Dissipation processes of $^{14}$C-carbofuran in soil from Northwest Morocco as influenced by soil water content, temperature and microbial activity

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In order to predict the fate of carbofuran in soil, this study was undertaken to evaluate the influence of soil temperature, soil moisture and soil microbial activity on mineralisation and dissipation rate of carbofuran on a typical sugar beet clayey soil of Loukkos area (Northwest Morocco). Results of incubation studies showed that soil temperature and soil water contents have great influence on mineralization and dissipation rates of carbofuran, and soil autoclaving process significantly increased the half-life of the insecticide. The degradation and mineralization rates in non-autoclaved soil increased with increasing soil temperature and soil moisture content. Carbofuran dissipation rates followed first-order kinetics with half-lives ranged from 26 to >90 days in natural soil. In the autoclaved soil however, the half-life increased 3.2-folds from 39 to 142 days. Mineralization was inhibited by autoclaving, indicating the importance of microorganisms in the breakdown process of the insecticide, and in mean time, the insecticide seems to have been degraded by non-biological process since bound residues were formed with high amount (19%) in the autoclaved soil. These data could be helpful for risk assessment studies of the insecticide and for validating pesticide dissipation models for clayey soils in Moroccan sugar beet-growing areas.

**Key words:** Carbofuran, $^{14}$C radiotracer, dissipation, soil.

**INTRODUCTION**

In Morocco, the environmental fate of pesticide residues is an issue that is now receiving more attention than ever before due to growing awareness of Moroccan authorities regarding, on the one hand, the dangers generated by pesticides use (17.519 tons in 2009; El Ouilani, 2011) and, on the other hand, to international residue limit requirements in food, drinking water supplies as well as in export products 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 millions tons of beets (Doukkali et al., 2009). With sugar cane, it allows production of 500,000 tonnes of sugar, approximately
54% of the national need in sugar (Bouziane, 2011). Therefore, in the aim to reach national self-sufficiency in sugar production, sugar beet cultivation has received great attention in Green Morocco Plan (GMP), a new Government Agricultural Development Strategy (ADA, 2009).

Development and productivity of sugar beet 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 plant protection (Aghbani and Jenane, 2002). Use of these chemicals in large amounts without consideration of potential human health hazards, resulting from pesticide residues accumulation in food and water (Looser, 2006; El Bakouri et al., 2008; Benicha et al., 2011a; Salghi et al., 2012) 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 (Trotter et al., 1991). Besides, it also exhibits relatively high mammalian toxicity (FAO/WHO, 1997), potential endocrine disrupting activity (Lau et al., 2007) and, therefore, has been classified as highly hazardous (FAO/WHO, 1997). The oral LD_{50} value of carbofuran in mice and other animals has been reported to vary from 2 to 9 mg/kg bodyweight (FAO/WHO, 1997). Due to its widespread use, high water solubility and lower adsorption coefficient (Sukop and Cogger, 1992), carbofuran has been detected in groundwater, surface and rain water, in soils, air, food and wildlife (Bouchway et al., 1992; Olieno et al., 2010; Devasia and Madhu, 2011). Due to its high toxicity, carbofuran has already been banned in many countries (Pashû, 2009).

In Morocco, as in many parts of the world however, carbofuran is readily available and still being used as insecticide-nematicide, at high levels, by farmers mainly in sugar beet and industrial tomato to control rootworms. It is applied at a rate of 20 kg/ha as Furadan, Diafuran 5G, or Axlera 5G (Ezzahiri et al., 2012). As biodegradation is the main cause of dissipation of most insecticides in soils (Yen et al., 1997), numerous studies have shown the ability of soil microorganisms to use carbofuran and its degradation products, as source of carbon and energy (Feng et al., 1997; Slaoui et al., 2007; Chanika et al., 2011). Thus, through mineralization, pesticides are completely degraded, in CO_{2}, by microorganisms reducing their persistence, and their risks to the environmental contamination (Chapalamadugu and Chaudhry, 1991).

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 (Fournier et al., 1997). Their effects on pesticide biodegradation have been the objective of numerous studies (De Silva et al., 2010) 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 (Ismail and Dan-linen, 2003). Fluctuations in moisture content and soil temperature would have considerable effect on biodegradation rate since water and temperature affect microbial metabolism as well (Parkin and Shelton, 1994).

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 (Getzin, 1973). Field studies showed that the half-life of carbofuran in soil ranged from about 6 weeks to 17 weeks (Caro et al., 1973) and even a year (Szeto and Price, 1991).

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. According to the national sugar beet crop surface (65,000 ha), it is expected that about 1300 tons of this insecticide should be spread annually in sugar beet fields. In Loukkos perimeter, potato, strawberry and sugar beet are the major cash crops for farmers community. It is therefore, expected that more than 100 tons of carbofuran should be applied annually only in sugar beet field at this area. With increasing population and increasing national sugar consumption, estimated at 34 Kg/person/year (Bouziane, 2011), efforts are being made to intensify sugar beet production to reach the national self-sufficiency in sugar production.

In the above context, with the implementation of the recommendations of the GMP, which provides increase of sugar beet agricultural areas at Loukkos perimeter, from 5400 to 8500 ha at 2013 and to 12000 ha at 2020 (ADA, 2009), it is therefore, expected to further 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 behaviour of pesticide in Moroccan environment requires in-depth studies of pesticide behaviour in soil in the aim to prevent human health and protect the environment. Among these pesticides the carbofuran is the least studied (Benicha et al., 2010, 2011b).

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. Detail studies of dissipation and degradation process 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...
14C radiotracer technique.

MATERIELS AND METHODS

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 use. Liquid scintillation counting technique was used to quantify the carbofuran residues in soil samples.

