MINERALIZATION, VOLATILIZATION, AND DE-GRADATION
OF 14C-U-RING CARBOFURAN IN A MOROC-CAN SOIL.

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ملخص

درس تعدين الكاربوفوران 14 C في تربة شمندرية وفي ظروف المختبر، إذا بللت التربة و دفنت تحت الماء لمدة 63 يوما. وقد حدد مدى تعدين الكاربوفوران بالقدر المتكون من 14CO2. وقد بينت النتائج درجة تعدين عالية للكاربوفوران : ٪19,2 في حالة التبلل و٪12,4 في حالة الدفن. كما أن سرعة تبدد الترسبات القابلة للاستخراج كانت أكبر في حالة الدفن منها في حالة التبليل. وتكونت ترسبات معقودة بكميات أكبر في حالة الدفن (٪33) منها في حالة التبليل (٪29). وأخيرا، فإن كمية المكونات العضوية القابلة للتبخر كانت أكبر في الحالة الاولى (٪18,2) منها في الحالة الثانية (٪5,6) والناتج الرئيسي عن التحلل هو الكاربوفوران فنول.

الكلمات المفتاحية : كاربوفوران، تعدين، تبخر، تحلل

Résumé

La minéralisation du carbofuran-14C a été étudiée dans un sol betteravier Marocain sous conditions de laboratoire. L'étude a été conduite sous conditions de sol humide et immergé sur une période de 63 jours. La minéralisation du carbofuran a été déterminée par la mesure de 14CO2 formé. Les résultats montrent une haute minéralisation du carbofuran avec 19.2% de la dose initiale sous conditions humides et 12.4% sous condition immergée. La vitesse de disparition des résidus extractibles a été supérieure sous condition immergée que sous condition humide. Les résidus liés ont été formés en grandes quantités avec un léger excès sous condition immérgée (33.3%) par rapport à ceux formés sous condition humide de sol (29%) La quantité des produits organiques volatils formée était très supérieure sous condition immergée avec 18.2%, alors que celle formée sous condition humide ne dépassait guère 5.6%. Le principal produit de dégradation est le carbofuranphenol.

Mots clés : Carbofuran, minéralisation, volatilisation, dégradation

Abstract

The mineralization of 14C-ring labelled carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-methylcarbamate) in an untreated Moroccan soil of sugar beet cultivation was studied under laboratory conditions over a period of 63 days. The experiment was conducted in flooded and non-flooded conditions. Carbofuran mineralization was determined by monitoring the 14CO2 production from soils amended with 14C-U-ring Carbofuran. The result showed that, under both conditions, higher mineralization rates of 14C-carbofuran were observed. The extent being more in moist (19.2%) than in flooded conditions (12.4%). In both conditions, the soil extractable pesticide residues decreased with time and the bound residues gradually increased. The extractable14C-activity disappearance rate was much higher/faster in flooded than in non-flooded conditions. At the end of the experiment, 15.8% and 31% of applied dose were recovered as extractable residues under respectively flooded and moist conditions. Soil bound residues were formed to the extent of 33.3% of the applied radioactivity in flooded soil, while 29.1% was formed in non-flooded soil. The amount of the organic volatiles was much higher in flooded condition (18.2%), as compared to 5.6% in non-flooded soil. The main degradation products formed was carbofuran phenol.

***Key words:** Carbofuran, mineralization, volatilization, degradation.*

Introduction

Microorganisms are considered to be one of the primary factors that determine the fate of organic xenobiotics in soil soil (Alexander, 1999). They influence directly, and indirectly, on the persistence of pesticides in soil (Farenhorst et al., 2000). Numerous studies have documented the ability of soil microorganisms to utilize pesticides as a carbon and energy source for their growth, either by the isolation of pure or mixed cultures through enrichment techniques, or by the demonstration of mineralization in soil (release of 14CO2 from labelled pesticides) (Chaudhry and Ali, 1988; Feng et al; 1997; Fournier et al., 1997).

