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Review article

Biodegradation of pesticide in agricultural soil employing entomopathogenic fungi: Current state of the art and future perspectives

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A R T I C L E I N F O

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A B S T R A C T

Pesticides play a pivotal role in agriculture for the effective production of various crops. The indiscriminate use of pesticides results in the significant bioaccumulation of pesticide residues in vegetables. This situation is beyond the control of consumers and poses a serious health issue for human beings. Occupational exposure to pesticides may occur for farmers, agricultural workers, and industrial producers of pesticides. This occupational exposure primarily causes food and water contamination that gets into humans and environmental pollution. Depending on the toxicity of pesticides, the causes and effects differ in the environment and in human health. The number of criteria used and the method of implementation employed to assess the effect of pesticides on humans and the environment have been increasing, as they may provide characterization of pesticides that are already on the market as well as those that are on the way. The biological control of pests has been increasing nowadays to combat all these effects caused by synthetic pesticides. Myco-biocontrol has received great attention in research because it has no negative impact on humans, the environment, or non-target species. Entomopathogenic fungi are microbes that have the ability to kill insect pests. Fungi also make enzymes like the lytic enzymes, esterase, oxidoreductase, and cytochrome P450, which react with chemical residues in the field and break them down into nontoxic substances. In this review, the authors looked at how entomopathogenic fungi break down insecticides in the environment and how their enzymes break down insecticides on farms.

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1. Introduction and scope of the review

In recent decades, there has been an extensive use of synthetic pesticides and anthropogenic substances, which has generated serious environmental issues. These chemical substances cause severe effects on humans and other living organisms. Insecticides are harmful substances that are present in soil and aquatic systems and can migrate into the human body and cause various diseases. The chemical compounds are categorized based on their mechanism of action [1]. Insecticides that enter target organisms disperse into the environment and may transfer from one area to another, increasing the likelihood of insecticides entering non-target organisms and causing harmful effects. Alkyl halide insecticides such as aldrin, dieldrin, heptachlor, lindane, and mirex are widely employed. In United States UN and many other countries, the use of these insecticides has been reduced or discontinued because they accumulate and are toxic to animals, soils, and humans [2].

The common organophosphorus insecticides used on corn, peppers, and tomatoes are chlorpyrifos as they control soil-dwelling pests [3]. Besides, malathion organophosphate insecticide is used to control sucking pests and chewing insects, and it also prevents mosquitoes as well as parasites. Indiscriminate use of chemical substances can cause damage to the gastrointestinal tract, skin, and lungs. It affects the central nervous system of invertebrates, the immune system of vertebrates, and the liver and blood of fish. Mutations have been found in humans when white blood cells, lymphocytes, and blood cells were cultured [4].

The pyrethroid-cyfluthrin insecticide leaves toxic residues on commonly consumed raw vegetables, and exceeding the maximum residue limit (MRL) causes harmful effects on human health and the environment [5]. Therefore, the development of pesticide degradation by microbial enzyme-based approaches has received great attention from environmental workers. Investigation of fungi as degradation of pesticides has not been well addressed before. The elimination of 2,4-D, a chemical herbicide, has been done by the fungus Umbelopsis isabellina, which was identified by Ref. [6].

The production of endosulfan (organochlorine) is still being produced by various industries, but endosulfan is a very toxic insecticide for soil application, and it has been banned in many countries, including Korea. It is more toxic and stays longer in the soil system [7]. Lindane (the organochloride) is one of the most widely used insecticides worldwide and is biodegraded by white-rot fungi, including Trametes hirsutus, Bjerkandera adusta, and Pleuotus sp [8,9]. Cyanobacteria in the irrigation water and soil degraded diuron. These encouraging findings suggest that using cyanobacterial mats and other microbial sources to remediate soil and water pollution could be a viable option [10–12]. This review mainly focuses on international reports on insecticide pollution in the agro-ecosystem system and the damage caused by insecticide compounds. The second important role of this review is to show how insecticide pollutants enter the soil system and are then transferred to humans through the food chain. The third goal is to find vital mechanisms to combat insecticide pollution in laboratories and in large-scale studies. In this paper, the authors looked into how entomopathogenic fungi help break down insecticide pollution and how their enzymes help fight pollution in the agroecological system.

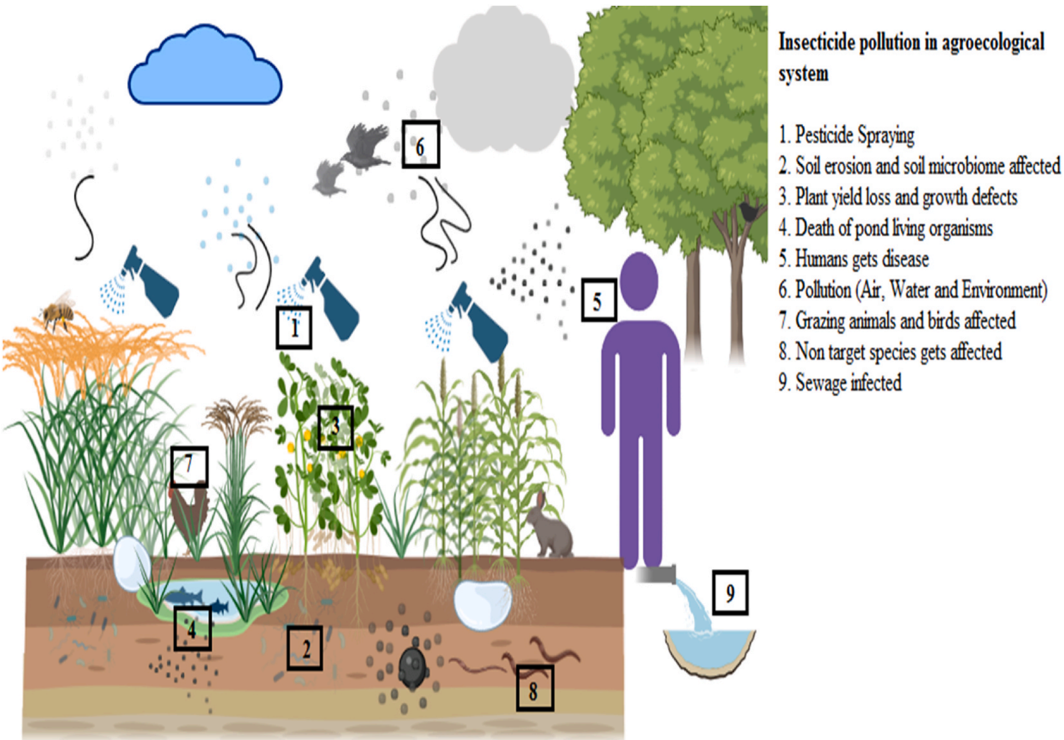

Fig. 1. Insecticide pollution in agro-ecological system and its causes.

Table 1
Types of Insecticide and insecticide pollution damages to the environment exhibiting toxic effects.

