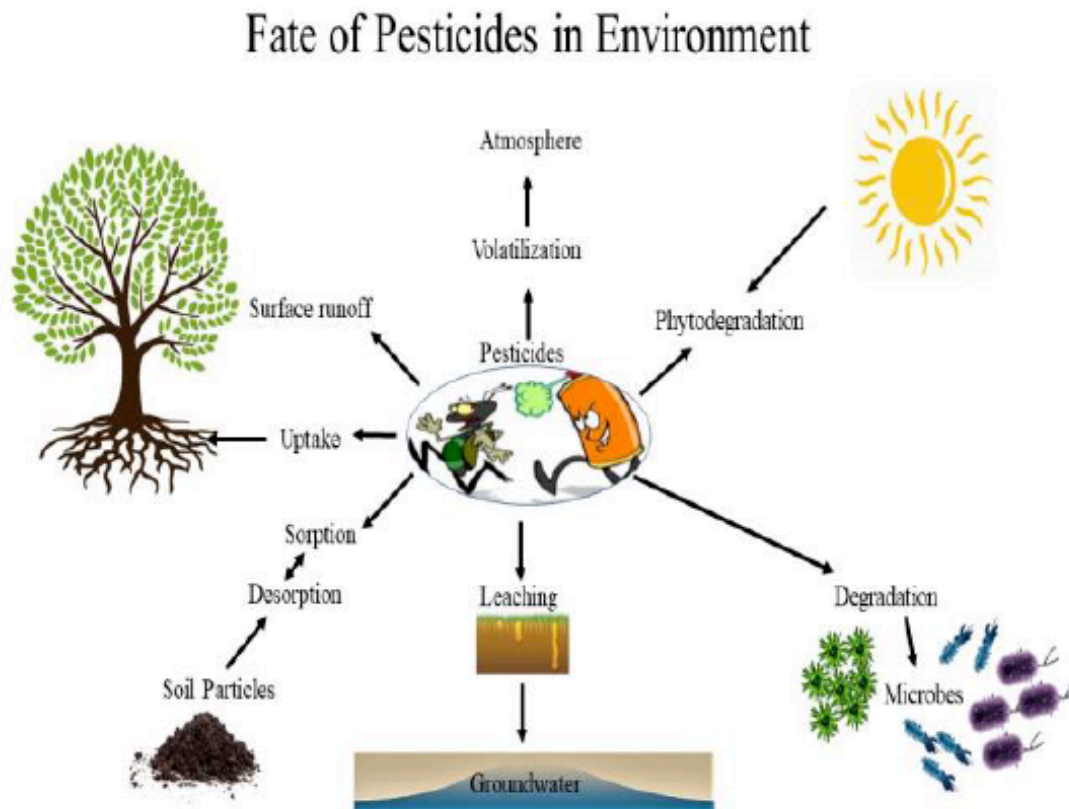


Fig 1. Pollution of pesticides in the environment [[15]

Pesticides trigger ROS production and lipid peroxidation by inhibiting photosynthesis when the concentration amounts in the plant are excessive. Thus, it can cause significant damage to the plant [16]. When plants are stressed by pesticide uptake, they have elaborate defence mechanisms to reduce pesticide uptake and transport [17]. These mechanisms remove reactive oxygen species by defensive antioxidants [18, 19]. The effects of this toxicity on plant soil and microorganisms and the detoxification mechanisms of plants should be investigated in detail[2, 20].

2. Relationship of pesticides with microorganism

It progresses through economically, efficiently and safely elimination from pesticide-contaminated areas. The aim is to clean contaminated sites before going into production [21]. Microbial bioremediation and phytoremediation applications are more effective in the improvement of pesticides. Rhizodegradation is a type of remediation that allows both plants and their growth to increase rhizosphere microorganisms by secreting plant root exudates [22]. For example, it was observed that the removal of

aldicarb pesticide, which contaminated the soil, was degraded by the plant. Bioremediation with microorganisms can also be done by stimulating inoculation of pesticides that disrupt the structure of microorganisms [23].

