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Study on Degradation Processes of ^{14}C-Carbofuran in Soil from Northwest Morocco as Influenced by Soil Water Content, Temperature and Microbial Activity

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ABSTRACT

This study was conducted to assess the impact of soil temperature, moisture, and microbial activity on the mineralization and dissipation rate of Carbofuran on a typical sugar beet clayey soil in the Loukkos area in order to predict the fate of Carbofuran in soil and environment (Northwest Morocco). The results of incubation investigations demonstrated that both soil temperature and water content have a substantial impact on the rates of Carbofuran mineralization and dissipation, and that the autoclaving of soil greatly lengthened the half-life of the insecticide. In non-autoclaved soil, the rates of mineralization and degradation increased as soil moisture content and temperature rose. In natural soil, Carbofuran half-lives ranged from 26 to more than 90 days, and dissipation rates followed first-order kinetics. Chemical degradation and microbial breakdown are the principal pathways of Carbofuran degradation which are highly dependent on soil temperature and moisture contents. However, the half-life increased 3.6 times in the autoclaved soil, from 39 to 142 days. Autoclaving prevented mineralization, demonstrating the significance of microbes in the pesticide's breakdown process. However, since bound residues developed in large concentrations (19%) in the autoclaved soil, it appears that the insecticide was also dissolved by non-biological processes. Insecticide risk assessment studies and the validation of pesticide dissipation models for clayey soils in sugar beet-growing regions of Morocco may benefit from these findings.

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Keywords: Carbofuran; ^{14}C radiotracer; dissipation; soil; Loukkos (Morocco).

1. INTRODUCTION

The environmental fate of pesticide residues in Morocco is a hot topic which is gaining more emphasis and attention than ever before owing to Moroccan authorities' increased awareness, on the one hand, to the risks generated by increasing use of pesticides to the environment and health; 17519 tons in 2009 [1] and 28773 tons in 2016 [2] and, on the other hand, to international residue limit requirements in food, drinking water supplies as well as in exported produce such as fruits and horticultural products.

Sugar beet cultivation occupies a very important place in Moroccan agriculture with on average acreage of 65.000 ha and produces 3 million tons of beets [3,4]. With sugar cane, it allows production of 500.000 tons of sugar, approximately 54% of the national needs in sugar [5]. Therefore, in the aim to reach national self-sufficiency in sugar, sugar beet cultivation has received great attention under Green Morocco Plan (GMP) [6] and by Generation Green (2020-2030); a newly launched Government Agricultural Development Strategy [7].

Development and productivity of sugar beet production system, however, are tightly linked to pesticide use in Morocco. In fact, the use of pesticides, mainly insecticides, remains one of the most important pest control measures for sugar beet health protection [8]. Use of these chemicals in large amounts without consideration of potential human health hazards, resulting from pesticide residues accumulation in food and water [9,10,11,12,13,14] however, can be dramatic.

Among these chemicals, Carbofuran (2,3-dihydro-2,2- dimethyl-7-benzofuranyl methylcarbamate) is a versatile broad spectrum systemic insecticide, nematicide and acaricide widely used for controlling many rootworms in sugar beet, sugar cane and many horticultural crops [15,16]. Besides, it also exhibits relatively high mammalian toxicity [17,18], potential endocrine disrupting activity [19,20] and, therefore, has been classified as highly hazardous [21,22]. The oral LD_{50} value of Carbofuran in mice and other animals has been reported to vary from 2 to 9 mg/kg bodyweight [17]. Due to its wide spread use, high water solubility and lower adsorption coefficient [23], Carbofuran has been detected in groundwater, surface and rainwater, in soils, air, food and wildlife [24,25,26,27,28]. Due to its high oxicity, Carbofuran has already been banned in many countries [29].

As biodegradation is the main cause of dissipation of most insecticides in soils [30], numerous studies have shown the ability of soil microorganisms to use Carbofuran and its degradation products, as source of carbon and energy [31,32,33,34,16]. Thus, through mineralization, pesticides are completely degraded to CO_2 by microorganisms reducing their persistence and hence lowering their contamination risks to the environment [35,36,22].

Generally, parameters which facilitate bacterial development also accelerate biodegradation processes. Soil temperature and humidity are the major parameters that control microbial degradation of pesticides in soils [37]. Their effects on pesticide biodegradation have been the objective of numerous studies [38] because they are, probably, key factors controlling microbial activity in soil. Generally, an increase of soil temperature and humidity corresponded to significant increases in biodegradation rate [39]. Fluctuations in moisture content and soil temperature would have considerable effects on biodegradation rates and hence fate since water and temperature affect microbial metabolism as well [40].

Carbofuran is a relative persistent insecticide with a half-life in soil ranging from 3 weeks to more than 50 weeks depending on soil pH [41]. Field studies showed that the half-life of Carbofuran in soils ranged from 6 to 17 weeks [42] and reached even a year [43].

