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Review Article

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Cypermethrin toxicity in the environment: analytical insight into detection methods and microbial degradation pathways

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Abstract

The unrestricted utilization of xenobiotic compounds has sparked widespread concern by the world's growing population. A synthetic pyrethroid called cypermethrin (CP) is commonly utilized as an insecticide in horticulture, agriculture, and pest control. The high toxicity levels of accumulated CP have prompted environmental concerns; it damages soil fertility, and an ecosystem of essential bacteria, and causes allergic reactions and tremors in humans by affecting their nervous systems. The damage caused by CP to groundwater, food, and health makes it imperative that new effective and sustainable alternatives are investigated. Microbial degradation has been established as a reliable technique for mineralizing CP into less toxic chemicals. Among the many enzymes produced by bacteria, carboxylesterase enzymes are determined to be the most efficient in the CP breakdown process. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) have been reported as the best methods for determining CP and its metabolized products, with detection limits as low as ppb from diverse environmental samples. The current study describes the ecotoxicological impact of CP and innovative analytical techniques for their detection. The newly isolated CP-degrading bacterial strains have been evaluated in order to develop an efficient bioremediation strategy. The proposed pathways and the associated critical enzymes in the bacterial mineralization of CP have also been highlighted. Additionally, the strategic action to control CP toxicity has been discussed.

Keywords: analytical techniques, cypermythrin, toxicity, microbial degradation, pyrethroids

Introduction

The Indian agriculture sector plays a crucial role in the economic development of the country, and each year about 45% of the outcomes are lost due to pest infestation. This has led to the widespread application of pesticides for the management of pests to increase agricultural yield. The insecticide pyrethroids, which are extracted from the dried flowers of the *Chrysanthemum cinerariaefolium* plant, are regarded as the most effective as it works even at low quantities. Cypermethrin (CP) [(+/-)-α-cyano-3-phenoxybenzyl (+/-)- *cis*, trans-3 (2, 2 dichloro vinyl)–2, 2-dimethylcyclopropane carboxylate] is a synthetic type-II pyrethroid pesticide used for controlling the insect population such as fruit moths, vegetable moths, and cotton moths in both agricultural and urban fields, especially in third-world countries (Sharma et al. 2016). It can also be used for household tasks, including home and garden pest management, and some of the most popular formulations exist in the form of chalk, which is frequently used in houses to ward off ants and cockroaches. Across India, the amount of CP produced was around 24 thousand metric tonnes at the end of the fiscal year 2020. In aquatic bodies, CP concentrations range from 0.01 to 9.8 mg L^{-1}, while after application, agriculture runoff can have amounts of up to 194 gL^{-1} (Zhao et al. 2021).The recent studies showed that the continuous and excessive use of CP creates massive environmental and health problems by contaminating the water bodies and soil. The compound accumulates in the soil as a result of its overuse and disrupts the soil microbiota and terrestrial invertebrates, and causes diseases in human beings. According to studies, it has extremely high toxicity, and carcinogenic effects and causes low levels of RBC and plasma proteins in aquatic animals like fish, zooplankton, and amphibians. It may also cause neurotoxicity, endocrine disruption, immunotoxicity, and developmental toxicity. There are four *cis* and four trans isomers of CP, and both forms are present in a 1:1 ratio. Trans isomers are alkylated two times more quickly than *cis* isomers. The *cis:* trans ratio exists in the ranged from 48:52 to 50:50 according to the papers that were submitted for assessment. The half-life of CP in soil and water is predicted to be 30–60 days; during that time, it could enter organisms that live in the polluted sites through the food chain and spread to nearby land and stagnant water resources. Depending on the properties of the soil, the half-life of CP in the environment ranges from 14.6 to 76.2 days (Chen et al. 2012a). The United States Environmental Protection Agency has also classified CP as possibly carcinogenic to humans.

For the eradication of this pesticide, various physicochemical procedures such as hydroxylation, photocatalysis, filtration, membrane technology, coagulation, dilution, photocatalytic degradation, and flotation have been developed (Bisaria et al. 2021; Affam and Chaudhary, 2013) However, concerns like process complexity, harmful byproduct formation, insufficient removal efficiency, technical limitations, and inflated costs demand for the development of novel wastewater treatment systems (Sinha et al. 2022, Solanki et al. 2022). Biological method is an appealing approach for CP degradation, which results in the mineralization of toxic components into a non-toxic form. This process is both effective and

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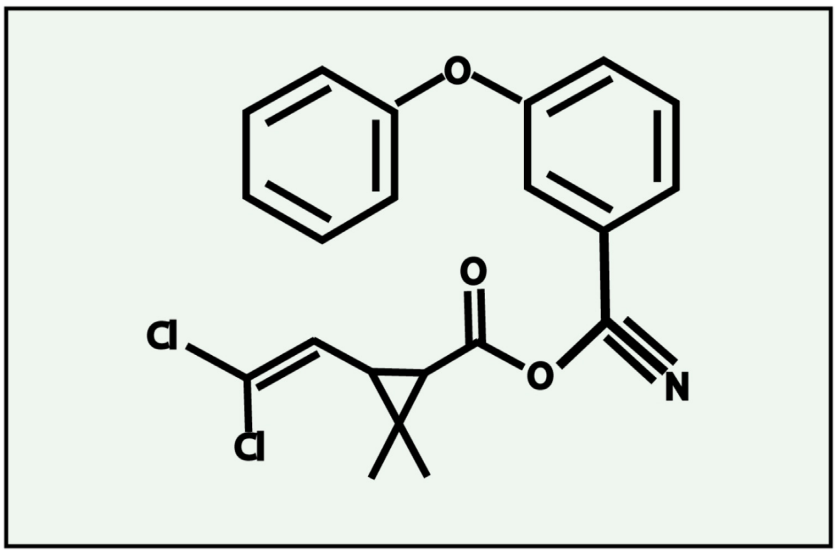

Figure 1. Chemical structure of CP.

environmentally beneficial. Various bacterial and fungal species, including *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Roultella*, *Aspergillus*, *Candida*, and *Trichoderma* have been reported to degrade CP. The metabolism of CP by microorganisms in soil determines the pesticide's fate and contributes to reduce it in nature.