2.1 Microbial degradation of pesticides

For pesticides to be taken into the plant, pesticides must first be dissolved or broken down. Soil is the medium most affected when we apply pesticides to agricultural products. Pesticide critical areas also include pesticide industry wastewater, activated sludge, sediments and sewage sludge. Many laboratories have collections of microorganisms with pesticide solubility. Isolation and characterization of these microorganisms are carried out to purify the contaminated area. The decomposed pesticide accelerates the metabolism of microorganisms by forming CO₂ and H₂O with the oxidation of the parent compound. These microorganisms' intracellular or extracellular enzymes play a vital role in the degradation of microorganisms [24].

The fraction of soil biota can be applied continuously to improve the degrading ability of some of the pesticides. These chemicals constitute the necessary electron donors and carbon source [25]. A pathway is emerging for the cleaning of contaminated areas [26]. Pesticide-degrading microflora should be added when the soil's natural microorganisms are insufficient for persistent pesticides in the soil [27]. Therefore, isolated microorganisms suitable for biodegradation may be preferred [28]. In addition, it depends on the available nutrients, water potentials, pH and temperature of the environment. Some pesticides are very difficult to degrade in the soil [24]. At the same time, it has a role in ecological, physiological, molecular and biochemical [29].

Microbial degradation (biodegradation) is the primary source of pesticide degradation in soils [30]. Catalyst enzymes in this degradation cause significant differences in pesticides' toxicological properties and structure [31]. It occurs when microorganisms use pesticides and soil nutrients as a source of C and energy.

Researchers say over 5000 bacterial populations in 1 gram of soil can be found. This population's large population offers a more straightforward, more environmentally friendly and inexpensive strategy [32].

They activate microorganisms increases in soils with neutral pH, moist and average ambient temperature. Although the biodegradation of pesticides has been known for a long time, the adaptation mechanism of pesticides is not understood. Microorganisms can obtain genetic material encoding the required biochemical mechanisms [30]. After the pesticide in the soil enters the microbial cell membrane, it can be metabolized through internal enzyme systems. While biochemical metabolism can be remarkably rapid through enzymes, the process can be prolonged for other compounds. Biodegradation of organic compounds; They decompose rapidly and begin as a source of energy and growth. Thus, organic compounds are easily used. Sometimes biodegradation begins slowly, followed by rapid degradation. It can be said that this is in a period of getting used to. On the other hand, persistent organic compounds biodegrade rather slowly [31].

3. Plant defence system against pesticide toxicity

3.1 Antioxidant defense mechanism relationship between plants and microorganisms against abiotic stress

Organic and inorganic pollutants (including pesticides, polycyclic aromatic hydrocarbons (PAHs), explosives, polychlorinated biphenyls (PCBs) and aromatic and halogen-containing compounds cause pollution of the earth's water and soil reserves, resulting in the deterioration of plant and food quality associated with them [33]. These deteriorations also threaten other organisms, including humans and animals [34]. These toxic organic pollutants mixed with soil and water affects humans and animals and affect the number, distribution, and functioning of rhizospheric and endophytic microorganisms in the soil. It also affects microorganisms by changing [35]. For example, it has been reported that pesticides and herbicides containing chemicals such as glyphosate, endosulfan, paraquat, fipronil and aldrin are

carcinogenic, endocrine disruptors, neurotoxic, and cause fatal damage to the reproductive system and various vital organs such as the liver and kidney. Although most of these organic compounds are forbidden or greatly restricted due to their harm [36], The application of these pesticides is widespread to continue[37].

Dichloro diphenyl trichloroethane (DDT), a member of the organochloride class, was the first synthetically produced pesticide. It was widely used to control insect and insect-borne diseases [37]. However, it has been reported that dichloro diphenyl trichloroethane (DDT) disrupts the central nervous system in animals and directly affects hormone production [38]. Although banned in 1988 [39], it was later converted to partly chlorinated and less toxic DDE and DDD [38]. Most countries forbade it in 2009 because it is carcinogenic to humans [40].

