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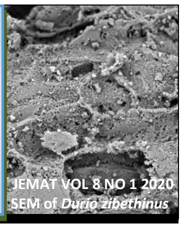

Biodegradation of Carbofuran; A Review

Fazillah Ariffin[1] and Sukirah Abdul Rahman[2]*

[1]School of Fundamental Sciences (PPSA), Universiti Malaysia Terengganu (UMT),
21030 Kuala Terengganu, Terengganu, Malaysia.
[2]Biotechnology Research Centre,Persiaran Mardi - UPM, Mardi,
43400 Serdang, SelangorSelangor, Malaysia.

*Corresponding author:

Sukirah Abdul Rahman
Biotechnology Research Centre,
Persiaran Mardi - UPM,
Mardi, 43400 Serdang,
Selangor,
Malaysia.
Email: sukirah@mardi.my

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ABSTRACT

Pesticides are substances used for the destruction or control of pest. They include insecticides, herbicides, fungicides, rodenticides rats and mice, molluscides and others. Carbofuran is an insecticide that is very toxic pesticide often used to control pests in agricultural areas. Its usage is still being reported despite worldwide efforts to ban the pesticides. Reported deaths and illness due to indiscriminate and excessive use of carbofuran is still being reported in developing and third world countries prompting the call of more research on isolating microorganisms that are able to completely mineralize this toxic pesticide. The presence of toxic carbofuran in various soil types that can persist for many months can be remediated via the use of carbofuran-degrading microorganisms which is a more feasible and economical methods of remediation compared to various physicochemical methods. The aim of this review is to highlight the toxicity of carbofuran, especially in the Malaysian perspective and the current body of knowledge on carbofuran-degrading microorganisms that are able to remediate this toxic threat.

INTRODUCTION

Pesticides may be of the broad-spectrum type that kills a wide range of organisms or the selective type, which destroys only one organism or a few specific organisms. In the early part of this century the increase in world population resulted in a greater demand for food. It has led to a substantial increase in the production and use for agrochemical such as pesticides and fertilizers [1–7].

Pesticides are very known to contribute significantly in reducing losses and thereby increasing food production and quality. Past trend indicates that most of the pesticides used in the developing countries have frequently been employed in the control of vectors of human disease and on industrial export crops. Roughly one thousand pesticide preparations are utilized around the world today. The yearly globally agriculture utilization of pesticides has been approximated to be in the order of five million tons and approximately seventy percent is utilized in farming and the rest by public health agencies and government departments for vector control and for household goals [5,6,8,9].

In the developing countries, particularly in North America, Western Europe and Japan, pesticides have come to play an extremely important role in the maintenance of high agriculture productivity. Despite the uses of pesticides, about 35% of the crops are lost from pests, diseases and weeds. Worldwide FAO estimates that an average of 38% of the cotton crop is saved from destruction by pests through the effective use of pesticides. However, pesticides have been known to affect a number of non-target organisms. Many experts have approximated that only a small percentage roughly about 0.1% of the pesticides used on crops get to their target pests and most 99% of the utilized pesticides have an effect on non-target organisms [8,10–16].

A good example is the decline in paddy field fish populations due to increased pesticide usage, causing severe economic hardship and nutritional deficiencies among the poorer paddy farmers. The rice growing areas of central Thailand, farmers use the food irrigation method where when they drain the water from the rice paddies into the rivers and canals, carrying pesticides residues to nearby agricultural farms. In December 1982, this practice resulted in the complete destruction of the aquaculture industry in central Thailand. Over US$10 million worth of fish were killed and about 5 million kilograms of fish were lost due to pesticides poisoning. This affected people throughout the country because fish is a major source of cheap protein [17]. Pesticides induced chronic toxicity is emerging as a public health concern including cancers, reproductive impairment and irreversible neurotoxicity. According to the World Health Organization (WHO), over half a million people are poisoned each year by pesticides and five thousand of the victims die [18].