Types of insecticides	Damage	Mode of action	Toxic effects	Reference
Organochlorine	Animals, Soil and Water	DDT and aldrin have lower water solubility, they would less likely be dissolved in waters of lakes, ponds, or rivers but would tend to bind readily with soils and sediments as well as bioaccumulate in fatty tissues of soil invertebrates and some plant tissues	Breast cancer, reproductive and endocrine-disrupting effects, alteration of immune function in birds and mammals, accumulates in adipose tissues, liver, and other organs, hypertension, reproductive damages, cardiovascular diseases	[29]
	Plants	Phytoextraction or phytoaccumulation, phytodegradation or phytotransformation, phytovolatilization, and rhizodegradation or phytostimulation	Cellular growth of plant sis damaged, reduction in IAA	[30,31].
Organophosphate	Animals	Inhalation, Direct contact, Ingestion	Memory, speech loss, lack of coordination, and impaired judgment, cancer, Respiratory System damages, Cardiovascular System, CNS, Gastrointestinal and Metabolic Systems	[32]
	Plants	OPs prevent the biosynthesis of catalase, perioxidase and δ-aminolevulinic acids (ALA), chlorophyl biosynthetic pathway by inducing Fe deficiency in plants	Cell division and elongation, plant growth and nitrogen metabolism and affects photosynthesis, plant mineral nutrition, carbon metabolism, photochemical reactions	[33]
Carbamates	Animals	Carbamylating of the active site of acetylcholinesterase leading to the inactivation of the enzyme	Nervous system damage	[34]
	Plants	Arylhydroxylation and conjugation, or hydrolytic breakdown	Cell division inhibitors	[35]
Formamidines	Animals	Affect biogenic amine regulatory sites and receptors	Cause local anesthetic action in the mammalian cardiovascular system	[36]
Dinitrophenols	Mammals	Oral exposure	Nausea, vomiting, sweating, dizziness, headaches, and loss of weight, formation of cataracts and skin lesions, weight loss, affects bone marrow and CNS	Pubchem, National library of medicine, NCBI
	Plants	IAA metabolism, or direct interaction with lAA; Respiration, Glycolysis, Oxidative phosphorylation, Photosynthesis and photosynthetic phosphorylation	Affects seed germination, growth and development, necrosis and loss of root hairs, plant leaf tip yellowing	[37]
Organotins	Animals	Exposure leads to lipid peroxidation, abnormal cellular function, and cell death; lowers low blood potassium;	Brain, liver, adipose tissue, and reproductive organs, as they are endocrine-disrupting chemicals (EDCs), hypokalemia, renal tubular acidity, and increased urinary pH, kidney stone	[38]
	Marine Environment (Plants, invertebrates)	Potential pollution source for the marine environment via intensive shipping, reception of various wastes from the ships, cargo loading and unloading operations, and ship building and repairing/remodelling	Severe reductions in growth of plants; Endocrine disruptor in invertebrates	[36]
Pyrethroids	Animals	Inhibit 35s-TBPS-specific ligand bind-ing	Causes mammalian central nervous system (CNS	[36]
Nicotinoids	Animals	Interference with acetylcholine neurotransmitter signalling causes continuous activation of the receptor	Symptoms of neurotoxicity	[39]
Pyrazoles	Plants	On exposure	Plant Necrosis	[40]
Quinazolines	Plants and Animals	Oxidative stress, up-regulated transcription	DNA damage, damages lipid membranes; liver cancer and enteropatia in humans and wildlife	[41]
Thiamethoxam Clothianidin Dinotefuran	Animals,	Hydrolysis, hydroxylation, N-acetylation, N-demethylation or nitro reduction	Development of liver cancer	[42]
	Plants,	Oxidative cleavage, hydroxylation, glycosylatio	Affects respiration, malnutrition, photosynthesis process affects, poor yielding	[42]
	Water,	Hydrolysis, photolysis	Movement of the pesticide in surface runoff	[42]
	Soil	Enters pathways like aerobic degradation, hydroxylation and reduction	Effect on soil microorganisms	[43]
Imidacloprid Nitenpyram	Animals,	Effective by contact or ingestion	Post-synaptic nicotinic acetylcholine receptors in the nervous system, DNA damage	[42]

(*continued on next page*)

Table 1 (*continued*)

Types of insecticides	Damage	Mode of action	Toxic effects	Reference
	Plants,	Translocates rapidly through plant tissues following application	Modifies mitotic kinetics, DNA damage	[43]
	Water,	Transport analyses are helpful estimate leaching potential from soils that could result in groundwater pollution	Causes skin irritation, eye irritation	[43]
Thiacloprid	Animals	Nitroreducase, hydroxylation, N-remethylation, decarboxylation, Oxidation, reduction, N-desmethylation.	Exhibits a toxicity to mammals, Neurotoxic, reproductive toxicity, uterine tumors and thyroid	[42,43]
	Water and Soil	Microbial degradation in soil, aerobic microbial processes and photolysis.	Variability in soil texture	[44]
Diamides	Plants and Animals	Oxidative stress, pro-oxidant/antioxidant homeostasis	Damage DNA, proteins, lipids, and carbohydrates	[45]
Derived from plants and microbes	Animals	–	No greater risk to human health	[46]

2. Global status of insecticides pollution in agroecological system

The World Health Organization classified methyl parathion (MP) as highly toxic, and its use has been restricted by the U.S. Environmental Protection Agency (EPA) [13]. Further, pyrethroids are significantly toxic to mammals and insects, disrupting the sodium chloride channels and, at higher concentrations, the GABA-gated chloride ion channels [14,15]. In India pesticide production started in 1952 in Calcutta. India is the second-largest manufacturer of pesticides in the world [16]. The steady increase in pesticides in India has been recorded since 1958. In 2005, about 5000 metric tons of pesticides were produced, and in 1998, it was around 102,240 metric tons [17]. reported that the value of pesticides went up by about $0.5 billion between 1996 and 1997. This is based on the fact that their demand went up by 2 %, which is how much they sold on the world market as a whole.

The global insecticide usage rate in 2014 was 7.50 %, and insecticide and active ingredient production peaked in 2007. And the use of insecticides significantly declined after 2007. The use of chlorinated hydrocarbons has declined since 1990. In 2014, organophosphate insecticides were widely employed after organophosphate pyrethroids, carbamates, botanicals, and chlorinated hydrocarbons were used. The average annual insecticide use from 2010 to 2014 in Japan was 6.634, in Mexico it was 2.313, and in India it was 0.09 [18] (Fig. 1). Approximately 2 million metric tons of pesticides have been produced and utilized, of which 29.5 % are insecticides [19]. [19] reported that the use of insecticides has increased 10 times from 1945 to 2000 in the USA. At the same time, the damage caused by the insects to the crops has increased by 7–13 %. Insecticides are being used less and less in the United States because biopesticides and natural plant products are being used instead [19].

3. 2 insecticides and their problematic issues

The application of pesticides causes adverse effects on different forms of life and ecosystems [20]. Certain insecticides and fungicides also cause damage to the plants [21]. About 90 % of pesticides used do not reach their intended targets and are instead distributed in the soil, food, vegetables, air, and water [22]. Many people and farmers have been affected by the wide-spectrum use of pesticides. It also causes adverse impacts on the environment, flora, and fauna [23]. These toxic compounds of insecticides lead to various disorders, including cancer, reproductive diseases, neuronal disorders, allergic reactions, and skin diseases [24]. Pyrethroid pesticides are restricted to use around children, pregnant women [25].

The US National Academy of Sciences indicated that the DDT metabolite of DDE causes eggshell thinning in eagles, and the US declined the use of DDT [26]. Malathion carcinogenicity has been reported at some doses, and it also causes maternal toxicity by lowering intake and weight loss [27]. In particular, Malathion also infected rats and mice [28] (Table 1). Globally, insecticide pollution is increasing as the use of insecticides in agriculture spreads to water bodies and agricultural fields, causing pollutants to stay for a long time, which in turn threatens humans, the marine environment, and animals. Another big problem with the widespread use of pesticides is that fresh fruits and vegetables that have pesticides on them can get into the food chain.