The microbes have a variety of enzymes that can partially or completely biodegrade CP and finally release non-toxic biproducts. The bacterial enzymes responsible for the deterioration of foreign substances are laccase, hydrolase, peroxidase, esterase, dehydrogenase, manganese peroxidase, and lignin peroxidase (Cycon and Piotrowska-Seget, 2016)), and their specificities vary from microbe to microbe (Yin et al. 2013). In a previously reported study, 95% degradation of CP was observed by using indigenous bacteria (*P. alcaligenes*, *B. amyloliquenfaciens*, and *P. aeruginosa*) from contaminated soil (Indratin et al. 2019). Based on the aforementioned literature, the current review aims to furnish a brief summary of the toxicological effects of CP along with their detection and degradation techniques. The review also consists of recent findings, highlighting the benefits and drawbacks of the use of CP pesticides and recent techniques for pesticide remediation. Moreover, factors that affect the efficiency of CP degradation have also been analysed to understand their role in influencing the rate of biodegradation of CP. Strategies to control and prevent the use of synthetic pyrethroids like CP are also discussed along with the future prospects of the study. In addition to providing a reference for other studies, this information could be used to assess the pesticide degradation efficiency of various bacterial species. This study can help researchers, environmental engineers, and scientists to bridge the gap in the available knowledge about the bacterial mineralization of CP.

Research methodology

Using different search engines of different databases like Web of Science, PubMed, SCOPUS, and ScienceDirect, inclusive literature of the pyrethroid majorly on CP was done from 1990 to 2020. The search was carried out using selected keywords such as pyrethroids, CP, mineralization of CP, degradation process of CP, mineralization of pyrethroids, quantification and analytical methods used for monitoring, and techniques used for detection of pyrethroid. Overall, 150 papers were selected and reviewed. The last 120 articles were incorporated in this review based on their importance to the selected review in agreement and peer-reviewed articles. Unpublished work was excluded from the study.

Toxicity of CP

CP, a toxic chemical synthesized in 1974, was first marketed as an insecticide in agriculture and public health in 1977. Due to low vapour pressure and Henry's Law Constant, it cannot be volatilized in nature. The chemical structure and physical properties of CP are illustrated in Fig. 1 and Table 1, respectively.

Impact on humans

In humans, CP toxicity occurs through accidental or intentional exposure through inhalation, skin contact, or ingestion. According to recent research, among 573 cases of acute pyrethroid poisoning, 229 and 344 cases of poisoning were caused by occupational and accidental exposures, respectively (Ramchandra et al. 2019). The toxic intermediate 3-phenoxybenzoic acid (3-PBA) of CP breakdown was found at significant concentrations in humans, for which diet was considered the primary route of exposure (Zhao et al. 2021). CP toxicity affects the nervous system by reducing antioxidant defence mechanisms and inhibiting acetylcholinesterase (AChE) activity. CP attacks the central nervous system, interacts with the sodium channels, and causes hyperexcitability (Indratin et al. 2019). CP toxicity arises two types of acute pyrethroid neurotoxicity: tremor-type (T)-syndrome and choreoathetosis-salivation (CS)-syndrome (Richardson et al. 2019). T syndrome can be developed by type I pyrethroids, which cause tremors throughout the body and hypersensitivity reactions. The CS syndrome is caused by pyrethroid type II classes. CP toxicity to humans is a type I hypersensitivity reaction, and the ECG shows the changes in ST-T channels and premature ventricular beats. The high dose of CP may lead to situations like a muscular switch, convulsion, coma, and even, in some cases,

Table 1. Physical parameter of CP.

IUPAC name	Molecular formula	C:N:P Ratio	pH	Temperature	Moisture content	Dissolved oxygen	Relative molecular	Melting point	References
Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate	$C_{22}H_{19}Cl_2NO_3$	100:10:1	6.5–8.0	25–30°C	60%–80%	10–12ppm	416.3	80°C	(Laskowski, 2002)

respiratory loss. Toxicity also enhances the saliva level, thickening of alveolar septa in the lungs, upper gastrointestinal bleeding, and even kidney failure. The sub-lethal dose of CP also affects the reproductive system (Das and Parajuli 2007) that can lead to the reduction of follicular cells and oocytes in the ovaries. It also reduces the total sperm count and produces sperm-less seminal plasma and tailless spermatozoa in the epididymis. Hence, CP toxicity induces changes in both man and woman reproductive systems (Ramchandra et al. 2019). CP toxicity also affects the immune system of the body by causing a mutation in the TP53 gene (Chrustek et al. 2018). Thus, they impair immunity and induce apoptosis of the cells. It also causes chronic effects like pathological changes in the thymus, liver, adrenal glands, and lungs. Toxicity can lead to changes in organ morphology and can increase the glucose and lipid concentration levels in the body. Children are more prone to neurotoxicity due to reduced metabolic activity (Anand et al. 2006). Hence, CP leads to devastating effects by creating neurotoxicity in an organism that majorly attacks the sodium channel of the nerve membrane and also enforces the functional and molecular systems. No antidote present can depress its poisoning effects (Ramchandra et al. 2019).