Through mineralization, the persistence of pesticides is reduces and, as a result, reduces the environmental risk (Chapamaladugu and Chaudhry, 1991). Among the major microbial processes that may involve pesticides are oxidation reactions, such as oxidative coupling, hydroxylation, and aromatic cleavage (Dec et al., 2002).

Mineralization and degradation of pesticide in soil is a very complex process. It is influenced by many factors, such as type of soil, temperature and soil moisture, concentration of pesticides in soil, nature of the chemical, population of soil microbial (Bujin et al., 1999; Ismail et Azlizan, 2003).

The insecticide carbamates have been developed as an alternative to the recalcitrant organochlorine pesticides, however many of these carbamates are highly toxic and inhibit acetylcholinesterase, an enzyme vital to the functioning of the nervous system (Barron, 1971). Carbofuran (2,3-dihydro-dimethyl-7-benzofuranyl methylcarbamate) belonging to the N-methylcarbamate class, is a versatile broad spectrum systemic insecticide, nematocide and acaricide, registered for use worldwide to control many soil insect pests (i.e. the green leafhoppers, rootworms, brown planthoppers, stemborers and whorl maggots) (Venkateswarlu et al., 1977; Fournier et al., 1997) on a variety of crops such as sugar beet, sugar cane, rice, corn, peanuts, cotton, strawberries, potatoes (Chapalamadugu and Chaudhry, 1992; Jaramillo et al., 2000). Consequently, carbofuran has attracted much attention as a potential alternative to the persistent and toxic chlorinated hydrocarbon insecticides. Unfortunately, carbofuran is of environmental concern because of its high mammalian toxicity having an oral LD50=2 mg/Kg for rat (Chaudhry and Ali, 1988) and LC50 = 0.2-0.8 mg/Kg for fish fish (Jinhe et al., 1989). Its use under granular formulations was banned by USEPA since 1994. This concern is heightened by the facts that Carbofuran residues have been detected in surface water, in groundwater (Trabue et al., 1997) and in wells (Bouchway et al., 1992).

Carbofuran is a relative persistent insecticide with a half-life in soil ranging from 3 weeks to more than 50 weeks depending of the pH of the soil (Getzin, 1973). A field study by Caro et al. (1973) showed that the half-life of carbofuran in soil ranged from about 6 weeks to 17 weeks. Persistence of carbofuran in soil has also been reported by Szeto and price (1991) who found 78 _g/g of carbofuran near a year after application. Miles et al. (1981) found that Carbofuran is relatively more persistent in sterile soil than in unsterile soil. Flooding results in faster degradation of Carbofuran has been shown by Venkateswarlu et al., (1977) The major degradation products of carbofuran in soil include carbofuran phenol, 3-hydroxycarbofuran, 3-ketocarbofuran and 3-ketocarbofuranphenol.

Mineralization is an important process affecting the fate of a pesticide in the environment. Because of their direct influence on microbial growth and activity, several factors are known to affect the mineralization and degradation of Carbofuran in soil such as type of soil (Getzin, 1973; Miles et al. 1981), soil pH (Getzin, 1973), soil microorganisms (Chaudhry and Ali, 1988; Ramanand et al. 1988), soil moisture (Ou et al., 1982; Mojàsevic et al. 1996), temperature (Ou et al., 1982), soil depth (Trabue et al., 1997).

Carbofuran was found to be degraded chemically (hydrolysis) and by microorganisms, mainly via hydroxylation at the benzylic carbon to give 3-hydroxy- and 3-keto-carbofuran and via hydrolysis to form phenols and bound residues under flooded-soil conditions (Jinhe et al. 1989). Chemical hydrolysis occurs more rapidly in alkaline soils as compared to neutral or acidic soils (Getzin, 1973). The slower degradation in acidic and neutral soils was dominated by microbial and chemical mechanisms (Getzin, 1973). Arunashalam and Lakshmanan (1990), Ramanand et al., (1988) and Trabue et al., (1997) reported that Carbofuran is primarily degraded through chemically or enzymatically mediated hydrolysis of the carbamate linkage, yielding Carbofuran-7-phenol and methylamine. Rajagopal et al. (1983) reported that hydrolysis at the carbamate linkage is the major pathway of degradation of carbofuran. Turco and Konopka (1990) suggested a two-step mechanism for the fate of carbofuran in soils: (i) hydrolysis of the carbamate linkage and (ii) coupling of the primary metabolite, carbofuranphenol, to soil.