Pesticide contamination in groundwater, which is used for drinking, is causing the most problems in developing countries [47]. EDCs are chemicals that interfere with the production, transport, metabolism, release, action, or elimination of hormones in the body [48]. Insecticides, in particular, have a primary mode of action that targets the nervous system. For example, organophosphate insecticides like chlorpyrifos stop the enzyme that breaks down acetylcholine from working [49]. This causes a buildup of this important neurotransmitter [49]. According to research [50], pesticide exposure in children and prenatal exposures have been linked to social and behavioral problems. Identifying a causative mechanism for cancer is frequently difficult due to multiple exposures and long latency periods. Pesticide exposure has been linked to leukemia, breast, prostate, liver, pancreas, lung, brain, kidney, and skin cancers [51]. Globally, insecticide pollution is increasing as the use of insecticides in agriculture spreads to water bodies and agricultural fields, causing pollutants to stay for a long time, which in turn threatens humans, the marine environment, and animals (Table 1).

4. Toxic effects of insecticides on living organisms

The use of insecticides and secondary poisoning from tainted food harms birds and mammals, and high pesticide dosages have an impact on their reproductive rates [52]. The pesticide evaluation of the following foods was conducted in the year 1996: apples, tomatoes, strawberries, and grapes. Seven pesticides were evaluated, such as chlopyriphos-methyl, methamidophos, iprodione, acephate, procymidone, chlopyriphos, and chlorothalonil. The study conducted by Ref. [53] tested the pesticide contamination in commodities like oranges, peaches, carrots, and spinach, analyzing them for 20 different pesticides, of which 32 % contained residues of those pesticides and 2 % were above the Maximum Residue Limit (MRL). Spinach, carrots, and peaches exceeded the MRL level. And it does not exceed the "ADI", acceptable daily intake limit.

A total of 4700 samples were tested for the 20 pesticide residues in 4 sets, including cauliflower, pepper, wheat, and melons, in 1999, and the number of residues of methamidophos exceeded the MRL by around 8.7 %, maneb 1.1 %, acephate 0.41 %, and benomyl 0.35 %. Methamidophos levels in peppers and melons are 18.7 and 3.7 % higher, respectively. Residues of maneb in cauliflower are 3.9 % higher [54].

A program was conducted on insecticide contamination in foods entitled "Monitoring of Pesticide Residues in Products of Plant Origin in the European Union" in 1996. Samples of 9700 were analyzed for pesticide residues. Approximately 5 % of the samples were found to be contaminated with residues, which is 0.31 % higher than the MRL. The highest pesticide residues are from the chemical mancozeb and in lettuce, at 118 mg per kilogram.

The first poisoning report in India was published in 1958. Consumption of parathion-contaminated wheat flour led to the deaths of 100 people [55]. The committee from ICAR started focusing on the problems caused by pesticide residues in food and the environment and how they are incorporated into the food chain from soil and environmental ecosystems [56]. The decomposition of chemical pesticides may even cause more damage than the original pesticides based on their chemical formula. Pesticides have an expiration date of 2 years, after which they are not considered safe for use unless tested and proven [57]. [58] say that some compounds may make pollutants that hurt almost every living thing on earth.

4.1. Insecticides and their problems in the soil system

The effect of the pesticide was global, and it has infected the subsoil and groundwater systems. Pesticides usually don't stay in the plants sprayed; they runoff into the surrounding ecosystem, and non-target vegetation and organisms have been affected. The drift of pesticides from one place to another is the most common issue faced [59]. Pesticide pollution is a serious issue all over the world; public safety is in danger because of these chemical sediments of pesticides (El-Ghany and Ibrahim, 2016). The overuse of pesticides and the accumulation of chemicals pose the most serious threat to public health [60]. say that the pollution caused by pesticides can be fixed by getting rid of the chemicals in the right way and getting rid of the residues in landfills through in situ incineration or in situ biological remediation.

Endosulfan is hydrophobic by nature, which causes it to absorb into the soil and sediments and persist longer in the soil and water [61]. These chemicals then accumulate in crop waste and aquatic systems, contaminating phytoplankton, fish, vegetables, and dairy products [62]. In many soils, pesticides are intentionally introduced into the soil to treat the phytopathogens and microbes in the soil [63] (Fig. 2). Pesticides hurt the soil, the environment around it, and the water when they are used too often or in excess of what is allowed.

4.2. Soil system into humans

According to Ref. [64]; pesticide poisoning caused by insecticide and rodenticide chemicals accounted for 7.16 % and 6.47 %,

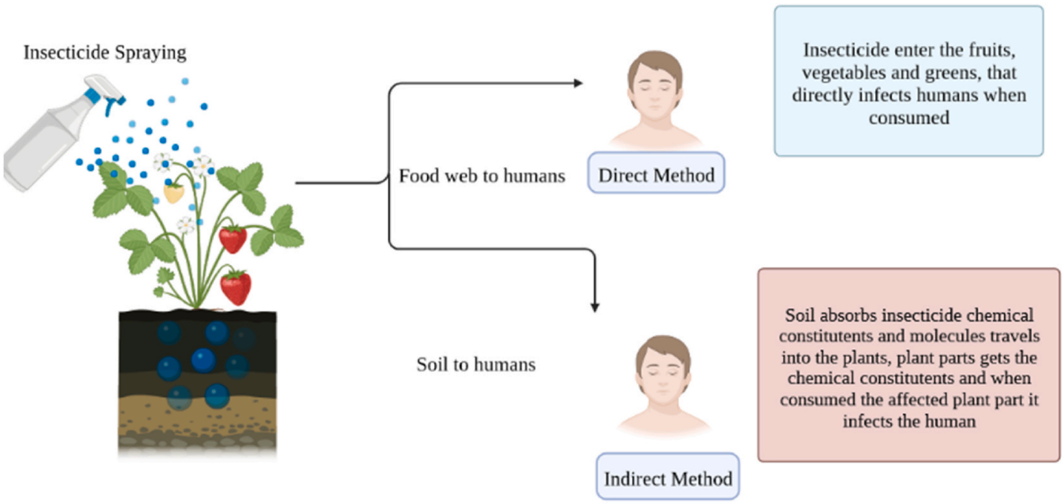

Fig. 2. Direct (Food web) and Indirect (Soil to food to humans) method of pesticide pollution into humans.

respectively. The fact that 86.02 % of poisoning reports were available is the main issue with organophosphorous pesticides. From 2006 to 2015, over 3000 children in Zhejiang suffered pesticide poisoning during the farming season [65]. Pyrethroid pesticides are toxic to both humans and animals [66]. The wide use of chlorpyrifos increases public health by causing dietary problems in humans. Macrophages are the cells that fight pathogens that get into the body [67]. found that using too much b-cypermethrin and cyhalothrin slows down macrophage phagocytic activity. In 1976, the Seveso disaster in Italy produced 2, 4, and 5-T-herbicides with chloracne and exposed 200 people to dioxin [67].

The persistence of the pesticide is very high in the environment. This pesticide gets incorporated into the food chain, and the ecosystem and humans get affected [68]. Pesticide residues are a major burden for developing countries, as no population is completely protected [69]. One million pesticide poisoning cases—that is the death and chronic infection rate in one year [70].

Cyfluthrin and chlorpyrifos are toxic to human fatal glial cells in the brain and spinal cord, human peripheral lymphocytes, and the number of chromosomes. They also cause apoptosis (Segura et al., 2018; Muzinic and Zeljezic, 2018). Using a Salmonella microsome mutagenicity test, chromatid exchange and chromosomal aberration were found to be signs of cyfluthrin's cytotoxicity [71]. Bifenthrin creates toxicological effects in human cells, like homeostasis and cell viability [72]. Pesticide contamination is generated by overuse of pesticides at a higher level than the recommended dosage, and waste that is generated by producing and formulating the pesticide. The characteristics of the pesticide are causing contamination worldwide [73]. Insecticides have an easy chance of getting into the food chain and causing serious health effects, and when farmers increase the dosage of insecticide usage simultaneously, it reflects on humans and soil health-related issues (Fig. 2).