Impact on animals

CP affects the nervous system of the animals equally. It blocks the sodium channel gates that lead to membrane depolarization (Indratin et al. 2019). Due to the hairy layer of the skin on animals, the CP is absorbed through the skin at very low concentrations, and in the liver, it is rapidly metabolized by the enzyme glucuronidase to form non-toxic compounds that can be easily excreted through urine. But some animals, like cats, cannot metabolize CP as they lack the enzyme glucuronidase. Arthropods exhibited hyperactive behaviours when exposed to low amounts of CP, while at high concentrations; they suffered paralysis attacks that could be fatal (Ramchandra et al. 2019). Exposure to CP in rats led to increased activity-related brain damage (Kumar et al. 2004). In laboratory investigations, it was found that even at low concentrations of the CP (0.3 to 4.3 mg L^{-1}), mice and rats experienced negative side effects. (Yin et al. 2013). Additionally, CP toxicity can also cause neurobehavioral-grabbing changes, burrowing movements, increased urination, and reduced metabolic activity (Ramchandra et al. 2019).

Impact on plants

CP toxicity produces negative effects on plants also. It increases the chromosomal abnormalities and inhibits the cell division of plant cells. It also decreases the time taken by plants for nitrogen fixation (Cox, 2002). Studies have been conducted to observe the CP effect in agricultural soil on the growth, cell division, and photosynthetic pigment of plants like onion, maize, and green pea. It is observed that with increasing concentrations of CP, there have been negative impacts on the overall growth of the plants (Ramchandra et al. 2019). Results showed that test concentrations of 0.2, 0.4, 0.6, and 0.8 gL $^{-1}$ of the CP remarkably lessened the growth of roots, and shoot length as compared to the control. A decrease in the concentration of the photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids was also noted. With the increase in the concentration of CP, there was a

significant reduction in the moisture content of the plants (Cox, 2002).

Analytical methods for CP detection

Conventional techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC) along with GC and mass selective detection (GC/MSD), GC with electron capture detector (GC/ECD), electron ionization and mass spectrometry (EI/MS), UV-visible spectrometry, and MS have been used for the separation and identification of CP and its residues from various samples such as crops, soil, and other environmental samples. These methods are popular because they can identify CP metabolites up to ng of sample concentration (Grimalt and Dehouck 2016). These techniques work on the premise of extracting CP residues with an organic solvent, cleaning the extract by solvent-solvent partition and adsorption column chromatography, and determining the CP residue with various chromatographic and photometric detectors. GC and MSD or TLC are commonly used to estimate CP residues. Even in the presence of other synthetic pyrethroids or other classes of pesticides and organochlorine insecticides, these methods are highly effective for determining CP. Nardelli et al. (2021) developed a sensitive screening method using GC in conjunction with an ECD to estimate up to six pyrethroids, including CP, in eggs and meat samples (Nardelli et al. 2021). The CP concentration obtained was in the range of 50–500 gL^{-1}, as determined by an instrument with a detection limit of 0.22–0.63 gL^{-1}. The GC/ECD methods are preferred tools for the analytical detection of CP due to their simplicity and sensitivity (Mekebri et al. 2008). Chromatographic techniques such as GC and HPLC have primarily been used to detect CP in soil (Nardelli et al. 2021). The detection limit for CP in GC was reported around 0.007 gg^{-1} (Hernandes et al. 2014), whereas 0.001 mg kg^{-1} with HPLC (Bissacot and Vassilieff 1997). Kanyika et al. (2020) employed a UV-visible spectrophotometer to estimate CP from soil and water samples by following Janghel et al. 2007 methods of CP determination with certain modifications, attaining a detection limit of 0.003 gL^{-1}, which was nearly equal to the previous study's detection limit of 0.0027 gL^{-1} (Janghel et al. 2007, Kanyika et al. 2020). In another study, solid-phase extraction techniques in conjunction with GC-MS were used to investigate the degradation of CP and its residues in soil (Kavvalakis et al. 2014; Garoiaz et al. 2012). The extraction and detection procedures were carried out in two steps: first, CP was recovered from the matrix using a mixture of 30% ethyl acetate in n-hexane by the Soxhlet extractor, and then it was analysed using GC and electron impact ionization MS. The detection limit for CP was determined to be 6.5 gkg^{-1}. The GC-MS method is highly specific and sensitive in order to detect very small amounts of CP in agricultural samples. This technique could also be used to identify metabolites and degradation products (Garoiaz et al. 2012). The high-performance thin-layer chromatography (HPTLC) approach also employed to identify CP after it was recovered from soil samples using the micro-assisted extraction (MAE) technique; the detection limit was reported to be around 2.1 $ngspot^{-1}$ (Acikkol et al. 2012). Therefore, the HPTLC method is also quite effective for detecting CP in soils because of its great sensitivity and speed.

Traditional methods of CP detection are being phased out in favour of newer techniques such as electrochemical sensors, optical sensors, etc. (Bouya et al. 2012; Kaur and Singh 2021). Some of the optical sensors used include chemiluminescence, Raman, fluorescence, and UV-visible. These advanced technologies are simple, fast, precise, less expensive, and allow on-site pesticide detection. A new approach named the Microfluidic Paper-Based Analytical Device is being used to identify type II pyrethroids in various water samples. This technique is rapid, low-cost, requires fewer amounts of samples, and provides semi-quantitative analysis of CP and other pesticide-contaminated samples. A smartphone-based dual-channel immunochromatographic test strip that enabled the simultaneous analysis of CP and its primary metabolite 3-PBA was proven to be a very precise device (Pengpumkiat et al. 2020). When the target sample was exposed to CP, a competitive immunoreaction occurred between the target sample and the antigens placed on the test line. Red fluorescence on the test line, which was recorded and processed by smartphone-based equipment, served as a visual indicator of the presence of CP. The device found quantities of CP and 3-PBA between 1 to 100 $ngmL^{-1}$ and 0.1 to 100 $ngmL^{-1}$, respectively. This gadget is reliable for pesticide exposure biomonitoring (Zhao et al. 2021). The advantages and disadvantages of different techniques for CP determination are summarized in Table 2.