Toxicology of Pesticides in General

Pesticides (whether insecticides, herbicides or fungicides) by their nature and purpose are poisons. Even if their amount is minimal in comparison to that of silt, their impact on the environment may be considerable. Since 1962, the use of pesticides in the Unites States has increased more than two-fold. It now endangers groundwater quality in most of the States [19]. Recently, concern about effects of pesticides on human health and on the ecosystem began to move beyond cancer. It appears that some chlorinated hydrocarbon pesticides exert a multitude of toxic effects. These pesticides are neurotoxic, mutagenic and teratogenic. They exert toxic effects on the reproductive system and suppressed the immune system. These compounds act by mimicking or inhibiting estrogen receptors. It also affects women's health but also believed to be responsible for a decrease in sperm count and a rise in testicular cancer in human as well as abnormal sexual development in some wildlife species [20].

Pesticides in Soil

Soil in the environment consist of numerous ecosystems where recycling of organic matter occurs. Soil microflora which consist of bacteria, fungi, protozoa and algae play a major role in nutrient cycle. This is because of the capability of using the organic matter as a source of nutrient. Examples of these process are nitrification, denitrification and carbon mineralization [21–27].

Several studies have shown that pesticides behavior in soil are influenced by the absorption, motility and degradation process. Adsorption of pesticides to soil particle is a vital course of action impacting on their migratory conduct in numerous soil environments. The characteristics absorption and desorption and the mobility of the pesticides 2,4-D, lindane, paraquat and glyphosate in the soils of two Malaysian agriculture show variability in absorption and desorption properties [28]. In the research, it was observed that the absorption-desorption attributes and leaching behavior of the above pesticides demonstrated little variations from the results documented in soils from other areas of world. The inclination of these pesticides to be absorbed by soil particles differs with their chemical and physical qualities. Absorption of paraquat in soil was very fast and binds tightly to the soil particle especially in clay and loam [29].

Pesticides absorption-desorption characteristics are measured by partition coefficient. Research carried out to the mobility of carbofuran in two soil types demonstrated only slight variations between adsorption coefficients. The study demonstrates that carbofuran exhibited a Kd value of 22.4 in clay loam (OC content 53%, pH 8.6) and a Kd value of 19.9 in silt loam (OC content 18%, pH 8.4). Therefore, higher adsorption of carbofuran occurs in the presence of a greater organic matter [30].

The absorption pesticides in soil are very important because it can lead to environmental problems. Pesticides that do not bind to the soil particle will be degraded to produce less toxic metabolites while in comparison, adsorbed pesticides will continue to be in the surroundings for a number of years and may build up into food chains many years after their use in soil [28]. Pesticides range of motion is important for pest management. For instance, particular pre emergence herbicides used on the soil surface area must shift several inches into the soil to arrive at the germinating weed seeds. There are actually five processes that may shift pesticides including runoff, volatilization, leaching, physical removal and absorption. Due to its good water solubility (351 mg/L at 25 °C), carbofuran is comparatively mobile in soil and in surface runoffs and is expected to partition into the water from soil [31]. As an example, it is estimated that carbofuran, which is widely utilized in paddy soil remains in the water at about 54% and in soil of about 46%.

Pesticides Degradation

During pesticides degradation, some of the metabolites can also become more toxic than the original compound. The vast majority of pesticides residues in the environment are turned into non-active or less poisonous or harmful chemical substances by means of primarily chemical degradation, photo degradation and microbial degradation [4,6,32–38].

Chemical Degradation

Chemical degradation of pesticides is a degradation process which do not include living organisms. Examples of chemical degradation of carbofuran include oxidation-reduction, hydrolysis and ionization which can be generally associated with pH. Just about the most common pesticides breakdown is hydrolysis, a degradation process in which the pesticides interact with water. It might happen in both acid and alkaline conditions. As soil pH gets to be highly acidic or alkaline, microbial activity generally reduces, but such circumstances may lead to elevated chemical degradation [4,6,32,33,38].

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Hydrolysis

The most crucial reaction involves hydrolysis, which is the splitting of a bond in a molecule by way of its reaction with a water molecule. Usually a compound is modified in a hydrolytic reaction by the replacing of chemical groups of the substance with a hydroxyl group. The hydrolytic reactions are generally catalyzed through the reactions of hydrogen or hydroxide ions and therefore the reaction rate is firmly determined by the pH of the environment. A number of functional groups that are prone to this hydrolytic reactions include lactones, carboxylic acids ester, amides, epoxides, carbamates, phosphoric- and sulfonic acids esters [4,6,38–40].