5. Mitigation of insecticides contamination using chemical and biological strategies

5.1. Combat of insecticides contamination, lab and large-scale investigations

High concentrations of pesticides cause soil fertility problems and generate several environmental issues. Food safety and security are major concerns for India because of its diverse climatic conditions and different crop protection and cultivation approaches [74]. Pesticides are popular all over the world for their immediate action against insect pests, but they cause serious health issues in humans when exposed [75]. Farmers are exposed to pesticides every day, and this may lead to food poisoning and have short-term and long-term effects. It also degrades the environment. Although there is a pesticide toxicity reference, it is uncertain due to its factors, and farmers may not know if the pesticide is safe or performs well under all conditions. Despite many pesticide studies on its fate and toxicity for long-term health issues.

Humans get easily infected with pesticides through exposure to occupations such as farmers, agricultural workers, or workers in industry [76]. The form of pesticide formulation may affect the worker. If it is a liquid pesticide, it has the possibility of splashing and reacting directly on the human skin or indirectly. When the pesticide is applied, it may have the possibility of spreading via dust particles in the air, resulting in respiratory problems. The packaging of pesticides is another major way of spreading them through the air and surroundings. Proper maintenance, such as wearing a mask and clothing, is a must when pesticides are packed.

Pesticide registration is one of the important steps to follow after production to determine the pesticide's purpose and its uses, minimal usage and maximum usage rates, packaging, labeling, and advertisement (WHO, 2010). The only way to reduce pesticides in the agricultural field is to use registered pesticides. When pesticides get legally registered, the use of unregistered pesticides in the environment drastically reduces. Here are the basic pathways to registering a pesticide: (1) conduct research prior to product

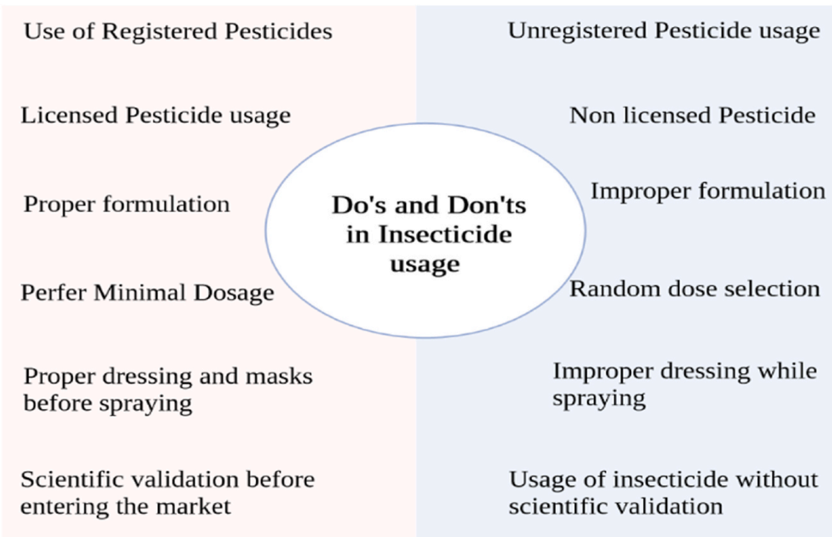

Fig. 3. Do's and Don'ts in Insecticide usage in Environment.

manufacturing; (2) submit research data to the registration authority; (3) the registration authority reviews the data submitted; and (4) the authority makes a registration decision based on the merits and demerits of the pesticide (FAO, 2002). The number of drastic effects caused by insecticides can be reduced if the overuse of insecticides is controlled, and mandatory insecticide registration in industries will help to prevent major disorders, as unregistered products will never reach the market for selling in commodities (Fig. 3).

5.2. Metabolism and the mode of action of biopesticides in insects

The metabolism of pesticides involves three different phases. In phase I, the initial properties of the parent compound are transformed through oxidation, reduction, and hydrolysis to produce water-soluble content that is less toxic than the parent compound. The second phase is the conjugation of pesticides and their metabolites to sugar and amino acids, which thereby increases their solubility in water, and the third phase is the conversion of phase II into secondary conjugates, which are nontoxic, and fungi or bacteria are involved in producing enzymes extracellularly and intracellularly [77].

Myco-biocontrol is one of the most widely used biological controls for pests. More than 700 species have been found in 90 genera that are pathogenic to insects. A few such myco-biocontrol species are discussed here, including Beauveria bassiana, a filamentous fungus that is highly host-specific, causing white muscardine disease in insects [78]. In addition, Lecanicillium lecanii, a widely distributed fungus, causes a massive decline in cerealcyst nematodes and has a very wide host range [79]. Another such species is Metarhizium anisopliae, a very potent biocontrol agent against insect pests [80,81],c,d; 2023a,b,c). Metarhizium rileyi is a dimorphic fungus that causes epizootic death in insects [82]. Purpureocillium lilacinum and Isaria fumosorosea are nematophagous fungi that kill nematodes causing disease and are widely used as bionematicides in soil (Seryczyska et al., 1975). According to Vivekanandhan et al. (2022a, [81]; Step 1: The fungi attach to the cuticle of the insect; Step 2: Formation of an infection structure in the cuticle; Step 3: Penetration into the cuticle; and Step 4: Once inside the insect body, fungal spores begin producing toxic metabolites.

At the moment, the main disadvantage of using microbial pesticide degradation is that strain separation and microbial degradation are limited to the lab scale and must be applied in the field. This could be primarily due to the requirement for degradation conditions for the effective use of microbial degradation of organophosphorus pesticides, which are difficult to achieve outside of the lab. Temperature, pH, organophosphorus pesticide concentration, and microbial essential nutrients will all change over time under natural conditions. It can't be sure that microbes will reproduce, and it may even stop them from doing so [83].

In comparison to traditional pesticide abatement methods, bioremediation technology is regarded as a feasible and cost-effective solution (savings of up to 85 %). The average cost of biological treatment for 4500 tons of contaminated soil is 55 USD/t, which varies (2963 USD/t) depending on the initial concentration of the pollutant. There are three types of treatment: physical, chemical, and biological. Incineration, for example, has an approximate cost of 250–500 USD/t, whereas a general biological approach has an estimated operational cost of 40–70 USD/t [84], which is nearly one-third less than that of conventional methods [85]. Furthermore, the average costs of biotreatment, incineration, and landfill disposal are 50–130, 300–1000, and 200–300 USD/m^3, respectively [86]. Microbe-assisted pesticide degradation in polluted sites employs a variety of biotechnological approaches (e.g., land-farming/engineered oil pile methods; on-site subsurface techniques, and fully blended soil slurry reactors for ex situ removal of excavated polluted soils).

6. Biodegrading capabilities of entomopathogenic fungi

6.1. General introduction of entomopathogenic fungi, their types, isolation and purification

Techniques.

Entomopathogenic fungi are naturally occurring ascomycota and entomophotoromycota. Entomopathogenic fungi are heterogeneous organisms that play an important role in the ecosystem. Several species of entomopathogens, such as Oomycetes (12 species), Chytridiomycota (65), Microsporidia (339), Entomophtoromycota (474), Basidiomycota (238), and Ascomycota (476 species), have been reported (Araujo and Hughes, 2016). The two most commonly found fungi in soil are *Metarhizium* sp and *Beauveria* sp. control arthropods and have a symbiotic relationship with plants. They help plants grow by acting as growth promoters and attaching themselves to the roots of pests [87].

Beauveria bassiana controls fungal pathogens and plant pests in 25 plant species and has been reported by Refs. [88,89]. Fungi colonize the root and the shoot and act as endophytes and epiphytes [90,91]. It suppresses the disease-causing pathogens and increases the plant's defense [92]. *Lecanicillium* prevents fungal diseases in plants by colonizing roots, staying on the surface of the leaves, and producing antimicrobial activity [92]. Isaria fumosorosea is effective against root knot nematodes, Meloidogyne javanica [93].