Methods for CP degradation

Concerning the molecular structure of CP, the three main proposed degradation channels are ester cleavage (the breaking of carbon and an oxygen double bond), isomerization (the conversion of one isomer to another isomer), and reductive dehalogenation (the elimination of chlorine, fluorine, or bromine atoms) (Ismail et al. 2013). For the mineralization of CP, several physicochemical and biological techniques have been established. However, the physicochemical approaches are inefficient because they have many drawbacks, such as their high cost and lack of environmental friendliness, and do not completely degrade toxic compounds into non-toxic compounds instead of aiding them in binding to the matrix or converting them from one phase to another. Therefore, newer studies are now focusing on the potential of microorganisms for the effective degradation of CP, which is a more cost-effective and environment- friendly alternative to traditional methods.

Microbial degradation

The use of microbes for degrading pollutants from air/soil/water in variable conditions is considered one of the practically feasible and appropriate methods at a low-cost (Mehrotra et al. 2019, Mehrotra et al. 2021a). Microbes also live in symbiotic relationships with other organisms that help in successful degradation (Mehrotra et al. 2020). The CP breakdown is primarily accomplished by bacteria, followed by fungal and algal strains. A key benefit of using bacteria for pesticide breakdown is their diversity, widespread distribution, and ability to adapt to different metabolic pathways (Huang et al. 2018). The gene clusters, genetic manipulation, and genetically engineered bacteria have also been used to completely degrade organic toxic pollutants into non-toxic compounds like carbon dioxide, water, and various other inorganic compounds (Mehrotra et al. 2019b). The two important conditions required for successful degradation are the effective microbial consortia in the polluted site and the

Table 2. Analytical Methods for CP detection: advantages and disadvantages.

Method	Advantages	Disadvantages	References
GC/MS	• High sensitivity and rapid • High resolution separation	• Significant sample preparation with chemical modification • Slow analysis time • limited number of molecules can be analysed	(Saied et al. 2021)
TLC	• Sensitive method • Easy method to separate components of a complex solution • Non-volatile components can be separated • Small sample size can also be analysed	• Results can be difficult to reproduce • Only qualitative analysis is possible not quantitative • Not automated • Only soluble components of mixture can be separated	(Kaur and Singh, 2021)
HPLC with UV detector (UV)	• Rapid and precise quantitative analysis • Automated operation • Highly sensitive detection • Quantitative sample recovery • Can be used for different types of samples • Low cost	• Less separation efficiency than capillary GC • Difficult to operate for first time users • Relatively low sensitivity	(Li et al. 2016)
GC-ECD	• Rapid Analysis • High resolution capacity • Automated systems • Can analyse small quantities of sample • Low cost • Highly accurate quantification • Sensitive Detectors	• Not suitable for thermally labile compounds • Requires extensive sample preparation • Requires spectroscopy to confirm peak identity	(Ouattara et al. 2013)

optimized environmental conditions. The pH, temperature, bacterial concentration, or CP concentration have a significant impact on the CP degradation. The largest amount of CP was decomposed by the *P. nitroreducens* bacterial strain CW7 at pH 7.0 and 32°C (Bhatt et al. 2020b), although *B. thuringiensis* Berliner metabolized CP in a liquid medium under ideal circumstances of pH 8.5 and 37°C temperature (Birolli et al. 2021). The steps involved in the biodegradation of CP are schematically represented in Fig. 2. Microbes utilize pesticides to meet their nutritional requirements as the sole sources of carbon and nitrogen and obtain energy from the degraded products (Bhatt et al. 2019, Guo et al. 2021). Enzymes guide all metabolic pathways, and bacteria are considered as the primary producers of enzymes such as lipases, proteases, alpha-amylase, hydrolase, esterase, lacases, transferase, and many others (Arora, 2020). The main enzymes for CP degradation belong to the hydrolase family known as esterase and carboxylesterase breaking the ester bonds of the pollutant (Bhatt et al. 2020a). Various studies showed that the breakdown of CP could be affected by the presence of certain energy-carrying molecules such as adenosine monophosphate (AMP), fructose 1–6 bisphosphate (F1-6BP), and NADH, and co-factors such as adenosine triphosphate (ATP), alanine (Ala), phenylalanine (Phe), and phosphoenolpyruvate (PEP) (Zhao et al. 2019). Pyruvate kinase enzyme has expressed a positive impact on CP biodegradation (Zhao et al. 2021). Nutrient regulation can help in the effective biodegradation of pyrethroids. Nutrients such as glucose, urea, peptone, and ammonium chloride helped to enhance the degradation of CP in cornflour (Zhao et al. 2019). The presence of nitrogen also has a positive impact on the degradation of CP in the soil. The maximum degradation of CP achieved after 14 days of treatment in existence of nitrogen was 80%, whereas it was 62% without nitrogen (Wen-Jun et al. 2008)). With the increase in nitrogen content in the soil, the microbial activity increased resulting in higher production of dehydrogenase enzyme activity that degraded CP in the soil. The list of bacterial strains investigated in CP degradation with their degradation efficiency are summarized in Table 3. It can be observed from Table 3 that under optimum conditions, 80%–90% degradation of CP was achieved primarily by *Bacillus* species after certain days of incubation.

Enzymes involved in CP degradation

In recent years, instead of using whole microorganisms for biodegradation, enzymes extracted from them are regarded as the most effective way for bioremediation. Enzymes are biological macromolecular catalysts that enhance the degradation rate by reducing the activation energy of molecules (Sharma et al. 2018). They have a wide specificity range and can convert harmful to non-toxic chemicals even under extreme conditions (Zhan et al. 2020).