The vast and intensive agricultural use of pesticides in some Moroccan zones such as Loukkos area (north-western) has important implications for the contamination of the environment. Considering that the average national production area of sugar beet (*Beta vulgaris*) is 65.000 ha, it is estimated that about 1300 tons of this insecticide are spread annually in sugar beet fields. In Loukkos perimeter, strawberry, potato, and sugar beet are the major cash crops for farmer's community. It is therefore, expected that more than 100 tons of Carbofuran should are applied annually only in sugar beet field in this basin. With growing population and related increase in national sugar consumption, estimated at 34 Kg/person/year [5], efforts are being made to further intensify sugar beet production to reach the national self-sufficiency in sugar production. The new agricultural strategy Generation Green (GG) (2020-2030) aims to reach a harvested sugar beet area of 120,000 ha in 2030 [7], it is therefore, expected for increased use of pesticides including Carbofuran. This may lead to the contamination of the environment and poses high risks to human health. Moreover, lack of knowledge on the fate and behavior of pesticide in Moroccan environment requires in-depth studies of pesticide pathways in soil in the aim to prevent human health and protect the environment. Among these pesticides, the Carbofuran is the least studied [44,45,46].

In order to predict the fate of Carbofuran in soils, it is essential to understand the various physical, chemical and biological processes that occur when the chemical is added to soils. Detailed studies of dissipation and degradation processes of Carbofuran in soil will essentially help in improving the efficacy of this pesticide in controlling target organisms as well as in minimizing the adverse effects on non-target organisms. Accordingly, this study was conducted with the main objective of investigating the influence of temperature, soil moisture and microbial activity on Carbofuran dissipation in a typical sugar beet soil under laboratory conditions using ^{14}C radiotracer technique.

2. MATERIELS AND METHODS

2.1 Preparation of Soil Samples and Treatments

A clayey soil (2.2% organic matter, 1.7% total nitrogen, 8.2% sand, 12.1% silt, 79.6% clay, pH 7.2, CEC: 33.2 meq/100 g) collected at 0 to 30 cm from sugar beet field at Loukkos perimeter (North-western Morocco) was used. This Vertisol, which has no history of Carbofuran treatment, was air-dried, ground and sieved through a 2 mm mesh screen before its use. Liquid scintillation counting technique was used to quantify the Carbofuran residues in soil samples.

2.2 Chemicals

[U-phenyl-^{14}C] Carbofuran (specific activity 1.12 GBq/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 thin layer chromatography. (U-phenyl-^{14}C) Carbofuran was diluted with unlabeled Carbofuran (to specific activity: 4.08 MBq/mmol) before application. All the reagents used were of analytical or scintillation grade. All the solvents used were residues or analytical grades.

2.3 Mineralization and Degradation Studies

50 g portions of dry and sieved soil were placed separately in sterile standard 250 ml biometer flasks. Each soil sample was treated with 0.25 µCi (0.5mg) of U-ring-^{14}C-Carbofuran (specific activity = 81.5 MBq (2.2 mCi)/mmol^{-1}, 98.62% purity). Every sample was slowly homogenized, in the rotary evaporator, at room temperature until total evaporation of the methanol. 20 ml of ethanolamine were placed in the arm side of every flask to fix $^{14}CO_2$ liberated, while the volatile products were trapped by polyurethane placed in the passage between ethanolamine and soil. The flasks were tightly closed and placed in the dark at the desired temperature and soil moisture during 63 days. At weekly intervals, the flasks were aerated for some minutes to ensure aerobic conditions. Three different temperatures: 10, 25 and 35°C and three different rates of soil water content (20, 60 and 100% water holding capacity (WHC)) were chosen. These conditions were maintained along the experiment by adding adequate fresh bi-distilled water and incubated at 25 ± 2°C in dark during 63 days. The effect of soil microbial activity was examined by conducting experiment on sterile and non-sterile soils at room temperature under 60% WHC. The sterilization was carried out by three consecutive autoclaving for 20 min at 120°C with an interval of 24 h. The soil samples underwent the same procedure as that described above. Freshly bi-distilled water was used for maintaining humidity of the sterile soil samples.

2.4 Sampling, Extraction, Clean-Up and Analysis

Immediately after application of the insecticide, flasks in duplicates were pooled out from each case, to determine the initially applied Carbofuran (100%). Other flasks were sampled out in triplicates at 15, 31, 45 and 63 days after Carbofuran application. The soil samples were air-dried under a ventilated fume hood, ground and homogenized then stored in plastic bags at -21°C until analysis.