To isolate a pure fungal strain of DSP, it was cultured in PDA plates containing chlorpyrifos (100 mg/L). Identification was done by the methods of [94,95]; and characterization can be followed by Ahren et al. (1998). Isolation of endosulfan-degradation microbes can be done in media enriched with endosulfan as the sole carbon source [96]. For soil microbes, the direct inoculation method and the serial dilution agar plating method are followed to isolate soil microbes. For fungal isolation, the aliquots were cultured on Czapek Dox Agar and Potato Dextrose Agar. Rose Bengal was added to the medium for the primary isolation of fungi [97].

The species of fungi are basically identified by describing their morphology and molecular identification with the standard procedures [94,98]. The fungal strains can also be isolated by using chlorpyrifos in the media as a sole carbon source, and the degradation of chlorpyrifos is then extracted. Fungi are suitable for bioremediation and waste treatment because of their metabolic and ecological processes, which make them potential in different fields [99]. Chlorpyrifos and pyrethroids were effectively degraded by fungal cell-free extracts [100]. The media was prepared with the supplements of DDD dissolved in DMSO, and qualitative screening was performed; fungi were inoculated into it and incubated for 5 days at 32 °C to check the tolerance efficacy of the fungi in DDD containing petriplates [101].

Entomopathogenic fungi can be isolated from the dead insect cadaver of the Oryctes rhinoceros beetle. In a study, the researchers isolated 10 different strains in PDA (Potato Dextrose Agar) at 27 °C for 30 days. The conidial suspension was prepared from the young colony by using L-rod and transferring the spores to the other tube containing 0.1 % (v/v) Tween 80 and autoclaved with distilled water. The stock suspension was then standardized at a certain concentration, and the number of fungal spores per ml was counted using a Neubauer hemocytometer and a microscope. The isolated fungal strain *Metarhizium anisopliae* was suggested how to prepare the sample by Refs. [102,103].

6.2. Entomopathogenic fungi and biodegrading of insecticides

Entomopathogenic fungi are found in the natural environment. They come into contact with different anthropogenic pollutants. Only a few studies have been reported on the interaction of EPF with toxic pollutants, whereas the pathogenic effect of EPF on insects has been widely studied. The most harmful Endocrine-disrupting chemicals EDCs are anthropogenic compounds that are both natural and synthetic. These fungi have structural similarities and can easily bind to cell receptors, disrupting cell function in humans and animals' endocrine systems. Some examples of EDCs are alkylphenols, synthetic estrogens, and pesticides (Rozalska et al., 2015; [104, 105].

It is known that entomopathogenic fungi are used in insect pest control techniques. Some of those are *Metarhizium* sp, *Beauveria* sp, *Purpureocillium* sp, and *Isaria* sp, which have a broad spectrum of activity [106]. Microorganisms have the potential to degrade and remove pesticides from the environment and have been attempted successfully [107]. [108] used entomopathogenic fungi to biodegrade the oils in the contaminated soil. *Cladosporium* fungi species have been shown to biodegrade diesel pollution in an aqueous environment (Li et al., 2008). Apart from this, there is a specific area of entomopathogenic fungi that is less studied, namely its ability to degrade chemical pesticides. Basically, fungi biotransform pesticides by introducing structural changes to the molecule, making it nontoxic, and releasing it back to the soil. Further degradation will be done by the soil bacterium [109] (Fig. 4).

For instance, very few studies have shown that entomopathogenic fungi were responsible for the degradation of various chemical

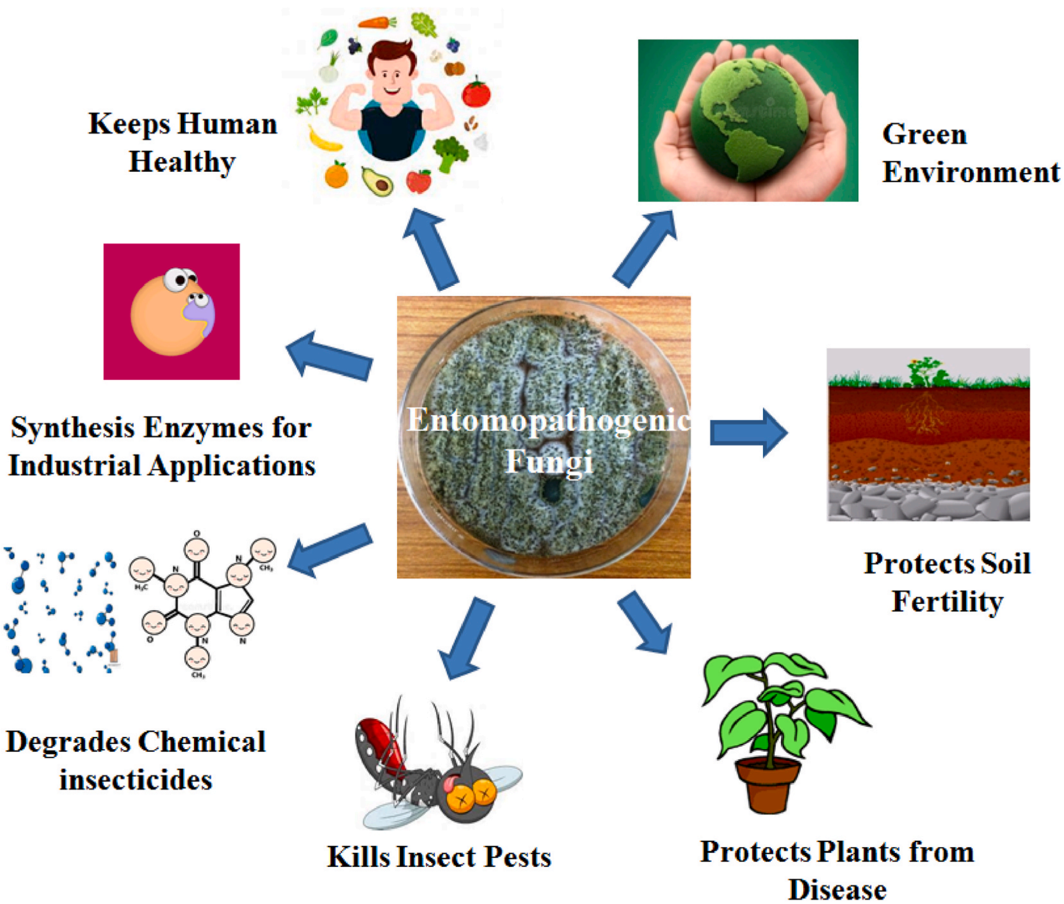

Fig. 4. Entomopathogenic Fungi and their benefits in different areas.

pesticides. When Lecanicillium sp. was added to the chlorpyrifos contaminated soil, the degradation activity was significantly increased. In the comparison study of sterilised soil and Lecanicillium fungal sp, inoculated soil, chlorpyrifos in inoculated soil shows more degradation activity than in sterilised soil. Penicillium camemberti degrades pentachlorophenol (PCP), 2-chlorophenol, and trichloroacetic acid (Taseli and Gokcay, 2006). In very old research by Ref. [110]; it was demonstrated that Trichoderma viridae has the ability to degrade toxaphene and aldrin, which come under organochlorine pesticides. Another *Trichoderma* fungi species degrades DDD, as identified in a study [101]. Phanerocharte chrysosporium and Phlebia sp degrade the organochlorine hepachlor [111].