A number of bacteria strains that capable of using carbofuran as source of C and N for their growth, have been isolated from soil and characterized (Chaudhry and Ali, 1988; Ramanand et al., 1988; Fournier et al. 1997). Feng et al. (1997) reported that plasmids may be involved in the complete mineralization of the insecticide. These bacteria are capable to mineralize both carbonyl group and the aromatic ring of Carbofuran and residues (Ou et al., 1982; Chaudrhy and Ali, 1988; Feng et al. 1997; Trabue et al., 1997 and 2001) leading to CO_2 and H_2O. Ramannand et al (1988) suggested that the microbially mediated hydrolysis of the carbamate linkage was the first step in complete mineralization of Carbofuran. There are likely to be three groups of microorganisms that may be involved in the degradation of Carbofuran in soils; Groups 1 and 2 are capable of hydrolyzing Carbofuran to give carbofuran phenol and methylamine. Group 3 utilizes the aromatic ring as a sole source of carbon (Chaudhry and Ali, 1988). Trabue et al., (2001) reported that the same microorganisms that mineralized the aromatic ring of Carbofuran were likely also to be responsible for carrying out mineralization of the aromatic ring of Carbofuran phenol.

The vast and intensive agricultural use of pesticides in some Moroccan zones has important implications for the contamination of the groundwater system. Areas such Loukkos, (northwestern) have high rainfall and intensive agricultural production and many pesticides are extensively used in these zones. Therefore, the contamination of aquifer systems is an increasingly serious problem because of high acute toxicity of these chemicals, improper applications exacerbate the problem. No studies on carbofuran behavior have been carried out in these zones with/on 14C labeled molecules. It is very expected that these areas can show high levels of pesticide residues (DPVCTRF, 1999). With the increasing population, efforts are being made to intensify sugar production to reach the self-sufficiency. This will result in an increase in the use of Carbofuran. Carbofuran residues and its toxic carbamate metabolites are therefore of great concern in terms of their persistence, mobility, dissipation pathways and toxicity to the human life.

The present work aimed at studying the rate of mineralization of Carbofuran in Moroccan soil of sugar beet cultivation under flooded and non-flooded conditions. For these investigations, radiolabelled laboratory soil mineralization studies were conducted, using pesticides labelled in the aromatic nucleus.

Materials and methods

Soil samples

Soil samples were taken randomly to a depth of 0-30 cm from different sites of a sugar beet field of Loukkos zone (northwestern) and mixed thoroughly. The soil, which has no history of Carbofuran treatment, was air dried, ground and passed through a 2 mm sieve before treatment.

Chemical

[U-phenyl-14C] Carbofuran (specific activity 1.12GBq/mmol) was purchased from the Institute of Isotopes of the Hungarian Academy of Sciences via the International Atomic Energy Agency (IAEA). The chemical was determined to be more than 98% radiochemically pure by TLC before use. [U-phenyl-14C] Carbofuran was diluted with unlabelled carbofuran (to specific activity: 4.08 MBq/mmol) before application. Carbofuran (98.8%), 3-OH-Carbofuran (>98%) and 3-keto-Carbofuran (>98%) and carbofuran phenol (>97%) analytical standards where purchased from Sigma Aldrich. All the reagents used in the study were of analytical or scintillation grade. All the solvents used were residues, HPLC, or analytical grades.