Mineralization rate was determined by counting $^{14}CO_2$ (trapped in the ethanolamine) in the LSC hp Tricarb 1100. While volatile products were extracted by Soxhlet apparatus with methanol during 4 h (15 min /cycle), and the extract was counted in LSC. Extracted soil was air dried, crushed and homogenized. Total and bound residues were determined by combusting soil samples (500 mg) in biological oxidizer material (Harvey OX-600) followed by counting in LSC. Extractable residues were determined by extracting soil samples with methanol in Soxhlet extraction apparatus during 6 h (4 to 5 cycles / h). An aliquot (2 ml) followed by counting in the LSC.

3. RESULTS AND DISCUSSION

3.1 Effect of Temperature on Carbofuran Degradation

In the present experiment, the degradation of Carbofuran was examined at three different soil temperatures (10, 25 and 35°C) in clayey soil of Loukkos perimeter, characterized by 60% WHC and 10 mg/kg insecticide concentration.

3.1.1 Effect on Mineralization

This experimentation aimed at determining the effect of temperature on Carbofuran mineralization and degradation rates. Fig. 1 illustrates the cumulative evolution of the $^{14}CO_2$ formed/liberated from the ^{14}C-U-ring Carbofuran at different temperature. Evolution and monitoring of $^{14}CO_2$ formation from Carbofuran degradation in soil show that substantial amount of the insecticide was mineralized to $^{14}CO_2$ after 63 days of incubation. The results showed an increase of Carbofuran mineralization rate with increasing time and temperature. Initially, we observed a low mineralization rate during first 15 days, which can be attributed to the lag phase (microbial adaptation period) [37,47], after what a gradual increase of mineralization rate with time was observed. At the end of experiment, 23.3 and 20.3% of the initial applied dose were mineralized, respectively at 35 and 25°C, while at 10°C, mineralization did not exceed 7%.

From Figure 1, we can observe that the increase of soil temperature between 10 and 25°C had greater influence on the mineralization rate than that between 25 and 35°C. The same tendency was reported by Ou et al. [48] who concluded that increasing soil temperature between 15 and 27°C presented higher influence on the degradation in comparison to that between 27 and 35°C. This could be explained by the fact that proliferation of microorganisms with temperature during Carbofuran degradation could be the reason of the fast increasing of insecticide mineralization rate. Similar results were obtained in literature [49]. Singh et al. [50] observed that Carbofuran is more quickly biodegraded at 35°C than at 25°C. Ou et al. [48] confirmed the dominating role of microorganisms in the degradation of the Carbofuran by showing that degradation is maximal between 27 and 35°C. Ramanand et al. [51] found that mineralization of

Carbofuran was more rapid at 35 than at 20°C. Parkin and Shelton [40] reported increasing mineralization rate with increasing soil temperature from 10 to 30°C.

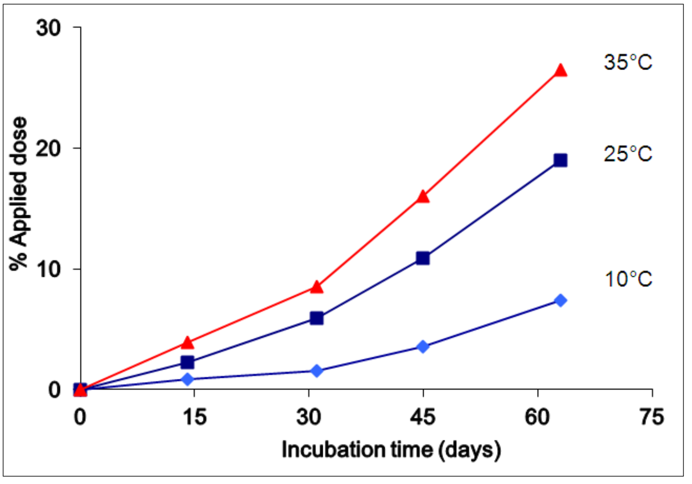

Fig.1. Effect of soil temperature on Carbofuran mineralization

3.1.2. Effect on Dissipation

Fig. 2 presents the influence of temperature on Carbofuran dissipation. The results showed that Carbofuran dissipated more quickly at 35°C than at 25 or at 10°C. Extractable residues decreased with time and temperature, while bound residues fraction increased. After 15 days of incubation, ^{14}C activity has degraded by approximately 33% at 35°C, while by only 26.4 and 14% respectively at 25 and 10°C. At the end of the experiment, balance sheet showed degradation of 82, 67.5 and 41% respectively at 35, 25 and 10°C with insecticide disappearance rates of 1.34, 1.12 and 0.68% respectively, showing a significant effect of soil temperature on insecticide dissipation rate.

The Carbofuran disappearance followed 1st order kinetics for the three temperatures, with correlation coefficients $r^2 > 0.97$. Calculated half-lives ranged from 29 days (at 35°C) to 41 days (at 25°C) and 67 days (at 10°C). These values are in agreement with those cited in literature.