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Oxidation –Reduction (Redox)

Oxidation –Reduction (Redox) reactions involve the transfer of electrons from the reduced species to oxidized species. It has been shown that mixed function oxidase (MFO) enzymes are capable of catalyzing aromatic hydroxylation, dealkylation, deamination, desaturation, expoxidation and N-or-S oxidation. Several of these mechanisms occur sequentially to form degradation products. For example, the reaction of N-dealkylation can occur at the carbamate amine. The oxidative metabolic of the ring hydroxylation is important in the metabolism of carbaryl and carbofuran. In this process it probably proceeds through epoxidation to the hyroxy compound and then to the keto and eventually to ring cleavage. Some compounds in which redox reactions have been observed to be important include mercury, toxaphene and DDT [41,42].

Ionization

The hydrogen ions in the water body determine the fate of toxic organics which are possibly acids or bases. The hydrogen ion concentration influences the ionic species of many compound including pesticides. For example, the ionized species of an organic acid is to a much lesser degree absorbed by sediments than its neutral form [43].

Photo degradation

The breakdown of pesticides by light especially sunlight is called photo degradation. It is a degradation process where energy in the form of photons smashes the bonds in a molecule (USEPA, 1987). It can demolish pesticides on vegetation, on the soil surface as well as in the air. Normally, the factors that affect pesticides photo degradation range from the level of the sunlight, especially its intensity, qualities of the application sites, the method of application and the attributes of the pesticides. Mill and Mabey (1985) illustrate the types of photolysis reactions impacting on many different substances such as chloroaromatics, ketones and aldehydes.

Research has shown that a quantity of pesticides is broken down at quantifiable rates of sunlight. Such is the situation that the number of pesticides not broken down by sunlight is very small. In fact, circumstances can be obtained to which almost all current day herbicides and plant - growth regulators will be decomposed by sunlight. Examples of herbicides that have been proved to be successfully degraded by sunlight involves 2,4-D, MCPA, 2,4,5-T, silvex and bromoxymil [44], simazine, monuron and diuron [45].

Microbial degradation

The breakdown of pesticides by microorganisms usually results in the pesticides becoming food source for growth and energy. It is now naturally revealed that microorganisms not just break down natural organic materials, but they are also accountable for the the breakdown of many other xenobiotics. These substances include petroleum hydrocarbons, pesticides, and organic solvents. Transformation of contaminants to less dangerous materials via biodegradation is highly likely in the case of mineralization while in under certain situations more often seen under anaerobic transformation, toxic products can also be produced [46–62].

Microbial degradation as a tool for bioremediation

Basically, the mineralization or total biodegradation of an organic molecule for example pesticides in soils is practically constantly a result of bacterial activity. As they transform the organic substrate to inorganic products, the accountable population utilizes a number of the carbon in the substrate and turns it to cellular components. Simultaneously, energy is produced, and the populations increase in quantities and biomass as they absorb a significant number of the carbon for energy and biomass. Carbofuran, like many other pesticides is a soil-incorporated pesticide which has been confirmed to be affected by microbial degradation. The majority of pesticides at present is being used and have been found in the biosphere for more than forty years but a number of these substances are speedily microbially broken down in soil [60,63–73].

Pesticides contamination in the Malaysian environment

Contamination of the Malaysian environment is becoming an escalating issue throughout the last one hundred year with the continuing development of industry and agriculture and with the advancement human population. Not long ago, there has been an increase reports of pesticides contamination from the agriculture use of fertilizers and pesticides. In Malaysia, pesticides are widely-used to manage pests that damage crops and broadcast diseases to people and animal [6,36,58,74–76]. At the moment, the effective use of pesticides is in depth and agriculture, horticulture, vector control and forestry and livestock production are the reason for the highest usage. The pesticide market in Malaysia is increasing over the years with herbicides still accounting for the highest rate at 75 % followed by insecticides at 16 percent, fungicides at 5.4% and rodenticides at 3.5 % [77]. The insecticides are used in Malaysia mainly for vegetables, rice, cocoa, fruits, oil palm and tobacco. The major insecticides used are BHC, endosulfan, chlorpyrifos, carbofuran and carbaryl in rice fields. The major herbicides used are gyphosate, paraquat, 2,4-D and lindane [78–88].