New pesticides are the pesticides of choice in the United States because they degrade faster than other pesticides [41]. For example, another pesticide, Malathion, is used for residential pest control and public health purposes and agriculture [42]. This pesticide in agriculture; has been widely used to protect the cotton crop, the extermination of Mediterranean fruit flies and mosquitoes, and the extermination of boll weevil [43]. However, widespread use of Malathion poses a significant risk to humans and other organisms [44]. It has been reported to be lethal, especially to honeybees and other beneficial insects, some fish, amphibians, and freshwater invertebrates [45]. Its effect has also been shown significantly in the aquatic ecosystem [46]. Although studies have shown that even low doses of Malathion adversely affect organisms and the carcinogenicity of this pesticide is high [47], it has been reported that it has several essential properties (genotoxicity and oxidative stress), incredibly similar to human carcinogens, and may be effective in humans [48].

Microorganisms are concentrated in the part of the soil defined as the rhizosphere, and they affect the physiochemical activities in the soil. Bacteria constitute the majority of them [49].

Many studies have reported that some bacteria were living in the rhizosphere support plant growth with different mechanisms of action. These beneficial bacteria were found by Kloepper et al. (1980) as PGPR (Plant Growth Promoting Rhizobacteria) and are also known as "Probiotic Rhizobacteria" due to the many benefits they provide to the plant. Furthermore, in most research on the uses of rhizobacteria, rhizobacteria support plant growth by breaking down pesticides and producing enzymes, among other benefits [50].

There are promising results that PGPRs have many benefits, such as eliminating the toxic effects of polluting pesticides and chemical fertilizers, ensuring the uptake of nutrients by the plant, and reducing biotic/abiotic stress in the plant [51].

The stresses that plants are exposed to are divided into two classes: biotic and abiotic stress. And heat, drought, salinity and heavy metal stress are listed as abiotic stress. It is known that abiotic stress factors affect biotic stress and reduce crop productivity. The most significant effect of abiotic stresses is decreased soil microbial diversity and soil fertility[52].

3.2 Types of abiotic stress caused by pesticides

3.2.1 Reactive oxygen species (ROS)

Hydroxyl radical (O.H.[•]), superoxide radical (O₂^{•-}), singlet oxygen (O₂), and hydrogen peroxide (H₂O₂) are different known types of ROS [57]. They are formed due to the natural aerobic metabolism of plants and microorganisms. They are produced in intracellular compartments [58] and cellular physiological and cellular metabolic processes (deoxidation of carbohydrates, denaturation of proteins, peroxidation of lipid membranes and DNA). It disrupts the plasma membrane by triggering events such as disruption of the plasma membrane, RNA structural modifications, reduction in enzyme production, and formation of photosynthetic and non-photosynthetic pigments [53].

The effects of ROS on the lipids of plant cells are in the form of oxidative degradation

of polyunsaturated fatty acids in the plasma membrane, and this phenomenon is called lipid peroxidation [54]. As a result of this lipid peroxidation, amino acid oxidation can also disrupt protein structures [55].

Excessive production of reactive oxygen species (ROS), which is a crucial stress indicator in plants, damages different cell structures. And it has been reported by many researchers that organic pollutants such as pesticides cause oxidative stress in plants by causing ROS synthesis [56].

When plants are exposed to oxidative stress, adverse effects occur on growth, and flower number [57], germination of seeds [58], gravitropism of roots [59], lignin biosynthesis in the cell wall of plants [60], polar cell growth [61] and cell senescence [62] are observed.

3.2.2 Malondialdehyde (MDA)

When the unsaturated fatty acids in the plant directly react with the free oxygen radicals, which is a reactive oxygen species, the oxidative stress process begins [63], and this reaction is called lipid peroxidation (LPO). This reaction; occurs in two ways: enzymatic lipid peroxidation by enzymes and non-enzymatic lipid peroxidation carried by free radicals.