In a study, it was reported that toxaphene in waste substrate was biodegraded by the fungus Bjerkandera sp. [112], imidacloprid and nicotine were degraded by Calocybe indica [113]. [114] discovered that brown rot and white rot fungi degrade 1,1,1-tricvhloro-2, 2-bis (4-chlorophenyl) ethane (DDT). Fungal genera such as Gloeophyllum, Daedalea, and Fomitopsis showed degradation activity for DDT and produced metabolic products like DDD, DDE, and DBP [115]. The presence of chemicals like Dextrin is a threat to human beings and disturbs the ecosystem [116]. Phanerochaete chrysoporium is a fungus that is widely studied for its pesticide biodegradation. A number of polyaromatic hydrocarbons, like pyrene, di and tribenzoic acids, DDT, lindane, and chordane, break apart during degradation [29].

The fungus Cladosporium cladosporioides degrades the pesticide chlorpyrifos in the soil [117]. Abd El-Ghany and Masmali [118] showed in a study that entomopathogenic fungi, Metahizium anisopliae, biodegraded 90 % of malathion chemical pesticides. The investigation was conducted to check the interaction between chlorpyrifos and the fungus Metarhizium anisopliae to control houseflies. The results showed that the residues of chloropyrifos were lower when compared to the control in the laboratory condition after 14 days of treatment. This led the researcher to the idea that the fungus M. anisopliae has the capacity to degrade chlorpyrifos in the soil.

Treatment involved soil, soil and insecticide, insecticide, and fungal inoculums. Each of the insecticides was added at 500 ppm, and two mL of fungal spore suspension was added to the Petri dish and incubated at 30 °C for 21 days. The insecticidal residual analysis was then conducted. Five concentrations of insecticides were taken with the solvents chosen, and modified solvent direct immersion extraction (SIDE) was used to extract insecticides from the soil [119]. The final residues were analyzed using the Acquity UPLC Water system with the photodiode array detector. Separation was achieved with an Acquity UPLC BEH C_{18} column and the emitted wavelength was read. The quantitative measurements of insecticidal residues were followed by the CDFA standards (CDFA, 2011).

The overuse of chemical pesticides such as chlorpyrifos and cypermethrin in the environment causes various hazards. M. anisopliae degrades the organophosphates in the soil [118]. In a study [120], used M. robertsii to degrade tributylin by estradiol, and a significant reduction of tributylin was observed. The successful degradation of chlorpyrifos was done by the fungus Lecanicillium sp [121]. [122]. also demonstrated that Aspergillus niger and Trichoderma viride degrade chlorpyrifos. In another study, it was shown that Aspergillus niger also degraded the malathion in the contaminated soil. After 24 h of treatment, 300 mg of malathion has been broken down, but 800 mg of malathion has been used in the field [123].

Nonylphenols are non-ionic surfactants used in detergents, paints, pesticides, and plastics. Nonylphenols are the most commonly used nonylphenols and occur in several dozen isomers. This notorious chemical is harmful to the environment; it persists longer, is bioaccumulative, and is toxic. These compounds have negative impacts on microorganisms and invertebrates and also inhibit the growth of plants [124]. *Metarhizium* species are able to degrade unbranched isomers of nonylphenol. Metarhizium robertsii and Metarhizium brunneum show a 90 % substrate loss in 24 h of incubation with these compounds (Rozalska et al., 2013). Metarhizium robertsii showed 35 derivatives after degradation of these chemicals (Rozalska et al., 2015; [125]. M. robertsii's degradation pathway suggests that it has some potential for reactions in both aliphatic and aromatic rings [125]. In the natural environment, M. robertsii can completely break down nonylphenol into CO2 and H2O (Rozalska et al., 2015; [125].

Table 2
Fungal species produce different enzymes and their potential degradation of chemical pesticide.

S. no	Fungal species	Enzymes types	Potential degradation of chemical pesticide	Reference
1	*B. bassiana*	chitinases	Synthetic Pyrethroid Cypermethrin	[132]
2	*B. bassiana*	chitinases	triazines	[1]
3	*T. harzianum* and *M. anisopliae*	phosphotriesterases	profenofos, diazinon and malathion	[118]
4	*P. ostreatus*, *R. solani*, *Mucor* sp, *Aspergillus* sp, *R. arrhizus*, *P. chrysosporium*, *T. hirsuta*, *L. edodes*, *T. versicolor*, *B. adusta*, *I. lacteus*, *A. bisporus*, *P. tuber-regium*, *P. pulmonarius*, *T.harzianum*	phosphotriesterases	Cypermethrin, organophosphate	[8]
5	*B. rhodina*	laccase	lubendiamide and thiamethoxam	[133]
6	*P. chrysosporium* and *T. versicolor*	phosphomonoesterase, protease, β-glucosidase, cellulase, laccase	simazine, dieldrin and trifluralin	[134]
7	*P. chrysosporium*	Cytochrome P450s	chloropyridinyl neonicotinoid	[135]
8	*T. versicolor*	Cytochrome P450	diuron and 3,4-dichloroaniline	[135]
9	*A. niger*	phosphatases and esterase	organophosphate	[136]
10	*A. niger*	phosphatases and esterases	organophosphorus	[122]
11	*F. oxysporum*	cutinase	malathion	[137]

It has now been reported that the nonlignolytic fungus Umbekopsis isabellina [126], Bjerkandera sp., Phanerochaete chrysosporium, and Pleurotus ostreatus [127] biodegrade nonylphenols. Bjerkandera sp. and T. versicolor removed tNP initially added at 100 mg/L-1 by 99 and 97 %, respectively, in the presence of glucose (Soares et al., 2005a). Candida aquaetextoris metabolized hazardous xenobiotics at 100 mg/L during the 14th day of incubation [128]. Entomopathogenic fungi are an ever-growing field for studying the biodegradation of insecticides. The use of chemical insecticides causes various ill effects. To reduce soil contamination from insecticide residues, researchers are looking to employ entomopathogenic fungi to degrade the pollutants caused by insecticides, as they are bio-based and have no chance of causing any toxicity to humans or the environment.

6.3. *Role of enzymes in biodegradation of insecticides*

6.3.1. *Fungal enzymes and their degradation capacities*

Fungi degrade extracellularly in two ways: one by hydrolytic enzymes and the second by producing lignolytic enzymes (Table 2). Hydrolytic enzymes produce hydrolase enzymes, which are responsible for degrading macromolecules and lignolytic enzymes. These fungi have complex and powerful extracellular enzymatic systems. In nutrient conditions, fungi are able to degrade lignolytic compounds like dyes and pollutants in the environment [129]; Sanchez, 2009). The specificity of fungal enzymes was low, which is the reason why fungi can metabolize diverse pollutant compounds. Phanerochaete chrysosporium degrades many compounds such as toluene, benzene, xylenes, nitroaromatic, organochlorines, pesticides, and dyes [130]. The white rot fungi are robust organisms, among others, and tolerate excess pollutants [129]. describes the main oxidative enzyme producers in the polluted environment. On the other hand, white rot fungidegrade lignin with their powerful enzymes [131]. White-rot fungi degrade polychlorinated compounds by suggesting the production of a variety of isoenzymes with diverse substrate specificities (Benitez et al., 2021).

In the laboratory, Trichoderma harzianum, Scopulariopsis sp., and Aspergillus niger strains were able to degrade glyphosate and aminomethyl phosphonic acid [138]. Several Aspergillus species, Trichoderma harzianum, and Penicillium brevicompactum have been reported to use chlorpyrifos as phosphorus and sulfur sources (Mohapatra et al., 2022). Similarly, extracellular release of alkaline phosphatase, inorganic phosphates, and ammonia was linked to the degradation of an organophophate insecticide, monocrotophos, by three fungal strains, Aspergillus flavus, Fusarium pallidoroseum, and Macrophomina sp [139]. Esterases have high catalytic activity, which enables them to degrade pyrethroids. Enzyme immobilization techniques have been used, but in limited numbers [71]. Phanerochaete acts on organic compounds as it has strong extracellular enzymes. H_2O_2 is produced by them, which catalyzes the reaction and cleaves into a number of compounds. In a study, it was found that Aspergillus niger synthesizes the enzyme phosphotriesterases, which hyrolyze organophosphate insecticides like chlorpyrifos. Many phosphotriesterases have been found, including organophosphate hydrolase, methyl parathion hydrolase, paraoxonase, carboxylesterases, and organophosphorus acid anhydrous from fungus [140].