Mineralization and degradation study

50 g samples of soil (oven-dry weight equivalent) were transferred into standard 250 ml Bellco biometer flasks placed in the laboratory. The experiments were carried out under moist and flooded conditions. The moist conditions were maintained by adding water equivalent to 60 % field capacity (FC) of the soil and kept at this levels throughout the course of the experiment. For the experiments under flooded conditions, the water level was maintained at 2 cm above the soil surface. 1.0 mg (20ppm) carbofuran equivalent to 0.50 μCi of 14C-Carbofuran was added to each flask and stirred to obtain a homogenous sample. In the side arm of each flask was added 20 ml of ethanolamine (scintillation grade) to trap the 14CO2 produced while the organic volatiles were trapped by polyurethane plugs placed at the passage between the soil sample and ethanolamine trap. Flasks were incubated at about 25°C in the darkness for 63 days. Each flask was tightly closed with a rubber stopper. At weekly intervals, the rubbers were removed for few minutes to ensure aerobic conditions.

Sampling of flasks

2 flasks were taken off immediately. after application of carbofuran, for analysis to determine the initial applied dose (100%). The other flasks were sampled in duplicate at 15, 31, 45 and 63 days after treatment. (2 flasks of each set). The soil samples were air-dried under a ventilled fumehood, ground and homogenate then stored in plastic bags at 0°C until analyzed.

Extraction and analysis

The trapped 14CO2 in the ethanolamine traps was quantified by liquid scintillation counting (LSC). While the organic volatiles were extracted with methanol and aliquot of the extracts were counted with liquid scintillation counter (LSC). For total and bound residues analyses, 500 mg soil samples in triplicate, were combusted in a Harvey Biological Oxidizer, OX 600 and counted (LSC) cocktail. For extractable residues, 10 g (dry weight basis) soil samples, in duplicate, were extracted in a Soxhlet extraction apparatus with methanol for 10 h (5-6 cycles/h). Methanol solution was then gently concentrated to 10 ml with Rotavapor, at 30°C and 1 mL, in duplicate, was mixed with toluene based scintillator and radioassayed with LSC. The nature of the 14C-residues in the methanol extract was determined by HPLC.

HPLC analysis

For determining the nature of the 14C-residus in the methanol extracts, samples of concentrated extracts were directly analyzed by HPLC Shimadzu, fitted with a Nucleosil C18 stainless-steel column (5 μm, 4.6mmx250mm), equipped with a guard column. The injection volume was 20μl. The mobile phase used was acetonitrile: water (50:50 v/v) with a flow rate of 0.7 ml min-1; ultraviolet absorption at 220 nm was measured and the peak area used for quantification. The identification was done by comparison the picks of samples with the authentic ones of standards

Results and discussion

The soil used was clay and its characteristics are shown in table 1.

Table 1 Physical and chemical characteristics of the soil used

pH (water)	pH (KCl)	Total Nitrogen(%)	Organic matter(%)	Clay(%) (<2_m)	Silt(%) (2-50_m)	Sand(%) (50-200_m)	texture
7.8	7.2	0.22	2.14	50.2	35.6	14.2	Clay

Figure 1. Cumulative evolution of 14CO2 from ring labeled carbofuran in soil samples (average of triplicate values. Applied radioactivity = 100%). (F: Flooded condition, NF: non flooded condition)
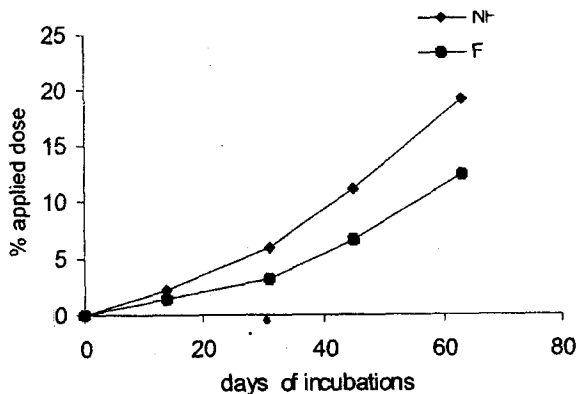
In the present investigations, monitoring of liberated 14CO2 from the degradation of ring labelled, carbofuran in Moroccan clay soil showed that considerable amounts of this insecticide are mineralized to 14CO2 during 63 days under both moist and flooded conditions. Figure 1 illustrates the cumulative evolution of 14CO2 from ring labelled carbofuran in Moroccan clay soil under flooded and non flooded conditions. The evolution of 14CO2 from Carbofuran labelled at phenyl ring, indicates the biological attack on the phenyl ring

The percentage of mineralization showed a consistent increase with time. The rate of mineralization was found to be more rapid in non flooded than in flooded conditions.