Lipid peroxidation causes the formation of highly toxic by-products by making poisonous effects on various biological pathways due to lipid damage (change of integrity, lipid fluidity, permeability, defect of delicate structures and loss of bio-membrane functions) [64]. The best known of these toxic products is Malondialdehyde (MDA) and is considered an indicator of membrane damage caused by lipid peroxidation.

It has been reported that there will be an increase in MDA due to oxidative stress and toxic effects when plants are grown in contaminated soil [65]. Furthermore, it has also been reported that this situation will change the formation of antioxidant enzyme production.

3.2.3 Hydrogen peroxide (H₂O₂)

Another essential type of ROS produced during oxidative stress in living organisms is H₂O₂. H₂O₂ has a vital role in strengthening antioxidant defense systems. H₂O₂ can also react with brassinosteroid (B.R.), auxins, abscisic acid (ABA) and ethylene gas, which are essential in plant growth. These are known to control leaf senescence. In addition, several studies have reported a gradual change in the production of CSDs and many other SOD family enzymes due to the production of H₂O₂ [66].

3.3 Antioxidant defense systems developed in microorganisms and plants against pesticides

It has been reported that pesticides and many organic pollutants of various types trigger stress by accelerating ROS production in microorganisms and plant cells. Conversely, many studies report that antioxidant defense systems in microbes and plants are activated against ROS production caused by these organic pollutants [67].

These antioxidant defense systems, which are responsible for scavenging ROS, consist of enzymatic or non-enzymatic chemical components. These defense systems can be found in different parts of the cell (mitochondria, endoplasmic reticulum, peroxisomes, plasma membrane, and chloroplasts) [68].

Antioxidant defense systems consisting of enzymes are mainly; protein such as catalase, superoxide dismutase (SOD) [80], ascorbate peroxidase (APX), guaiacol peroxidase (GPX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione S-transferases (GST), and glutathione reductase (G.R.) are units. Non-enzymatic antioxidants are substances such as glutathione, ascorbic acid, glycine betaine, tocopherols, carotenoids, flavonoids, and proline [69].

Many bacterial strains have been reported, including *Azotobacter*, *Flavobacterium*, *Caulobacter*, *Erwinia*, *Micrococcus*, *Serratia*, *Pseudomonas*, *Agrobacterium*, *Azospirillum*, and *Chromobacterium*, which contribute to this defense system by producing antioxidant enzymes [70].

3.3.1 Major enzymatic defense system elements

3.3.1.1 Catalase (CAT)

Catalase is the first oxidative stress scavenging enzyme discovered in the antioxidative defense system. It consists of a ubiquitous heme group containing a tetrameric enzyme that can catalyze two H_2O_2 molecules into oxygen and water. CATs are more unique than any other enzyme found in plants, as they catalyze H_2O_2 without the need for cellular reduction equivalents [71].

When cells are exposed to organic pollutant stress, they face energy shortages, and as a result, large amounts of H_2O_2 are produced metabolically. The breakdown of this H_2O_2 occurs thanks to CATs that require low energy [72].

A study reported that catalase activity increased GSSG accumulation and decreased ascorbic acid production fourfold in 10% wild-type transgenic tobacco plants. These data showed that catalase is essential for regulating the average growth of plants during stress from organic pollutants [73].

3.3.1.2 Ascorbate peroxidase (APX)

APX, an essential component of the enzymatic antioxidant defense system in plants and microorganisms, is an active element of the Ascorbate-Glutathione (GSH) cycle and plays a vital role in inactivating ROS in the cell. The presence of this enzyme varies depending on the intensity of H_2O_2 and redox signals. This enzyme is present in the parts of the cell where H_2O_2 is produced. Therefore, it is the most active and most abundant antioxidant enzyme in the cell compared to all other antioxidant enzymes for the clearance of H_2O_2 . Studies report that APX production increases due to abiotic stress caused by pesticides [74].