The monooxygenases are involved in the metabolic removal of xenobiotics. These monooxygenases have several structures, have catalytic activity, and have a wide range of substrates [141]. In the presence of a xenobiotic environment, Metarhizium sp produces more cytochrome P450 (CYP450), and CYP450 is involved in the elimination of nonylphenol, as research has shown [125]. Metarhizium species utilize 4-*n*-Nonylphenol, and this has been studied among the strains. M. anisopliae was more effective than other species after 24 h of incubation; it removed 30 % of NP. M. majus and M. lepidiotae were less efficient in the removal of the chemical 2, 4D at 48 h; they removed only 39 % and 34 % respectively. White rot fungi and their oxidoreductase enzymes are used in treating AP-contaminated wastewater, soils, and sludges [142]. [143] showed that the application of white rot fungal enzymes removes NP from contaminated soils.

[133] found that *Botrysophaeria rhodina* and JUANT070 fungi produced enzymes when they were grown on a liquid medium with Actara®, Belt®, flubendiamide, and thiamethoxam. CytochromeP450 activity increased three to fourfolds when M. brunneum and M. globosum were supplemented with 4-*n*-NP. *Metarhizium* has CYP450 monooxygenases that degrade and penetrate the insect cuticle rich in alkanes and hydrocarbons, which act as a barrier in protection against infections [144]. The cytochrome P450 enzyme hydroxylates the compounds in the insect and then catabolizes them by b-oxidation [145]. The fungi *M. robertsii* and *M. brunneum* break down nonylphenols, triazines, tin, synthetic estrogens, and industrial dyes [1], (Fig. 4; Table 2).

Pesticide removal using enzymes is thought to be a quick and efficient method (Table 2). Most enzymes are substrate-specific, and only certain pollutants can be degraded using specific enzymes. Entomopathogenic fungi, on contact with insecticide residues, produce various enzymes that have the ability to degrade the pollutants by means of enzymatic mechanisms. The enzymatic mechanisms of EPF are discussed in this review below.

6.4. *Enzymatic mechanism*

Entomopathogenic fungi, Beauveria bassiana, can assimilate n-alkanes. Metabolic pathways and enzymes are involved in decomposing these chemicals [146]. The compounds n-alkanes are hydrocarbons, which are xenobiotics present in the environment and most popularly in the insect cuticle, where fungi first overcome these compounds by utilizing them as a carbon source to enter the insect body. Extracellular oxidoreductase enzymes in fungi help to attack organic compounds. These enzymes support fungal growth on recalcitrant substrates such as lignocelluloses, which are not accessible by bacteria [147]. In the hydrolysis of organophosphates, two compounds are involved: phosphotriesterases (PTEs) and carboxylesterases (CBEs), whereas detoxification is done by carboxylesterases [148]. PTEs are important enzymes in the hydrolysis of organophosphates, which leads to the detoxification of organophosphates. The PTE enzymes involved in the reaction are organophosphate hydrolase, organophosphorus acid anhydrolase, paraoxonase, and diisopropylfluorophosphate. These metabolites are less toxic when compared to chemical pesticides [140].

Enzymes are required for the catalysis of various metabolic reactions, such as the addition of oxygen to a double bond, dehalogenation, hydrolysis, side chain metabolism, oxidation, reduction of a nitro group to an amino group, and sulfur replacement [149]. [150] PTEs produced by fungi from the marine environment show unique properties when their natural habitat is brackish or saline. These enzymes may act as biocatalysts with high tolerance to salinity, hyperthermostability, and cold adaptability [151]. Pesticide-containing esters can be degraded by enzyme. This enzyme acts as a regulator to degrade pyrethroid pesticides [71]. The degradation of 4-*n*-NP has four different pathways, which allows a better understanding of its mechanism. Group I derivatives possess a

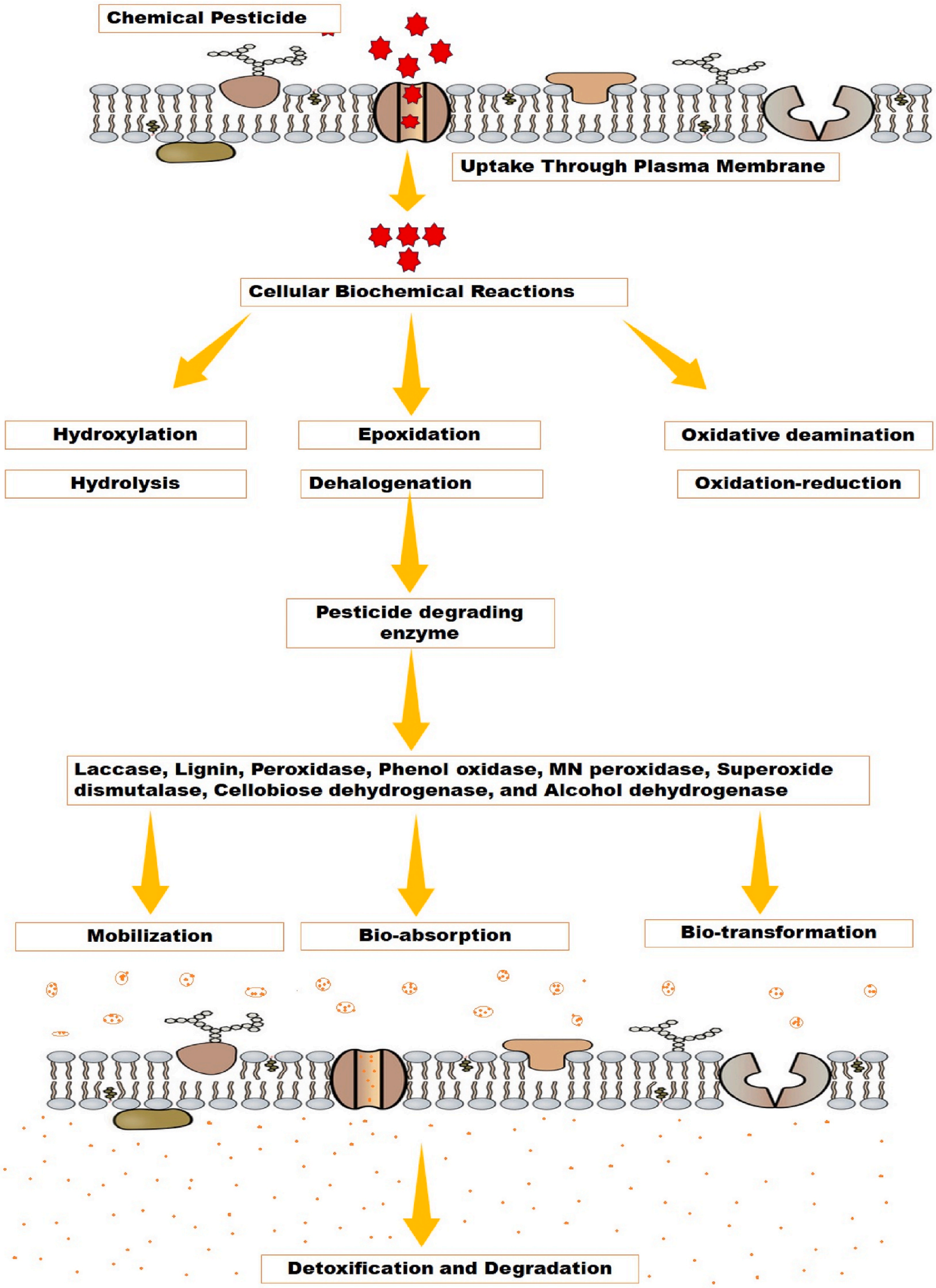

Fig. 5. Mode of action of fungal enzyme on chemical pesticide degradation.

hydroxyl group near the aromatic ring in the nonyl moiety, and some have two hydroxyl groups in the nonyl chain (Rozalska et al., 2015b). Group II includes monohydroxylated metabolites with a hydroxyl group in the nonyl chain. *Metarhizium* fungal species has the capability of oxidising the methyl group, which leads to the formation of carboxylic acid and removes carbon from the terminal chain. The most common mechanism of microorganism degradation is Group III (Krupi_Nski et al., 2013). Group IV derivatives belong to group III, hydroxylation of aromatic rings.