Regarding bound residues, the results of incubation studies generally showed a relatively high adsorption capacity of Carbofuran to soil which augment with increasing time and temperature. This adsorption took place quickly during the first 30 days of application (15, 19 and 22% at 10, 25 and 35°C respectively). After this time, bound residues formation progressed slowly and reached 19, 29 and 32% at 63rd day of incubation, respectively at 10, 25 and 35°C. This phenomenon is termed aged bound residues that become more stable the longer they are in the soil [45]. Similar results were obtained by Kale and Raghu [52].

They obtained 48.64 and 23.08% as bound residues of Carbofuran respectively under moist and flooded conditions over a period of 30 days.

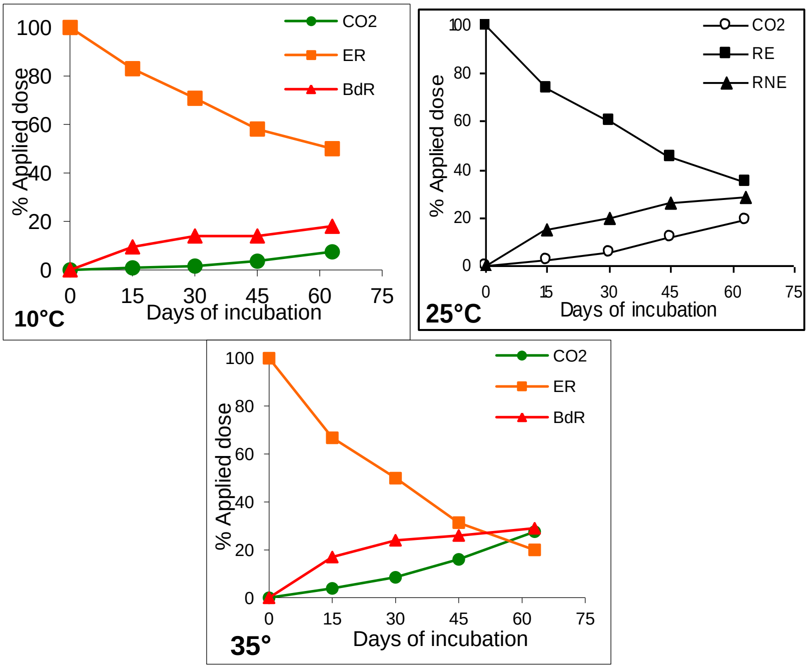

Fig. 2. Effect of temperature on Carbofuran dissipation in soil (E: extractable residues ; BR : Bound residues)

The decrease of extractable residues with time is a classical result obtained during incubation of soil with pesticides [53,45]. During the first two weeks, the disappearance rate was higher; 18, 23 and 35% were degraded respectively at 10, 25 and 35°C (Fig. 2). Extractable residues decrease with time to reach 52, 33 and 20% at 63 days, respectively at 10, 25 and 35°C. The lag phase associated with Carbofuran degradation progressively decreased with increasing temperature, suggesting that is the time required for adaptation within communities-controlled degradation rates [40].

The dissipation values obtained in this study generally agreed with the published data, which showed that Carbofuran degradation is positively correlated with soil temperature [48,49]. Yen et al. [30] observed that Carbofuran disappearance rate increased with soil temperature and concluded that Carbofuran seems to be capable to contaminate groundwater. Parkin and Shelton [40] observed that Carbofuran dissipated faster at 30°C than at 10°C in a loamy soil. Ramanand et al. [51] and Yen et al. [30] obtained the same tendency by increasing soil temperature. In a laboratory study, Yen et al. [30] found that Carbofuran's half-life in silty clay loam varied from 105 to 35 days by varying temperature from 15 to 35°C. While Deleu and Copin [54] found that Carbofuran degradation was effective only at a temperature higher than 25°C with soil humidity of 19% WHC. Ralph and Harris [55] observed that Carbofuran did not undergo any degradation in soil maintained at 3°C, but did at 15 and 28°C. It exhibited only a slight degradation process when heated at 15 and 3°C compared to 28°C. Howard [56] reported half-lives in soils ranging between 30 and 120 days depending on soil pH and temperature. Caro et al. [57] found that half- lives of Carbofuran varied from 46 to 117 days in soil depending on pH, temperature and soil moisture.

Singh et al. [50] observed accelerated degradation of Carbofuran at 35ºC compared to 25ºC. Likewise, Mora et al. [58] corroborated the findings revealing reduction in Carbofuran concentration when temperature was altered from 30 ºC to 40 ºC. Typically, the degradation rate of pesticides is promoted at a higher temperature, which in most cases reflecting escalated microbial activity in soil [59,60]. In microbial degradation, the mechanism involves adsorption of pesticides by bacteria, followed by enzymatic reaction and decomposition into carbon dioxide and water. The process is profoundly affected by temperature and moisture where the increase in temperature would promote growth and reproduction of bacteria, boosting the degradation rate [61].