3.3.1.3 Superoxide dismutase (SOD)

This enzyme is another essential component resulting from oxidative stress in plants and microorganisms. And it provides the breakdown of free oxygen radicals into O_2

and H_2O_2 . Therefore, it is produced in all cell organelles where the oxygen radicals formed as a result of stress is produced[75].

In other words, the increase in SOD concentration and activity indicate organic stress tolerance in plants and other organisms. It has also been reported that an increase in the intensity of SOD improves oxidative stress tolerance in microorganisms and plants[76].

3.3.1.4 Glutathione reductase (G.R.)

Glutathione reductase (G.R.) has an essential role in the antioxidant defense systems of plants and microorganisms and takes part in both enzymatic and non-enzymatic oxidation-reduction processes. With oxidation, GSH turns into GSSG. Glutathione reductase (G.R.) maintains a high intracellular GSH/GSSG ratio through NADPH for catalysis of GSSG to GSH [93]. There is a strong correlation between G.R. activity in microorganisms and plants growing at high H_2O_2 concentration due to oxidative stress, and G.R. production will increase as H_2O_2 increases [70].

3.3.2 Non-enzymatic antioxidant defense systems

Carotenoids are an essential antioxidant found in both microorganisms and plant cells and detoxify stress-causing ROS. A study reported that the plant showed better growth and yield when the carotenoid concentration was increased in sugarcane grown under organic pollutant stress. In addition, it has been reported that carotenoids increase the growth of plants by coping with abiotic and biotic stresses[77].

Another non-enzymatic antioxidant is tocopherol. Tocopherol forms belong to a group of antioxidants involved in the destruction of free oxygen radicals and peroxy lipid radicals [96]. Their primary function is to protect the lipid membrane and other components of the membrane against oxygen radicals [78].

On the other hand, ascorbic acid (ASA) is an antioxidant compound with a smaller molecular structure, produced in response to ROS activity, and found in higher

concentrations in the cell compared to other antioxidants. In addition, since this compound can donate electrons to other molecules in both enzymatic and non-enzymatic reactions, this property makes it a powerful antioxidant [68].

While close to 90% ASA is found in the cell cytoplasm, only a tiny amount of ASA is transferred to the walls and extracellular spaces of the cell. And this apoplasmic ASA formed the first line of defense against exogenous toxic oxidants[79]. In other words,

it plays an essential role in protecting macromolecules sensitive to ROS caused by organic pollutants.

Since non-enzymatic antioxidant molecules are protective against the adverse effects caused by oxidative stress, including ROS, we can say that these molecules indirectly affect the relationship of the plant with microorganisms.

The stress factors that the plant is exposed to and the antioxidant defense systems it develops are shown in Fig 2.