The first step in pyrethroid biodegradation is the hydrolysis of ester bonds. There are multiple enzymes that are involved in the cleavage of esters, including oxidoreductase, carboxylesterase, monooxygenase, and aminopeptidase (Zhan et al. 2020). The carboxylesterase enzyme is a carboxylic-ester hydrolase with a molecular weight of 31 KDa and an isoelectric point of 4.85 that degrades CP without the use of cofactors. Carboxylesterase catalyzes the degradation of pyrethroids by cleaving the carboxyl ester linkage. The catalytic mechanism depends on three amino acid residues present in the active sites, namely glutamine, histidine, and serine of the carboxylesterase enzyme (Zhan et al. 2020). Pyrethroid carboxylesterase catalyzes the acylation reaction by releasing the alcohol from a carboxyl ester and forming an acylated intermediate. The acylated intermediate hydrolyzed via a nucleophilic attack mechanism in which water molecules

Table 3. The bacterial strain used for CP degradation.

Microorganisms	Degradation (%)	Optimal conditions (pH, Temp.)	Time (Days)	References
Bacillus sp. AKD1	47	8, 37.8	7	(Tiwary and Dubey, 2016)
Bacillus sp. DG-02	89.2	7.5, 30	3	(Chen et al. 2014)
Bacillus sp. ISTDS2	99	7, 30	3	(Sundaram et al. 2013)
Bacillus licheniformis B-1	50	7.5, 30	3	(Liu et al. 2014)
Bacillus subtilis BSF01	93.9,89.4, 84.7	6.7, 34	7	(Xiao et al. 2015)
Bacillus thuringiensis ZS-19	81	7.5, 30	3	(Chen et al. 2015)
Bacillus cereus ZH-3	78.4	7.5, 28	3	(Chen et al. 2012a)
Acinetobacter calcoaceticus MCm5	84.7	7, 30	10	(Akbar et al. 2015)
Azoarcus indigens HZ5	70	7, 30	6	(Ma et al. 2013)
Brevibacteriumaureum DG-12	78.3	7, 27	5	(Chen et al. 2013)
Brevibacillusparabrevis FCm9	95	7, 30	10	(Akbar et al. 2015)
Brevibacillusparabrevis JCm4	28	7, 30	10	(Akbar et al. 2015)
Catenibacterium sp. CC-5	90	7, 30°C	7	(Zhao et al. 2013)
Pseudomonas aeruginosa JQ-41	87.2	7, 30	7	(Song et al. 2015)
Pseudomonas aeruginosa JCm8	46	7, 30	10	(Akbar et al. 2015)
Pseudomonas aeruginosa CH7	90	7, 29.4	12	(Zhang et al. 2011)
Stenotrophomonas sp. ZS-S-01	86	7, 30	5	(Chen et al. 2011)
Rhodococcus sp. JCm5	100	7, 30	10	(Akbar et al. 2015)
Streptomyces aureus HP-S-01	69.3	7.5, 28	3	(Chen et al. 2012b)
Streptomyces sp. HU-S-01	90	7.5, 28	1	(Lin et al. 2012)
Ochrobactrumlupini DG-S-01	90	7, 30	5	(Chen et al. 2011)
Ochrobactrumtritici pyd-1	100	7, 30	6	(Wang et al. 2011)
Ochrobactrumhaemophilum Cm7	78	7, 30	10	(Akbar et al. 2015)
Sphingobium sp. JQL4-5	36.5	7, 30	2	(Yuanfan et al. 2010)
Sphingomonas sp. JCm3	34	7, 30	10	(Akbar et al. 2015)
Sphingomonas sp. RCm6	92	7, 30	10	(Akbar et al. 2015)
Serratia sp. JC1	92	7.6, 31	10	(Zhang et al. 2010)
Serratia sp. JCN13	89	8, 34	10	(Zhang et al. 2010)
Serratia nematodiphila CB2	98	7, 30	7	(Tyagi and Prashar, 2015)
Bacillus licheniformis B-1	85	2.5, 30	5	(Li et al. 2016)
Bacillus sp. SG2	81.6	7.0, 32	15	(Sharma et al. 2016)
Bacillus subtilis 1D	95	-	15	(Gangola et al. 2018)
Pseudomonas fulva P31	75	7.3, 29.5	10	(Yang et al. 2018)
Acinetobacter baumannii ZH-14	85	-	9	(Zhan et al. 2018)
Pseudomonas alcaligenes, Bacillus amyloliquenfaciens and *Pseudomonas aeruginosa*	95	-	101	(Indratin et al. 2019)
Bacillus thuringiensis SG4	83.3	7.0, 32	15	(Bhatt et al. 2020a)
Bacillus sp. SG2, *Azoarcus indigens* HZ5 and *Streptomyces aureus* HP-S-01	-	-	-	(Kaur et al. 2021)
Lysinibacillus cresolivuorans HIS7	86.9	7.0, 35	8	(Saied et al. 2021)

serve as a nucleophile (Bhatt et al. 2020a). The CP-degrading aminopeptidase from *P. aeruginosa* GF31 is an extracellular hydrolase, unlike most pyrethroid-degrading endoenzymes. Oxidoreductase enzymes improve the transfer of electrons from one molecule to another, that is, from the reductant to the oxidant (Scott et al. 2008), releasing chloride ions, carbon dioxide, and methanol in exchange. Monooxygenases are oxidoreductase enzymes that catalyze biomineralization reactions by transferring one oxygen atom and reducing the other with electrons from cofactors, resulting in water as a by-product. Monooxygenases are also involved in aromatic chemical dehalogenation, desulphurization, denitrification, and hydroxylation reactions, which increase the reactivity of biomineralization reactions (Sharma et al. 2018).