In the microbial breakdown of any pesticide molecules, it has been suggested, that there is an initial lag phase, during which no significant breakdown of the pesticide molecule occurs (Fournier et al. 1997). This is the adaptation period of soil microbes to the new substance. This may be the fact that no significant increase amount of mineralization has occurred during the first 14 days (only 2.2% and 1.4% of applied dose was mineralized respectively under moist and flooded conditions). Afterward, a rapid increase in the rate of mineralization was observed; at 45 day, the 14CO2 activity amounted to 11.8 % and 7.4 % respectively under moist and flooded conditions. The amount of mineralization increased more and reached more than 19% for moist soil and 12% for flooded condition at the end of the experiment; 63 days after incubation. This is in accordance with the pattern of microbial breakdown of a pesticide, i.e. a period of rapid degradation follows the initial lag phase (Fournier et al. 1997).

Similar high levels of mineralization have been reported by many researchers; Lalah et al. (1996) reported mineralization of 13 % and 11% of applied dose, from 14C-U-ring-Carbofuran after 33 days of incubation respectively, under flooded and moist conditions. In experiments using a continuous flow system, Kale and Ragu (1996) have found that about 30% of carbofuran was mineralized under moist condition after 30 days of incubation. Trabue et al. (1997) reported significant mineralization of the aromatic ring structure of carbofuran that exceed 58% during 28 days of incubation. Zayed et al., (2001) reported that 13.9% of applied activity of 14C-carbofuran was mineralized during 90 days of incubation under moist soil. Kale et al.,

(2001) reported that carbofuran undergoes extensive mineralization; after 30 days, 29.9% and 33.7% of applied dose was mineralized under respectively moist and flooded conditions. High mineralization rate was also reported by Hussain et al., (1986). They found that 35.6% of applied dose was lost as 14CO2 after 20 days in a clay loam soil. In contrast to these results, some authors have reported lower extent of Carbofuran mineralization (Willems et al. 1996; Arunashalam et Lakshmanan, 1990; Turco and Konopka, 1990) presumably temperature may be behind these results (Kale et al., 2001).

The relative low mineralization obtained in flooded conditions as compared with the moist conditions can be explained by the following: in flooded soil, which could give rise to anaerobic conditions, the Carbofuran is subjected to hydrolysis to give Carbofuran phenol as main metabolite that undergoes immediately strong adsorption reaction with soil constituents and slowly metabolized by microorganisms (Getzin, 1973). This is the fact that bound residues were high in flooded conditions (table 3). Inversely, in aerobic conditions, carbofuran phenol is instable (Venkateswarlu and Sethunathan, 1978) and tends to be degraded farther through aromatic ring cleavage yielding CO2 and H2O (Ou et al, 1982; and Trabue et al, 1997). Therefore, the same microorganisms that mineralized the aromatic ring of Carbofuran were likely to be responsible for carrying out mineralization of the aromatic ring of Carbofuran phenol (Trabue et al., 2001; Feng et al., 1997). Ramanand et al (1988) suggested that the microbially mediated hydrolysis of the carbamate linkage was the first step in complete mineralization of Carbofuran. Turco and Konupka (1990) concluded that the biodegradation of carbofuran in the soil is a two-step process: hydrolysis of the carbonyl side-chain is followed by the adsorption of the primary metabolite, causing a decrease in ring breakdown. Thus, we suggest that there is a competition between mineralization of Carbofuran phenol leading to 14CO2 in aerobic conditions (Venkateswarlu and Sethunathan, 1978) and its adsorption to soil components and bound residue formation (Willems et al. 1996; Getzin, 1973). Venkateswarlu and Sethunathan (1978) and Ramanand et al (1988) reported that the first suggestion is more likely to be in aerobic condition. Therefore binding of carbofuran phenol to soil component is more likely to occur in flooded conditions than to its metabolization by microorganisms, hence the difference in the amount of bound residues formation in moist and flooded soil conditions.