In Malaysia, the main legal guidelines for the control of pesticides is the Pesticides Act of 1974. Normally the principal purpose of this act is the control of the production and import of pesticide by means of registration and licensing of premises marketing, storing, selling, labeling and management of the import of unregistered pesticides for research and academic applications [86].

The health impacts of pesticides on human and other organisms in Malaysia are expected to rise as the use of pesticides in the agriculture area increases. Nowadays, the use of pesticides in Malaysia is a cause of serious concern. Some pesticides have been shown to be toxic to the environment as well as human himself. Between the years 1970 and 1982 there were about one hundred cases of organophosphate poisoning needing admission to the University Hospital Intensive Care Unit in Kuala Lumpur [2]. Data from the Ministry on pesticides poisoning from 1979 to 1986 were associated with the pesticide paraquat with 49.1% of the cases are intentional and the remaining 37.8% are accidental. In the paddy field areas in Tanjong Karang, it was reported that about 72% of rice farmers experienced poisoning signs and symptoms when dealing with pesticides. It has been found that in these places, proper attire and clothing including googles, shoes and respiratory masks were hardly ever worn [89].

The levels of pesticide found in the blood serum of the general Malaysia population are much higher than that of the United States. The total mean DDT concentration in Malaysia farmers was 0.11 ppm, the level for rubber estate workers was 0.09ppm and the level for the general population was 0.066 ppm. The impact of this unhealthy trend of excessive pesticides usage in Malaysia will result in adverse effects in the environment and the wellbeing of the people [81].

It was also observed that the high accidental and occupational exposure of workers and the general population to pesticides further confirm this unhealthy trend. Estate workers formed the majority of all pesticide's poisonings in Peninsular Malaysia. There is a wide use of organophosphorus insecticides and rodenticides in the agricultural sector especially in oil palm plantations. Problem arise because estate workers are generally unaware or not made aware of the color coding of these hazardous chemicals.

Long term exposure has led to several illnesses such as pulmonary, eye, skin, and neurological problems [90]. Various factors have contributed to the acute poisoning cases which include the use of pesticide (by farmers) in concentration in excess of requirements, poor knowledge and understanding of safe practices in pesticide use, lack of protective clothing suitable for tropical climates, the poor maintenance facilities of spray equipment, giving rise to hazardous contamination and the use of pesticide mixture [91].

Carbofuran ($C_{12}H_{15}NO_3$)

Carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranol N methylcarbamate) is a broad-spectrum carbamate acaricide, insecticide and nematicide. It is widely used towards quite a few unwanted pests of agricultural products including rice, corn and on other agriculturally important plants. Carbofuran is utilized to manage soil-dwelling and foliar feeding pesky insects for example wireworms, corn rootworm, boil weevils, alfalfa weevil, mosquitoes, white grubs and aphids [3,7,92–94].

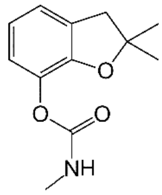

Fig. 1. Chemical structure of carbofuran

Carbofuran was initially utilized in a commercial sense in 1967 and its particular use is continuing to grow speedily more than following years. Its half-life in soil is from thirty to one hundred and twenty days. In sandy soils, the half- life is approximately thirty days while the intermediate half-life in loamy soils is about forty days. Its half-life in muddy soil is about eighty days [94]. Its molecular weight is 221.25 with a melting point of between 150 and 152°C. Under neutral or acidic conditions, it is comparatively stable but breakdowns rapidly in alkaline media. Carbofuran is soluble in water at concentrations of up to 700 mg/L in water but in organic solvents its solubility reduces to less than 30 mg/L [93].