Filamentous fungal species are characterized by the presence of cytochrome p450. It is involved in metabolic processes and the removal of xenobiotics [141]. This enzyme in M. robertsii detoxifies and eliminates dibutylin DBT, whereas U. isabellina detoxifies and eliminates 2,4-dichlorophenoxyacetic acid, the pesticide 2,4-D (Nykiel-Szyma_Nska et al., 2018b). Pleurotus ostreatus and Cunninghamella elegans degrade phenenthrene [152]. Trichoderma koningii detoxifies alachlor (Nykiel-Szymanska et al., 2018a). M. robertsii and M. anisopliae have two genes, cytochromeP52 and cytochrome P53, in the process of assimilation of alkanes. Cytochrome P52 from Metarhizium sp. is involved in pesticide degradation [153]. [154] used laccase and peroxidase enzymes to measure the biodegradation of phenol by Lentinus tigrinus in a liquid medium.

Extracellular fungal enzymatic systems typically include ligninolytic peroxidases, laccases, and reductases. Endosulfan exposure was also shown to increase laccase enzyme activity in groups of white rot fungi, *Pleurotus eryngii* and *Coprinus comatus* [155], compared to uninoculated and individually inoculated sets. This suggests substrate induction and a possible role for laccase enzyme in biotransformation (Fig. 5). Other extracellular enzymes, such as lignin modifying enzymes and lignin peroxidase activity, have been reported in Conidiobolus sp. Even though the activity was low, the breakdown of lindane was sped up under carbon- and nitrogen-limiting conditions and in the presence of veratryl alcohol, as has been seen with other white rot fungi [156].

[157] tested the ability of lignin peroxidase (LiP), manganese peroxidase (MnP), horseradish peroxidase (HRP), and laccase to break down glyphosate and other pesticides using two mediators ($MnSO_4$ and Tween 80). The results showed that in the presence of MnSO4 and Tween 80, MnP completely degraded glyphosate with and without wiON removal. On the other hand, Tween 80 removal reduced the rate of glyphosate degradation. Even though enzyme systems aren't used for bioremediation on a large scale yet, cell-associated or cell-free extracellular enzymes can be the most important biocatalysts for breaking down xenobiotic compounds like malathion (Fig. 5). Cell-free enzyme extracts have several advantages over cellular systems [158].

[159] recently identified and described a CCA gene cluster in a fungal strain, F. oxysporum, that contains duplicated cyanase and carbonic anhydrase and has the potential to detoxify a general-purpose pesticide cyanate. This gene cluster was discovered to have recently evolved independently in different fungal lineages and was linked to either the ability to use cyanate or resistance to plant-produced cyanates. Researchers are looking deep into the mechanisms that help degrade insecticide pollutants. In the future, EPF enzymes will be synthesized in large quantities to degrade existing pollutants in the environment. This green mechanism will be the way to generate a greener world.

7. Recommendations and future perspectives

7.1. Recommendations for large scale methods

According to numerous laboratory reports [160], pesticide susceptibility varies between fungal species and even strains of the same species [160]. Furthermore [161], worked on the myco-degradation of Malathion using *A. niger* and obtained an 86.72% degradation. Recent research in this field has revealed that a wide range of microorganisms can degrade malathion [162]. After a 21-day incubation period, *Trichoderma harznaium* and *Rhizopus nodosus* were able to degrade 70–80% of the chloropyriofos and ethion [163]. Ullivan et al. (2019) found that pyrethroids were absorbed by humans and were also found in their urine samples.

Esterase applications are hampered by their low biochemical stability. Large-scale bioremediation applications of esterases necessitate 1) the availability of suitable esterases for hydrolyzing pyrethroids and 2) the development of a method for stabilizing esterase enzymes for reuse in pyrethroid degradation 3) Esterase engineering to improve reaction efficiency [164]. Immobilization of enzymes is the most effective way to improve the stability of the esterase family (Fig. 5). Among the various methods for enhancing enzyme stability, carrier enzyme interaction and immobilization via adsorption on solid beads are widely used [165]. Enzyme immobilization aids in the recycling of biocatalysts, allowing for easier separation and improved enzyme performance in pyrethroid degradation. This method can be used to boost the activity of pyrethroid hydrolases found in bacteria, fungi, or heterologously expressed esterases [166]. Enzymes purified from various microbes may be a better option for rapid, large-scale pyrethroid bioremediation (Fig. 5). Large-scale bioremediation has begun, and when industries stop synthesizing chemical insecticides that cause environmental damage, the earth will be greener and no human-related health issues will be identified.

7.2. Future perspectives

More research should be done on insecticidal microorganisms to: (a) find new insecticidal microorganism resources, (b) broaden the insecticidal spectrum, (c) shorten the time it takes to grow, (d) make sure they can spread vertically and horizontally, and (e) make the environment more sustainable. (Zhang, 2018). The stability of microbes in the natural environment is of major concern and needs to be studied more broadly. As a result, future research on organophosphorus pesticide microbial degradation ought to emphasise the following points: (1) investigate degradation strains grown in natural environments; cultivate, develop, and employ high-efficiency fungi for organophosphorus pesticides; and establish a seed (gene) bank for high-efficiency degradation entomopathogenic fungi. (2) extract the related genes and transfer them to a common species, such as E. coli, for degradation enzyme gene cloning and expression, constructing engineering bacteria, improving degradation ability, and preparing degradation enzymes based on the identification of the degradation enzyme gene (3) Field application of biological pesticides, which can help reduce the use of high-toxicity pesticides; and (4) Promotion of more applied research on the microbial method for dealing with contaminant degradation in the real world [83].

8. Conclusions

The entomopathogenic and filamentous fungal species such as M. robertsii, M. brunneum, M. globosum, M. anisopliae, and other Metarhizium species, Beauveria species, Pleurotus ostreatus, Cunninghamella elegans, Trichoderma koningii, Lentinus tigrinus, Botrysophaeria rhodian, Aspergillus flavus, Fusarium pallidoroseum, Macrophomina sp., Phanerocharte chrysosporium, Phlebia sp., Gloeophyllum, Daedalea, Fomitopsis sp., Phanerochaete chrysoporium, and Cladosporium cladosporioides can effectively degrade the chemical synthesis pesticide in water and soil conditions. Insecticides like chlorpyrifos and cypermethrin, pyrethroids, organochlorines, and other organic insecticides could be the focus of future research on how to get the molecules and enzymes out of fungi. Pesticides can be degraded in the environment through microbial, chemical, and photodegradation processes. Still, microbial degradation is thought to be the most important factor in determining what happens to organophosphorus in the environment. It is often the main way that pesticides break down in soils, making it the safest, least disruptive, and most cost-effective way to treat the problem. There needs to be a lot of research in this area to make the catalytic enzymes that break down the insecticide pollutants.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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