Fig. 3 shows that the loss of Carbofuran by volatilization is influenced by soil temperature. At 10 and 25°C, volatilization seems to be low and not exceeding 1.6 and 5.8% respectively, suggesting that volatilization probably could not constitute an important pathway of Carbofuran dissipation in soil under 60% WHC at ≤25°C. While at higher temperature, volatilization increased and reached 17% at 35°C and could be another way of Carbofuran dissipation beside mineralization and degradation at high temperatures. Thus, volatilization is a contributing dissipation process.

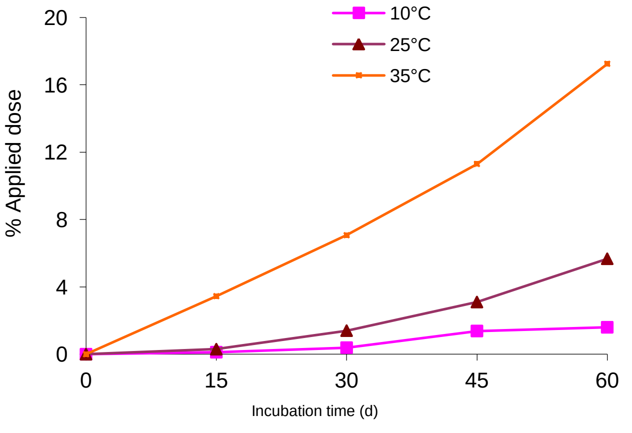

Fig. 3. Effect of temperature on Carbofuran volatilization

These results confirm that dissipation of Carbofuran is strongly influenced by soil temperature, which determines soil biological activities and chemical processes leading to an increase of insecticide disappearance rate (decrease of half-life with increasing temperature). Indeed, enzymatic reaction rate increases with temperature and facilitates the microbial population development and consequently, their action on insecticide mineralization. At low temperature (10°C), the activity of microorganisms however, is reduced becoming optimal between 25 and 35°C. The fast decrease of extractable residues observed during this study was made for the benefit of the mineralization and bound residue formation (Fig. 2). On the basis of these results, formation of bound residues with high amounts could be attributed to the activity of soil microorganisms, capable to transform Carbofuran, and to the chemical hydrolysis of the substance (demonstrated by autoclaving soil).

3.2 Effect of Soil Moisture on Carbofuran Degradation

3.2.1 Effect on Mineralization

The aim of this experimentation was to evaluate the effect of soil water content on the mineralization of Carbofuran. The evolution of the $^{14}CO_2$, formed during Carbofuran mineralization by soil microorganisms under different soil moisture contents, is presented in Fig. 4. The results showed that Carbofuran mineralization rate increased with soil water content; at 100% of WHC (flooded conditions), Carbofuran presents the highest mineralization amount (21.4%) as compared to 60% (17.1%) or 20% WHC (4.7%). Thus, soil maintained at this humidity level, reached a very high rate of $^{14}CO_2$ with regard to those obtained at 60% or at 20% WHC. A relatively low mineralization rate was obtained during the first 15 days of incubation. After this period, mineralization rate increased with time and soil water content, and reached at 63rd day, more than 23% at 100% WHC, while it was only 20.3 and 8.3% at 60% and 20%WHC, respectively.

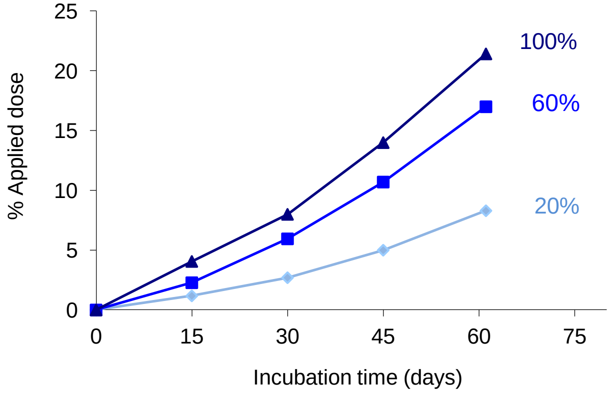

Fig. 4. Influence of soil water content on Carbofuran mineralization

Similar results were obtained by Shelton and Parkin [62]. These authors explained that low soil water content has, for consequence, to delay or inhibit the soil microbial metabolism. Shelton and Parkin Shelton [62] showed that the mineralization rate of Carbofuran decreases with decrease of soil humidity. Kale et al. [63] found that Carbofuran mineralization rate in a Vertisol soil during 60 days of incubation was higher under flood conditions with 35% mineralized, than at 60% WHC with 29%. Kühr and Dorough [64] explained this variation in mineralization rate of Carbofuran by the fact that, under conditions highly moisturized, soil microflora becomes more active in the degradation of the substance.