Previous research has found that pyrethroids can be eroded or metabolized by microbes and pyrethroid-resistant insects via oxidation by P450 monooxygenase coupling with glutathione S-transferases, and hydrolysis by phosphodiesterases or carboxylesterase (Cycoń and Piotrowska-Seget, 2016)., Ross et al. 2006). Numerous carboxylesterases for pyrethroid breakdown have been investigated, including permethrin carboxylesterase from *B. cereus* SM3, EstP from *Klebsiella* sp. strain ZD112, and pyrethroid-degrading carboxylesterases (PytH) from *Sphingobium* strains JZ^{-1} and JZ^{-2} (Wang et al. 2009, Xu et al. 2020). A study was conducted to understand the mechanism of degradation of beta-CP by monooxygenase enzymes secreted by *Streptomyces* species. At pH 7.5 and 30°C, enzyme activity was maximal. The enzyme activity was significantly stimulated by Fe^{2+} but strongly inhibited by Ag^{+}, Al^{3+}, and Cu^{2+}. During the degradation of beta-CP, this enzyme formed five products via hydroxylation and diaryl cleavage (Chen et al. 2013).

Hydrolases, also known as pyrethroid hydrolases, are another type of enzyme that is commonly involved in pyrethroid degradation. Bacteria naturally produce pyrethroid hydrolase enzymes in areas with high levels of pyrethroid contamination. They have a monomeric structure with a molecular weight of 31 KDa and an isoelectric point (pI) of 4.85 (Zhan et al.

Table 4. Intermediates produced in CP biodegradation by different microbial strains.

Microbial Strain	Source	Detected Intermediates	References
Acinetobacter baumannii ZH-14	Sewage sludge from wastewater	• 3- Phenoxybenzenemethanol • 3-Phenoxybenzaldehyde • 1,2-Benzenedicarboxylic acid • bis (2-methylpropyl) ester	(Zhan et al. 2018)
Bacillus licheniformis B-1 and *Aspergillus oryzae* M-4 (Co-culture)	Tea garden soil	• Chrysanthemic acid • 3-Phenoxybenzoic acid • Gallic acid • Phenol • Catechol	(Zhao et al. 2016)
Brevibacillus parabrevis BCP-09	Activated sludge from wastewater	• Methyl salicylate • Catechol • Pathalic acid • Salicylic acid • 3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid • 3-Phenoxybenzadehyde • 3-Phenoxybenzoic acid • Phenol • 4-Methylhexanoic • Benzoic acid	(Tang et al. 2018)
Eurotium cristatum ET	Soil	• α-Cyano-3-phenoxybenzyl alcohol • 3-Phenoxybenzaldehyde • 3-Phenoxybenzoic acid • Phenol • Catechol	(Hu et al. 2019)
Pseudomonas fulva P31	Activated sludge	• 3-Phenoxybenzaldehyde • (1R,3R)-trans-2,2- Dimethyl-3-(2-methyl-1-propenyl)cyclopropane-1-carboxylic acid • 1,2-Benzenedicarboxylic butyl dacyl ester	(Yang et al. 2018)

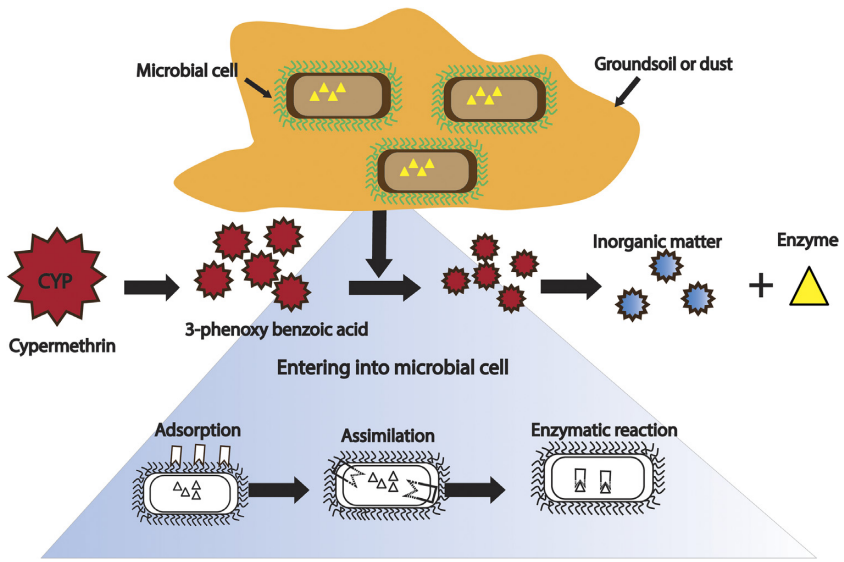

Figure 2. General mechanism of CP biodegradation.

2020). These enzymes do not require any cofactors and can catalyze the hydrolysis of a variety of bonds, including ester bonds, peptide bonds, and carbon-halide bonds (Scott et al. 2008). There are several other enzymes reported for CP biodegradation, including carboxylesterase PytZ and PytY from *Ochrobactrum anthropi* YZ^{-1} (Zhai et al. 2012, Ruan et al. 2013), carboxylesterase PytH from *Sphingobium* sp. JZ^{-1} (Wang et al. 2009). PytH has only 20% sequence identity and belongs to the α/β-hydrolase protein class (Xu et al. 2020). Metagenomic DNA coupled with activity-based functional screening from soil led to the discovery of a novel pyrethroid-hydrolyzing esterase gene Pye3. Pye3, a pyrethroid hydrolyzing esterase enzyme with a molecular mass of 31 kDa, is a monomer (Li et al. 2016). Due to its superior catalytic prop-

erties, the Pye3 enzyme is regarded as the best enzyme for hydrolyzing pyrethroids (Chen et al. 2013). These enzymes have a higher substrate specificity, making them a good choice for *in situ* pyrethroid detoxification.