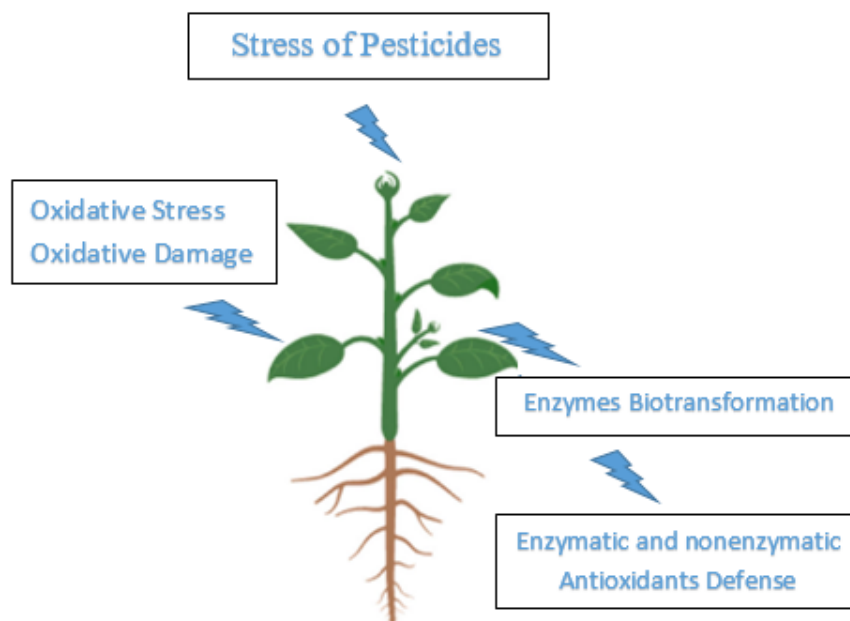


Fig 2. Mechanisms of the antioxidant activity of plants under pesticide stress

4. Enzyme biotransformation and Bioremediation

Enzymes (preferably immobilized) are used instead of microorganisms in biotransformation. This set of enzyme-catalyzed reactions can eliminate or alleviate many biotic and abiotic stresses. Microbial enzymes act as bio-oxidation catalysts as well as provide biodegradation. Optimum temperature and pH are very important for these specific enzymes. This enzyme reaction increases the reaction rate by lowering the activation energy, similar to other enzyme reactions [80]. The numbers, species and environment of microorganisms affect biodegradation, and these interactions are very complex. For these confusions: The geochemical structure of the groundwater

volume, the bioenergy of the process and the biodegradation processes are effective. Site conditions and the design, operation and monitoring processes of this improvement are essential for physical rehabilitation[81].

Generally, it is possible to destroy the pollutants that cause environmental pollution by transferring them to a different environment from their environment. Bioremediation can be applied to many other pollutants that cause pollution. Contaminants can be indifferent masses (solids, liquids and gases). Dangerous products under government supervision control can be rendered harmless. The resulting residues are harmless and consist of H_2O , CO_2 and biomass. Applications made with bioremediation are more economical than other environmental

cleaning applications and less affect the deterioration of the environment. In the transportation of waste materials, the damages to the environment and people are also eliminated. However, it is estimated that when the pollutants in the environment begin to dissolve, they may become more dangerous and permanent than their initial state. For the bacteria to reach a specific density; Appropriate conditions should be provided by considering the nutrients, electrons and the amount of carbon source (minimum) in the soil. It is necessary to develop new techniques for bioremediation because pollutants and pesticides do not distribute evenly in the environment. The applicability of bioremediation is challenging to assess in advance how effective it is [30].

5. Conclusion and future prospective

Globally increasing pesticide application and production in agricultural lands and pesticide residues in soils will pose a potential threat to ecosystems and living things. This review aims to understand the ecological damage caused by pesticides, antioxidant defense systems developed by plants and microorganisms against this damage, and effective remediation strategies by external intervention. Current research; reported that enzyme performance for bioremediation of pesticide-contaminated soils is affected by factors such as source, concentration, chemistry, the toxicity of pollutants, solubility, transport, adsorption, dispersion, and volatility polluting compounds. And also; Soil chemistry, physics, microbiology, detection, monitoring and limitations of environmental standards are essential. Pesticide accumulation adversely affects seed germination, growth and development, photosynthesis, enzyme activities of microorganisms, and uptake of macro and microelements from the soil. This accumulation can cause oxidative stress by inhibiting biochemical activities in plants and microorganisms. To reduce the threat of pesticides to the soil-plant systems, it is essential to determine the conditions in the enzyme transformation that activates the defense system of the plant, to investigate the positive and negative effects of the bioremediation event, which is the result of

this, on the plant and soil, and to apply it in agriculture. This review shows that issues are not fully understood.

Therefore, a lot of research is needed to review the studies focusing on pesticide uptake and accumulation by soil-plant systems, understand the metabolic and detoxification mechanisms of pesticides, analyze how they regulate interactions in plant cells, and understand the mechanisms of pesticide tolerance and detoxification in plants.

Abbreviation

Not applicable

Conflict of interest

The authors declare there is no conflict of interest in this research study.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Ethics approval and consent to participate

No human or animals were used in the present research.

Ethics declarations

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Source of funding

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