Similar high levels of Carbofuran mineralization rates, with increasing soil water content, have been reported in the literature; Kale et al. [63] reported that Carbofuran underwent considerable mineralization under moist and flooded conditions. The extent has been greater under flooded conditions (33.74%) than moist conditions (29.96%). Lalah et al. [65] have reported 13 and 11% of mineralization of Carbofuran under flooded and moist conditions respectively. Mojasevic et al. [66] observed that Carbofuran biodegradation increased significantly from 12 to 25% soil moisture, while Ralph and Harris [55] reported that Carbofuran did not undergo mineralization process in soil maintained at a moisture level of less than 9%.

Our results can be explained by the fact that soil moisture content determines the activity of microorganisms in soil. When it is high, the degradation of pesticide is generally facilitated. This explains the positive effect of soil moisture content on the increase of microbial population which was responsible for greater pesticide mineralization under 100% of humidity. According to Massoud [67] and Benicha et al. [68], soil water content affects adsorption of insecticides and their movement through the soil. In dry soils, or in low soil moisture content, insecticides are strongly bounded and are much less available for mineralization. This could explain why reduced Carbofuran mineralization rate was observed, and residue formed (15.6%) as bound at 20% WHC (Fig. 5).

3.2.2 Influence on Dissipation

Results of Carbofuran dissipation at different soil moisture contents are presented in Fig. 5. These results show that Carbofuran dissipation increased with increasing soil moisture. Under high moisture level (100% WHC), extractable residues decrease till 18% of the initial applied dose at 63rd day as compared to 32 and 59% at 60 and 20% WHC respectively. Inversely, bound residues increase with soil humidity and time; high quantities were formed at 100% WHC (32%) as compared to those formed at 20% WHC (28%) or at 60% WHC (30%). This could be explained by the fact that under high moisture content (100% flooded condition) which could give rise to anaerobic conditions, Carbofuran is subjected to hydrolysis to give Carbofuran phenol as main metabolite that undergoes immediately strong adsorption reaction with soil constituents [41,69]. Accordingly, bound residues were higher in flood compared to moist conditions. In a previous study on the fate of Carbofuran in soil Benicha et al. [11] observed under moist conditions, that 29.1% of initial Carbofuran was bound to the soil after 63 days of incubation at 25°C, against 33.3% under flooded condition.

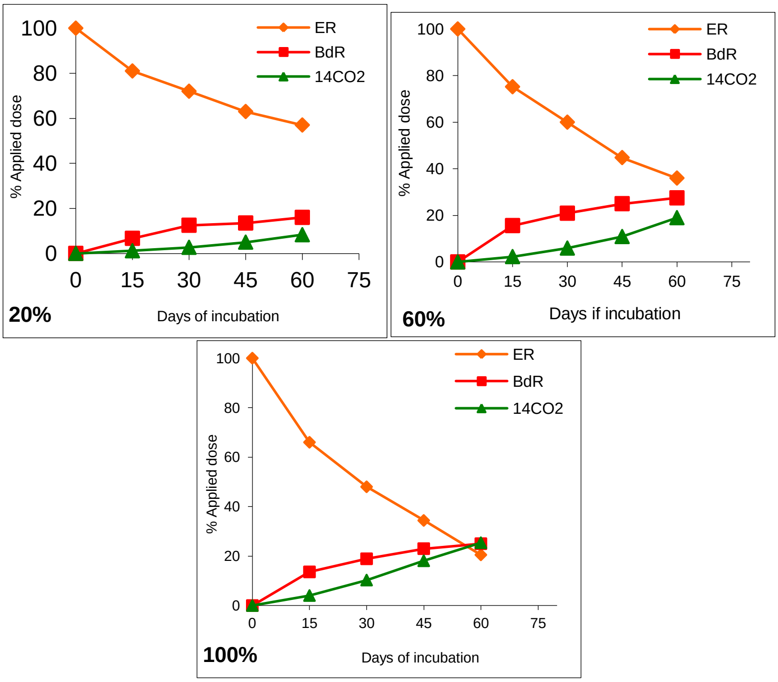

Fig. 5. Influence of soil water content on Carbofuran dissipation (ER: Extractable residues; BdR: Bound residues; $^{14}CO_2$: mineralisation)

Our results are in agreement with those cited in the literature. Yen et al. [30] found that Carbofuran dissipated easily at 100% WHC, than at 40 or 60% WHC in clayey and clayey-loamy soils. Kale et al. [63] reported that insecticide dissipation was quicker under flooded conditions than at 60% WHC in a clay soil. Gorder et al. [70] observed also a fast Carbofuran disappearance rate after important precipitation. Mojasevic et al. [66] showed that the degradation rate of Carbofuran increased greatly between 12 and 25% of soil humidity, while it remained constant between 25 and 35% in a Codorus silt loam soil. Deleu and Copin [54] however, did not observe degradation of Carbofuran at soil humidity lower than 19% in a loamy soil during 67 days of incubation. Carbofuran degradation in some tropical soils was reported to be faster under flooded conditions than under non-flooded conditions [52]. Kale et al. [63] and Tariq et al. [71] showed that temperature and moisture contents significantly reduced the half-life values of pesticides in soil. Kazemi et al. [72] found that Carbofuran estimated values of the degradation rates were approximately 40 to 49% lower in dry soil than in wet soil. Farahani et al. [25] concluded that soil moisture content, micro-organisms and the organic matter content (OM) affected the degradation of Carbofuran in soils.