Due to the low amount of enzymes produced, enzymatic bioremediation was previously thought to be ineffective. It has low stability and productivity, and any change in its activity can lead to a loss of enzymatic activity. Advances in recombinant DNA technology, enzyme engineering, and immobilization techniques, on the other hand, have significantly improved optimal growth conditions, catalytic activity, stability, and enzyme production (Sharma et al. 2018). Metagenomics and whole-genome sequencing (WGS) are considered the most appropriate approaches as they enhance the efficiency of enzymatic biodegradation (Zhan et al. 2020). The metagenomic library helps to identify and classify pyrethroid-degrading genes, such as *estP (Klebsiella* sp JD112) and *pyt H (Sphingobium* sp JZ-1), whereas the WGS provides the complete genome sequence information of pyrethroid-degrading microbial strains. WGS was performed on the illumina solexa sequencing platform and then the predicted genes were obtained using various databases, such as Gene Ontology (GO), the kyoto encyclopaedia of genes and genomes (KEGG), and clusters of orthologous groups of proteins (COG). A total of 5291 coding genes ranging in length from 38 to 11 199 bp were predicted in the *Citrobacter freundii* CD-9 genome. Among the annotation results, GO, KEGG, and COG database annotations accounted for 79.35%, 44.87%, and 77.77% of genes, respectively. Gene expression was tested using RT-qPCR, and consequently, the results obtained provide better insight into the mechanism of microbial degradation of pyrethroids (Zhou et al. 2022).

Pathways involved in CP degradation

The mechanism of CP biodegradation varies from species to species, as different organisms produce different intermediates (Zhan et al. 2020 Table 4). Because of variances in the incubation period and the chemical and biological features of the microorganisms, the mineralization routes differ (Cycon and Piotrowska, 2016). In comparison to the type-I class of pyrethroids, the pathways involved in the biomineralization of type-II pyrethroids have gain more attention (Bhatt et al. 2021a). The CP degradation involves the hydrolysis of ester bonds, which results in the generation of alcohol and carboxylic acid. The most common group of enzymes that mediates the hydrolysis of ester linkages is carboxylesterase (Sogorb and Vilanova 2002). The most common intermediates formed in degradation pathways are 3-phenoxybenzoic acid (3-PBA) and 3-phenoxybenzaldehyde (Zhan et al. 2018). Some microorganisms, such as *Bacillus sp*. DG-02 (Chen et al. 2014 Table 4), *Staphylococcus aureus* HP-S-01 (Chen et al. 2012b), and *Streptomyces sp*. HU-S-01 (Lin et al. 2012) were reported to degrade both parent and 3-phenoxybenzoic acid (3-PBA). The conversion of CP to 3-phenoxybenzaldehyde, which is more persistent and poses a greater environmental risk than CP, is the most widely recognized route of degradation. Further, 3-phenoxybenzaldehyde is transformed into 3-phenoxybenzyl alcohol and 3-phenoxybenzoic acid (3-PBA by reduction and oxidation processes, yielding a less harmful end product than the parent CP (Quiroz et al. 2011). Gangola et al. (2018) proposed a CP degradation pathway in *B. subtilis* strain 1D

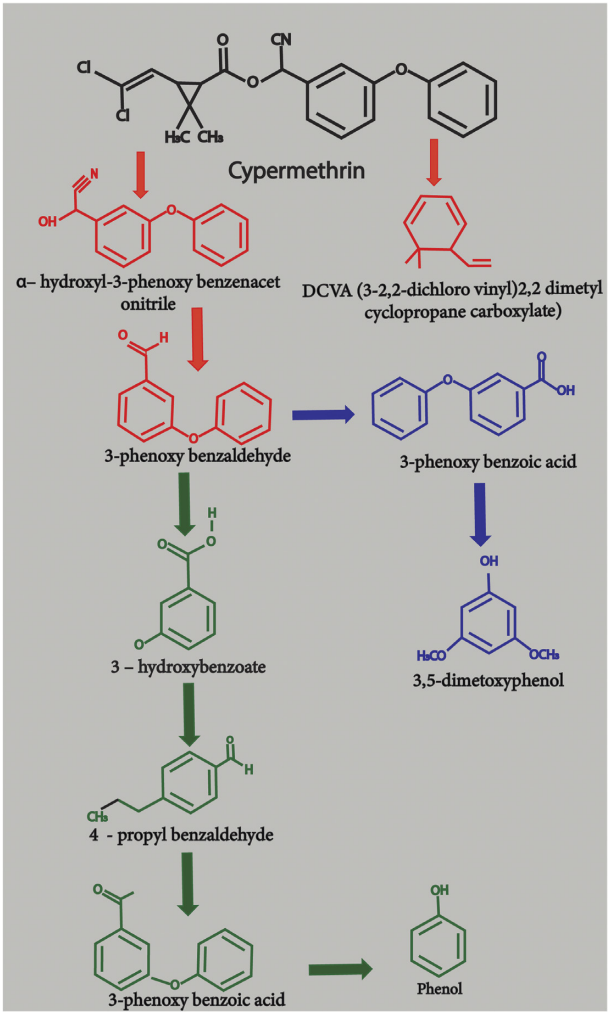

Figure 3. Metabolic pathways involved in microbial degradation of CP (*Bacillus* SG2-Red arrow, *Bacillus* BSFO1-Blue arrow, and common in both strains-Green arrow).