Sign and symptoms of poisoning

Carbofuran is a highly toxic compound when inhaled and moderately toxic through dermal absorption. Symptoms of acute toxic exposure include nausea, excessive salivation, abdominal cramps, vomiting, sweating, weakness, diarrhea, imbalance, breathing difficulty, blurring of vision, increased blood pressure and incontinence. The rapidity, with which these symptoms appear, is dependent on the amount of toxicant administered, chemical structure of the toxicant, mode of entry into the body, biochemistry and physiology of the animal treated [95–101].

Mode of action cholinesterase inhibition by carbofuran

Generally speaking, carbofuran successfully manages insects by means of its action on as an anticholinesterase activity. Acetylcholinesterase (AchE) inhibition by carbofuran is substantially studied in the past years. In both invertebrates and vertebrates, carbofuran act by suppressing the enzyme acetylcholinesterase (AchE), which is an enzyme important for regular nerve functionality inside the peripheral and central nervous systems. Cholinesterases are the enzymes that carry out neuromuscular and interneuron transmission and switch on various intracellular reactions in a number of tissues and cell varieties [102–107]. Death can happen consequently coming from asphyxiation resulting from extreme activation in nerves due to the buildup of acetylcholine at the nerve synapses of the central nervous system and resulting in the subsequent failure of the respiratory process [31,96,108,109].

Other effects of carbofuran toxicity

Organophosphate and organocarbamate pesticides including carbofuran can cause oxidative stress in erythrocytes. As the erythrocytes harbor polyunsaturated fatty acids and hemoglobin, they are prone to oxidative stress where the latter increase may correlate with an increased osmotic fragility of erythrocytes. [110]. In female Swiss albino mice, the interruption of estrous cycle and follicular toxicity caused by carbofuran exposure has been documented [111] where the estrous cycle was effected with a significant decrease in the duration of each phases of the estrous cycle and its number as well as a concomitant significant increase in the diestrus phase [112]

Bioremediation

Bioremediation is using microorganisms or plants to clean or detoxify the environment. It is an option which offers a solution to detoxify or at least lessen the toxicity of the substances in the environment. It is a natural process using living organism, primarily microorganisms to remediate the environment contaminants into less harmful forms. It is a course of treatment that employs microorganisms to degrade substance that are detrimental to human health and environmental surroundings. Typically, microorganisms make use of organic compounds to produce energy and nutrients for their growth. Bioremediation can happen under aerobic and anaerobic circumstances.

In aerobic situation, microorganisms make use of atmospheric oxygen and will transform numerous organic pollutants to CO_2 and H_2O. Whilst under anaerobic condition, reactions take place solely without molecular oxygen [26,27,62,113–120]. Bioremediation can be performed either ex-situ or in-situ. The ex-situ process requires excavation of contaminated soil before they can be treated. While, in the in-situ process, remediation is carried out in the location without excavation or removal of contaminated soil. In the case of in-situ bioremediation several successful cases have been reported and the target pollutants remediated include chlorinated pesticides, halogenated aliphatics, nitroaromatics, polychlorinated biphenyls, and polycyclic aromatics [26,27,62,116–118].

Bioremediation can be carried out by four basic techniques. To begin with, the microbes may end up being immediately employed to degrade pollutants. Secondly, in-situ spiking nutrients may be utilized to stimulate the growth of indigenous microbes capable of decontamination. Thirdly, cell extracts or purified enzyme products of microbial origin could be used for decontamination. Finally, plants can often be used to eliminate or transform pollutants. Presently, scientific studies are being carried out on the utilization of microorganisms to break down chemicals.

Methods and resources for the design of new microbes are already been created. Included in this are the design of regulated gene expression circuits that limit catabolic activities and the assemblage of gene blocks coding for chosen metabolic modules to produce new metabolic routes. As with many chemicals, pesticides are subject to both biotic and abiotic transformation process. Many of the abiotic transformations result in partial degradation to products that can be further degraded by microorganisms. Hydrolytic reactions are the most significant abiotic transformations. The reactions can be base or acid and occurred through interactions surface, reactive organic compounds and inorganics metal such as Cu^{2+} [26,27,62,113–120].