Table 2. ^{14}C-Organic volatiles trapped from the soil samples

Incubation Period (days)	Non-flooded soil		Flooded soil	
	_g carbofuran equivalent	%	_g carbofuran equivalent	%
14	1.30	0.29(_0.038)	24.50	3.45(_0.44)
31	3.60	1.37(_0.32)	82.70	7.07(_1.09)
45	13.80	3.08(_0.62)	121.30	11.13(_0.88)
63	26.70	5.67(_0.85)	198.20	18.25(_2.34)

100% corresponds to 1 mg=1.000_g

Table 2 shows the extent of organic volatiles under flooded and non flooded conditions. The results indicated that carbofuran undergoes considerable volatilization in flooded than non flooded conditions. At the end of the experiment, more than 18 % was lost by volatilization in flooded soil, as compared to only 5.6 % in non flooded conditions. This can be explained because carbofuran is very water-soluble (700 mg/l), therefore it is lost rapidly by co-evaporation with the water on the surface of the soil. Lalah et al., (1996) in a similar study, found that 50% and 13% of initial dose was recovered as volatile products respectively under flooded and moist soils 33 days after incubation. Caro et al. (1976) reported that 36.2% of the applied carbofuran volatilized from a sandy soil after 60 days of incubation. Venkateswarlu et al. (1977) reported that carbofuran under flooded conditions, is subjected to hydrolysis, rapid adsorption and volatilization.

Table 3. Masse balance of 14C-carbofuran dissipation in flooded and non-flooded clay soil as percent of applied activity (values are mean of triplicate, applied activity= 100%).

Incubation Period (days)	Extracts (%)		Bound (%)		14CO2(%)		Volatilization (%)		recovery (%)	
	F	NF	F	NF	F	NF	F	NF	F	NF
14	65,88	76,9	18,1	14,22	1,43	2,28	3,45	0.29	88.86	93,69
31	43.37	60,6	28,3	22,76	3,22	5,92	7.07	1.37	81.96	90.75
45	27.21	46,01	32,6	27,4	7,44	11.87	11.3	3.08	78.55	88.36
63	15.78	31.05	33,3	29.11	12.41	19.23	18.25	5.67	79.74	85.06

F: flooded

NF: non flooded

Table 3 represents the percentage dissipation of carbofuran in soil. The result showed that Carbofuran underwent extensive degradation under both moist and flooded conditions. The degradation is being more rapid in flooded than in moist soil. With increasing time, the extractable residues in general decreased. Within the first 2 weeks, the extractable residues decreased rapidly and reached about 33% and 77% of applied activity under flooded and moist soil respectively. With the passage of time, the amount of extractable residues showed a continuous degrease with time to reach about 57 % under flooded conditions as compared to 39.4% in moist conditions. At the end of the experiment, the extractable residues decreased to just 15.8% and 31% respectively under flooded and moist soil showing an extractable residues disappearance rate of carbofuran more rapid in flooded than non flooded conditions.

The bound activity in both conditions were high, the extent being more in flooded soil (33 % of applied activity) than in moist soil (29 % of applied activity). In both conditions, the binding of residues occurred very rapidly within the first 2 weeks after application and reach more than 18 % and 14 % of applied activity were formed as bound residues respectively under flooded and moist soil. With increasing time, the bound residues increased more: at 31 days, they amounted to 28.3 % and 22.7% respectively under flooded and moist soil. There after, binding progressed slowly to reach 33% and 29% of applied radioactivity at the end of the experiment, respectively

under flooded and moist soil, indicating a relative high binding tendency of carbofuran to this type of soil.