Carbofuran kinetic disappearance rates (^{14}C free) followed a first kinetic order for the three rates of soil humidity with correlation coefficients of $r^2 > 0.97$. It was faster at high soil humidity (100% WHC) with dissipation rate of 1.27%/days, followed by 0.71%/day at 60% WHC, while at 20% WHC, it was only 0.71%/day. At 25°C, the calculated half-lives for Carbofuran were 90, 41 and 26 days respectively at 20, 60, and 100% soil water content as was observed by other researchers [73]. In general, these values were within the reported half-life range of 30 to 120 days by Extoxnet [74].

Graebing and Chib [75] observed that Carbofuran exhibited 2.2 times longer half-lives when less moisture was available in the soil; from 34 to 15 days. Farahani et al. [25] observed half-lives of Carbofuran, ranging from 57.28 to 203.87 days with varying soil moisture from 100 to 60% field capacity. Lalah et al. [76] reported in temperate soils half-life values ranging from 66 to 115.5 days. Farahani et al. [25] reported that Carbofuran half-lives varied from 38.51 to 203.87 days. These authors found, that among the major factors that contributed to less persistence of Carbofuran, soil moisture content and microbial activity. With regard to our experimental results, it is interesting to note that the increase of Carbofuran dissipation (mineralization and degradation) varies according to the soil water content; more the soil water content is high, more this phenomenon is important.

In conclusion, Carbofuran mineralization and degradation are strongly influenced by soil humidity which determines the activity of soil microorganisms; when it is high, pesticide degradation is generally facilitated. This could be explained by the humidity effect on increase of microbial population which is at the origin of the mineralization of Carbofuran.

3.3 Effect of Soil Sterilization on Mineralization and Dissipation

3.3.1 Mineralization

To confirm whether Carbofuran mineralization is a biotic phenomenon or not, mineralization was measured in natural and autoclaved soil conditions. The results presented in Fig. 6, showed that the sterilization inhibited the mineralization process; 20.3% was mineralized in natural soil against only 1.2% in autoclaved soil condition. This is due to the elimination of microorganisms by autoclaving. This result confirms the biological aspect involved in the degradation process of Carbofuran as reported by Porto et al. [77].

3.3.2 Dissipation

Dissipation of Carbofuran has been studied in autoclaved and non-autoclaved soils under laboratory condition. The results presented in Fig. 7, showed that in non- autoclaved soil condition, Carbofuran dissipation was found to be higher than that in autoclaved soil. Carbofuran degradation disappearance rate, during the whole experimental period, was significantly reduced under sterile condition with regard to that under normal condition (Fig. 7). At the end of the experiment, 67.5% of initial Carbofuran was disappeared under natural condition, whereas only 28% was degraded under sterile soil by that time. This means that Carbofuran disappearance rate was 3.5 times lower under sterile conditions as compared to that under natural soil with calculated half-life exceeding 20 weeks (142 days) as compared to 41 days under natural condition (Fig. 8). Obviously, this suggests the possible microbial role in degrading Carbofuran. On the other hand, however in autoclaved soil, bound residues were formed and reached 19% at 63 days of incubation vs. 32% in non-autoclaved soil. This might be due to chemical process leading to adsorption of Carbofuran to soil. These results provided evidence that Carbofuran dissipation is both biotic and abiotic processes, as reported by Rajagopal et al. [69].

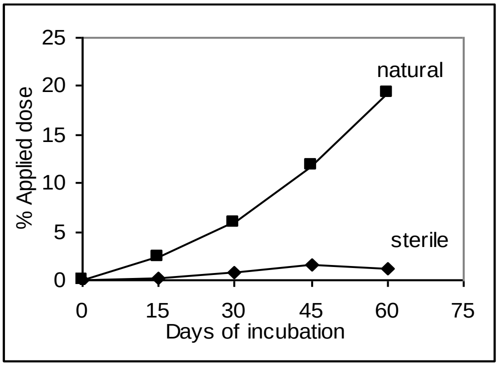

Fig. 6. Effect of soil sterilisation on Carbofuran mineralisation

Our results are in accordance with Miles et al. [78] who reported that Carbofuran was relatively more persistent in sterile soil than in non-sterile soil. Arunachalam and Lakshmanan [79] reported the degradation of only 20% of the initial dose of Carbofuran in sterile soil 60 days of incubation. Getzin and Shanks [80] observed that Carbofuran half-life in autoclaved soil overtakes 16 weeks, and that 85 to 90% of the initial dose are still in soil, 112 days after insecticide application. Kale and Raghu [52] found that Carbofuran did not undergo any degradation, in a sterile clay soil during 30 days of incubation, while considerable quantities of bound residues (29%) were formed. Farahani et al. [25] reported half-lives of Carbofuran in autoclaved soil, ranged from 147.5 to 301.37 days depending on soil organic matter content at 100% field capacity and 30°C, as compared to 57.28 and 192.54 days respectively in natural soil in the same conditions.