Carbofuran-degrading microorganisms

Carbamates and organophosphates are currently, the most employed insecticides for indoor and agricultural purposes. Even though they are biodegradable, they display neurotoxicity in mammals. Carbofuran, a class of carbamates is usually applied at the time of planting to control numerous insects in various agricultural plants. It is important that the carbofuran persist in the soil for 3-5 weeks after planting to control the feeding larvae [98,101,121–125]. Microbial deterioration of this compound continues to be analyzed and the effectiveness of carbofuran in the field is diminished and reapplication needs to be carried out as a result of the phenomenon of increased microbial degradation. It was suggested that bacteria contained in the area have the ability to make use of the bacteria as a substrate for growth [98,123–125].

It has become increasingly possible to isolate microorganisms that are capable of degrading xenobiotic and recalcitrant compounds from environments polluted with toxic chemicals. Several microorganisms are responsible for the metabolism pesticides in soil. The degradation of several carbamates including carbofuran in soil is mediated by several microorganisms that play major roles in degrading and utilizing carbamates as a sole source of carbon and included *Achromobacter* spp. [63,126,127], *Arthrobacter* sp. [128], *Sphingomonas* spp. [65,129,130], *Pseudomonas* spp. and *Chrysobacterium* spp. [131,132], Bacillus brevis [133], *Gliocladium* sp. [134], *Novosphingobium* sp. FND-3 [67], *Burkholderia cepacia* PCL3 [135], *Rhodococcus* sp., *Sphingobium* sp., *Bosea* sp. and *Microbacterium* sp. [136] and *Cupriavidus* sp. ISTL7 [73].

Carbofuran hydrolase

Chaudhry *et al.* [137] isolated and purified from *Pseudomonas* sp. 50432 a soluble carbamate hydrolase that had a wide specificity. The best characterized carbamate hydrolyzing enzyme was obtained by Kearney and Kaufman when they isolated a strain of *Pseudomonas striata* from soil which could metabolize phenylcarbamate chlorpropham (isopropyl m - chlorocarbamate) as a sole source of carbon and energy [138]. The enzyme isolated from *Achromobacter* sp. WM111 appeared to be a hydrolase after numerous purification and dialysis steps [126]. This carbofuran hydrolase was detected based on their activity and ability of *Achromobacter* sp. WM111 to utilize carbofuran as a nitrogen source [63]. Chaudhry *et al.* showed that the hydrolase has a wide substrate range can be potentially useful for the decontamination of wastes containing mixtures of hazardous compounds than those enzymes with a narrow substrate range [137] and the carbofuran hydrolase activity was found to be present predominantly in the cytoplasmic fraction (86%) and only a minor activity was associated with membranes (14%) [137].

Ultracentrifugation was a method used in separating the cytoplasmic proteins from the membrane fraction. Upon ultracentrifugation, 86.5% of the total carbofuran activity in a crude cell extract of *Achromobacter* sp. WM111 was found in the supernatant fraction while the remaining 13.5% of the activity was contained in the unwashed membrane pellet [126]. Hydrolase is stable at 4 ºC for 1 week and its activity is lost completely after 1 month. To overcome from losing enzyme activity, Chaudhry suggest the enzyme being stored at –20º C or –70º C in the presence of 20% glycerol. It may remain for a period of 1 month [137]. The enzyme was also stable in Triton-X-100 and other detergents such as CHAPS. The enzyme showed only 10% of the activity in the presence of 0.1%2-mercaptoethanol and 0.1% DTT. Almost 58% of the active enzyme is trapped in Triton-X-100, which separated as a floating layer. The optimum pH was broad (9.0 to 10.5) and the optimum temperature was between 45º C and 53ºC. [126]. The presence of 2-mercaptoethanol and DDT will inactivate the hydrolase. Hydrolase activity was determined to be optimum at pH 8.5 and 37ºC [137].

In conclusion, carbofuran, an important pesticide has many uses in agricultural and nonagricultural purposes. Bioremediation can provide a cost-effective method compared to existing physicochemical methods provided that a suitable microorganism can be found to completely mineralize carbofuran completely. The purified enzyme shows diverse substrate specificity and stability conditions also varied considerably. Novel carbofuran-degrading enzymes need to be isolated and characterized specifically from developing and underdeveloped countries where carbofuran is still being used despite worldwide ban.

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