that involved hydrolysis of ester linkages, resulting in the formation of two products that is, 3-(2, 2-dichloro ethenyl)-2,2-dimethyl-cyclopropane carboxylate and cyclododecylamine, which dissociated further into chloroacetic acid and phenol, respectively (Gangola et al. 2018). Because phenol is an unstable molecule in the environment, it interacted with water to generate cyclopentane, which subsequently transformed to the aliphatic compounds acetic acid and decanoic acid. In another study, *B. subtilis* BSF01 and *Bacillus sp*. SG2 were reported to erode CP primarily into two compounds: α-hydroxy-3-phenoxy-benzene acetonitrile and 3-(2,2-dichloroethene)-2,2-dimethyl-cyclopropanecarboxylic acid) (Xiao et al. 2015; Bhatt et al. 2021b). Fig. 3 depicts the CP degradation pathways of *Bacillus* SG2 and *Bacillus* BSFO1. The unstable compound, α-hydroxy-3-phenoxy-benzene acetonitrile degraded to produce 3-phenoxybenzaldehyde. Additionally, 3-phenoxybenzaldehyde transformed through different routes in *Bacillus sp*. SG2 and *B. subtilis* BSF01; *B. subtilis* BSF01 metabolized 3-phenoxybenzaldehyde into 3-phenoxybenzoic acid and 3, 5-dimethoxyphenol (Fig. 3). It was subsequently changed to the 3-phenoxy benzoic acid phenyl ester, and thereafter into phenol-M-tert-butyl, phe-

nol, and aliphatic hydrocarbon molecules (Xiao et al. 2015; Bhatt et al. 2021b). Tallur and coworkers proposed the CP degradation pathway in *Micrococcus sp*. strain CPN 1 that began with ester linkage hydrolysis, which produced 3-phenyl benzoate, and the hydrolysis replenished CP insecticidal activity, leading to detoxification (Tallur et al. 2008). Protocatechuate, phenol, and catechol were intermediates in the subsequent degradation of 3-phenoxybenzoate via diphenyl ether cleavage. It was claimed that CP degraded into two metabolites, namely, 3-(2,2-dichloroethene)-2,2-dimethyl-cyclopropanecarboxylic acid (DCVA) and 3-phenoxybenzoic acid (3-PBA) by *Serratia sp*. JC1 and JCN13. The 3-PBA further converted into phenol via diphenyl ether bond cleavage (Zhang et al. 2010). Thus, the associated CP degradation pathways and metabolites differ due to the diversity of microorganisms in their chemical and biological behaviour, but their primary degradation mechanisms are nearly identical across species (Zhan et al. 2020).

Control and prevention of CP toxicity

The use of synthetic pyrethroids can only be minimized by implementing appropriate strategies and practices. There is no specific antidote for the prevention of pesticide toxicity; management of pesticide use is the only largely supportive method (Ramchandra et al. 2019). Rather than relying solely on pesticides, multi-level approaches to crop production and safety should be implemented. Instead of industrial agriculture, ecological farming should practice to reduce crop risks. To make crops resistant to pests and to increase soil fertility, crops should be rotated in a type-wise and cultivar-wise manner (Voigt, 1992). Pesticides should be used in accordance with regulations, and biotechnological innovation in agricultural fields should be extensively incorporated into plans. Wetland construction is the most dependable management strategy. Pesticides can eliminate through biological, physical, and chemical processes such as plant absorption, sedimentation, precipitation, hydrolysis, photolysis, oxidation, ozonation, and reduction (Al-Dabbas et al. 2018; Ali et al. 2010). The medications like benzodiazepines can work in controlling pesticide toxicity. Lidocaine and tetracaine can antagonize the effects on sodium channels and reduce the toxicity level (Ramchandra et al. 2019). It is advisable to utilize biological pest control methods and pesticides with reduced hazard profiles. Organic farming and integrated pest management should be promoted in public places such as schools, parks, and hospitals. To sensitize children, farmers, pesticide dealers, and pesticide users about the health risks of pesticide overuse, a number of awareness initiatives, including workshops, rallies, and seminars, should be organized. Environmental and public health organizations should regularly monitor pesticide use, and banned chemicals should be avoided. To monitor pesticide toxicity and restrict its usage within the specified limit, a pesticide poisoning control and emergency centre should be established.

Conclusion and prospects

CP insecticide, while important for crop preservation by killing infectious pests, has drawbacks such as bioaccumulation in the soil, disruption of soil microbiota, and terrestrial invertebrates, and the ability to induce diseases in humans. CP and its intermediates are measured using spectroscopic and chromatographic methods. Recently developed techniques such as microfluidic paper-based analytical techniques, electrochemical sensing, and optical sensing are highly accurate, sensitive, cost-effective, and reliable for the detection of CP. The carboxylase enzyme produced by bacteria hydrolyses the ester bonds in CP and consider as the main enzyme responsible for its breakdown. For the successful detoxification of CP, different bacterial genera require diverse natural conditions, and 3-phenoxybenzaldehyde is the primary intermediate produced after breakdown. This study looks at the ways for quantifying and detoxifying pesticide traces in the environment. This review can provide new insights into CP removal from the environment through microbe-mediated technologies. According to reports, CP has high levels of toxicity, thus the government must outlaw its use and look for less expensive, environmentally friendly substitutes. Future investigations must focus on cutting-edge techniques for the selective detection of CP and its metabolites in environmental samples. Future studies should focus on developing engineered microbes with enhanced degradative enzyme production, which would remarkably escalate the efficacy of CP degradation. A number of molecular approaches are still need to be investigated for analysing the detailed mechanisms associated with CP mineralization.

Declarations

Ethics approval and consent to participate: not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare no conflict of interest.

Consent for publication

Not applicable.

Author contributions

Ishita Kansal (Data curation, Formal analysis, Writing – original draft), Arushi Kapoor (Writing – review & editing), Swati Solanki (Writing – review & editing), and Rachana Singh (Conceptualization, Funding acquisition, Supervision, Validation)

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