According to our experimental results, the inhibition of the mineralization, under sterile condition, confirms that biological degradation of Carbofuran is considered as an important dissipation pathway in soil. Furthermore, formation of bound residues with high amount (19%) under sterile conditions gave evidence that chemical degradation is another pathway. In absence of any biological activity, the reaction of physical and/or chemical agents can lead to degradation of the molecule indicating that Carbofuran dissipation in soil results from both biotic and abiotic phenomena. On the other hand, Carbofuran degradation rate in the autoclaved soil suggests that the insecticide could persist much longer in sub-soil where microbial activity is reduced [81]. Consequently, the probability of groundwater contamination greatly increased particularly in zones characterized by high rainfall rates, coarse- textured soils and shallow groundwater tables. All these, are characteristics of Loukkos area. A lot of care must therefore be taken prior to minimize environmental contamination and human health effect, particularly when the rain or irrigation arises a little period after insecticide application.

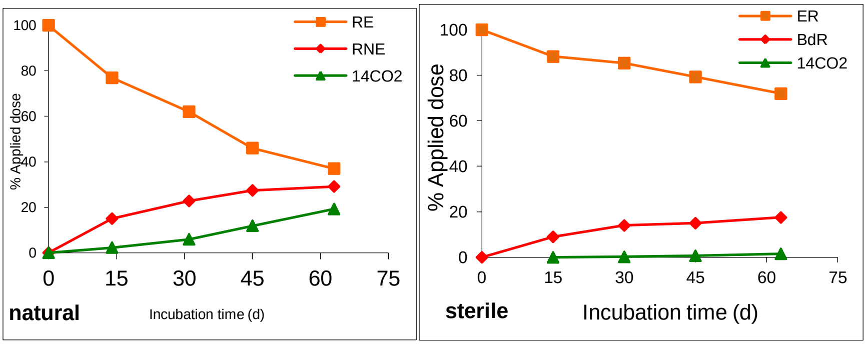

Fig. 7. Carbofuran dissipation under natural and sterile conditions (ER: extractable residues; BdR.: Bound residues)

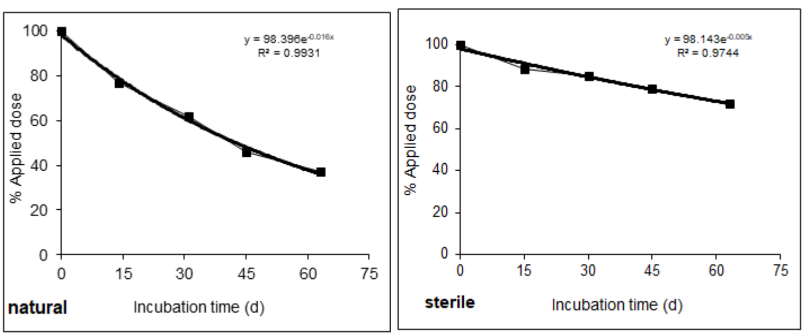

Fig. 8. Dissipation kinetic of Carbofuran under natural and sterile conditions Carbofuran half-lives=41 day in natural condition, >142 day in sterile condition

4. CONCLUSION

In the present study, we conclude that soil temperature, along with soil moisture and micro-organisms; play a major role in the degradability of pesticides in clayey soil of the sugar beet-growing area. This study showed that soil temperature and soil moisture are very important environmental parameters controlling Carbofuran biodegradation and dissipation in soil. Generally, increase of soil temperature and humidity increase Carbofuran biodegradation and dissipation rate. Fluctuations in moisture content and soil temperature would have considerable effect on biodegradation rate since water and temperature affect microbial metabolism as well. In absence of any biological activity (autoclaving), the action of physical and/or chemical agents can lead to a transformation of the insecticide (formation of bound residues). Carbofuran dissipation in soil results from both biotic and abiotic phenomena.

Chemical degradation and microbial breakdown are the principal pathways of Carbofuran degradation which are highly dependent on soil temperature and soil moisture content. Differences in the effect of soil water content and soil temperature on mineralization and dissipation kinetics could be attributed to the ability of soil indigenous microorganisms to transform the insecticide. Similar studies should be conducted on coarse textured soils and other crops to further judge on potential impacts of Carbofuran fate in the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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