Similar tendency was obtained by many researchers; Kale and Raghu (1996) found, in a clay soil, 20% and 31% of applied dose as extractable residues and formation of 48% and 23% as bound residues, respectively, under moist and flooded conditions, Kale et al.(2001) obtained in a Vertisol soil 8.5% and 10.0% as extractable and 55% and 42% of applied activity as bound residues under moist and flooded conditions respectively after 60 days of incubation of carbofuran. Venkateswarlu et al. (1977), and Venkateswarlu and Sethunathan (1978) reported that carbofuran degraded more rapidly in flooded soils (anaerobic conditions) than in non-flooded soils (aerobic conditions). Lalah et al. (1996) reported formation of 40% of bound residues in flooded soils after 40 days of incubation. In studies on fate of 14C-Carbofuran in a rice fish ecosystem, Hussain et al. (1986) reported formation of 59% of bound residues after 20 days of incubation. Ou et al. (1982) reported formation of bound residues of 14C-carbofuran from 5.1 to 94.5% depending of type of soil. Greater amounts of bound residues were formed in soils with high clay and soil organic matter content. Soil adsorption of Carbofuran and its 14C-residues was suggested to cause the increase of unextractable Carbofuran with time in the soil (Isensee and Tayaputch, 1986; Wilems et al., 1996). Moreover, Carbofuran is known to be persistent in clay and in clay loam soils (Ahmed et al., 1979). Willems et al. (1996) suggested that formation of carbofuran phenol likely plays a key role in the incorporation of carbofuran residues into organic matter.

A good balance sheet was obtained for non-flooded condition and the percentage recovery was generally between 85-93%. This suggests that volatilization probably does not represent a significant percentage in the dissipation of carbofuran from soil under this soil condition. While in flooded soil the balance sheet obtained was lower than in non-flooded conditions suggesting that there was some loss during water evaporation (co-evaporation). This was explained by the high percentage (18.25 %) obtained for 14C-organic volatile products compared to only 5.67 % under non-flooded conditions.

HPLC analyses of the extractable residues after 63 days of incubation showed that 78.24 % and 67.31% was in the form of carbofuran respectively under moist and flooded soils. In both conditions, carbofuranphenol was the major significant metabolite observed with 18.3% and 8.2% of applied activity under respectively flooded and moist soil (table 4). Similar results were obtained by Venkateswarlu and Sethunathan (1978), Arunashalam et al., (1990) and Lalah et al., (1996). They reported substantial formation of carbofuran phenol as the major degradation product.

Table 4. Percent metabolites identified in flooded and non-flooded soil sample extracts at 63 days after application.

Compounds	Retention time (min)	% metabolites	
		Moist soil	Flooded soil
3-ketocarbofuran	5.45	4.08	6.24
Carbofuran	6.42	78.24	67.31
3-ketocarbofuranphenol	6.61	3.46	4.38
Carbofuran-7-phenol	8.30	7.22	18.37

Literature had shown variable results on carbofuran metabolite recovery from soils, ranging from no metabolite (Isensee and Tayaputch, 1986) or only Carbofuran phenol up to 46% (Brahmaprakash et Senthunathan , 1985) to the presence of the three metabolites Carbofuran phenol, 3-hydroxy- and 3-Keto Carbofuran (lallah et al., 1996).

Carbofuran was reported to be subject to hydrolysis, specially in alkaline conditions, and its hydrolysis occurs at the carbamate linkage yielding Carbofuran phenol (Ou et al., 1982). This is the fact that high amount of carbofuran phenol was observed in flooded conditions. The alkaline nature of the soil (pH=7.8) in our study may be responsible also for the significance presence of carbofuran phenol. While Kale et al., (2001) reported that the near neutral soil pH may be responsible for the absence of carbofuran phenol. 3-hydroxycarbofuran metabolite was not detected. This may be due probably to its rapid conversion to 3-keto carbofuran metabolite (Camper et al., 1987).

Conclusion

In conclusion, these results confirm that carbofuran is more rapidly mineralized under flooded than moist soil, likewise it is important to note the difference in degradation rate of Carbofuran in moist and flooded soils. In moist soil, mineralization was formed to a considerable extent (19.2%) as compared to that in flooded soils (12.4%), whereas, soil bound residues were formed with high amount. The extent is being more in flooded (33.3%) than in moist soil (29.1%) indicating high potential for soil binding. Considerable dissipation was occurred through volatilization in flooded soil. Chemical hydrolysis and volatilization are an important dissipation way in flooded soil.

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