Chemicals

[U-phenyl-14C] 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-14C) carbofuran was diluted with unlabelled 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.

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-14C-carbofuran (specific activity = 81.5 MBq (2.2 mCi)/mmol, 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 14CO2 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 were chosen. These conditions were maintained along the experiment by adding adequate fresh bidistilled 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 bidistilled water was used for maintaining humidity of the sterile soil samples.

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 14CO2 formed (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.

RESULTS AND DISCUSSION

Effect of temperature on carbofuran dissipation

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.

Effect on mineralization

This experimentation aimed at determining the influence of temperature on carbofuran mineralization and degradation rates. Figure 1 illustrates the cumulative evolution of the 14CO2 formed/liberated from the 14C-U-ring carbofuran at different temperature. Evolution and monitoring of 14CO2 formation from carbofuran degradation in soil show that substantial amount of the insecticide were mineralized to 14CO2 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) (Fournier et al., 1997; Diez, 2010), 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%. We observed 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. (1982) 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 (Sahoo et al., 1990). Singh et al. (1990) observed that carbofuran is more quickly biodegraded at 35°C than at 25°C. Ou et al. (1982) 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. (1988) found that mineralization of carbofuran was more rapid at 35 than at 20°C. Parkin and Shelton (1994) reported increasing mineralisation rate with increasing soil temperature from 10 to 30°C.
studies generally showed a relatively high adsorption those cited in literature. 67 days (at 10° C). These values are in agreement with ranged from 29 days (at 35° C) to 41 days (at 25° C) and reach 52, 33 and 20% at 63 days, respectively at 10, 25 and 35° C. Similar results were obtained by Kale and Raghu (1996). They obtained 48.64 and 23.08% as bound residues formation progressed slowly and reached 14 and 22% at 10, 25 and 35° C respectively). After this time, bound residues fraction increased. After 15 days of incubation, approximately 33% of 14C activity was degraded at 35° C, while only 26.4 and 14% were registered, 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% days respectively, showing a significant effect of soil temperature on insecticide dissipation rate.

The kinetic of carbofuran disappearance rates followed 1st order kinetic 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.

Concerning bound residues, the results of incubation studies generally showed a relatively high adsorption capacity of carbofuran to soil which increased with increasing time and temperature. This adsorption took place quickly during 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. Similar results were obtained by Kale and Raghu (1996). They obtained 48.64 and 23.08% as bound residues of carbofuran respectively under moist and flooded conditions over a period of 30 days.

The decrease of extractable residues with time is a classical result obtained during incubation of soil with pesticides (Barriuso and Houot, 1996). During the first two weeks, the disappearance rate was fast; 18, 23 and 35% were degraded respectively at 10, 25 and 35°C (Figure 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 the time required for adaptation within communities controlled degradation rates.

The dissipation values obtained in this study generally agreed with the published data which showed that carbofuran degradation is positively correlated with soil temperature (Ou et al., 1982; Sahoo et al., 1990). Yen et al. (1997) observed that carbofuran disappearance rate increased with soil temperature and concluded that carbofuran seems to be capable to contaminate groundwater. Parkin and Shelton (1994) observed that carbofuran dissipated faster at 30°C than at 10°C in a loamy soil. Ramanand et al. (1988) and Yen et al. (1997) obtained the same tendency by increasing soil temperature. In a laboratory study, Yen et al. (1997) 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 (1995) found that carbofuran degradation was effective only at a temperature higher than 25°C with soil humidity of 19% WHC. Ralph and Harris (1986) 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 (1991) reported half-lives in soils ranging between 30 and 120 days depending on soil pH and temperature. Caro et al. (1976) found that half-lives of carbofuran varied from 46 to 117 days in soil depending on pH, temperature and soil moisture.

Figure 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. Ralph and Harris (1986) observed that at high temperatures, 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.

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 (Figure 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).

**Effect of soil moisture on carbofuran dissipation**

**Effect on mineralization**

The aim of this experimentation was to evaluate the effect of soil water content on the dissipation of carbofuran. The evolution of the $^{14}$CO$_2$, formed during carbofuran mineralization by soil microorganisms under different soil moisture contents, is presented in Figure 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 63$^{rd}$ day, more than 23% at 100% WHC, while it was 20.3 and 8.3% at 60% and 20% WHC, respectively.

Similar results were obtained by Shelton and Parkin (1991). These authors explained that low soil water content has, for consequence, to delay or inhibit the soil microbial metabolism. Parkin and Shelton (1994) showed that the mineralization rate of carbofuran decreases with decrease of soil humidity. Kale et al. (2001) 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 (1976) 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. (2001) 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. (1996) have reported 13 and 11% of mineralization of carbofuran under flooded and moist conditions respectively. Mojasevic et al. (1996) observed that carbofuran biodegradation increased significantly from 12 to 25% soil moisture, while Ralph and Harris (1986) reported that carbofuran did not undergo mineralisation 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 (1976), 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. This could explain why reduced carbofuran mineralization rate was observed, and residue formed (15.6%) as bound at 20% WHC (Figure 5).

**Influence on dissipation**

Results of carbofuran dissipation at different soil moisture contents are presented in Figure 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 (Getzin, 1973; Rajagopal et al., 1984). 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. (2011b) 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.

Our results are in agreement with those cited in the literature. Yen et al. (1997) found that carbofuran dissipated easily at 100% WHC, than at 40 or 60% WHC in clayey and clayey-loamy soils. Kale et al. (2001) reported that insecticide dissipation was quicker under flooded conditions than at 60% WHC in a clay soil. Gorder et al. (1982) observed also a fast carbofuran disappearance rate after important precipitation. Mojasevic et al. (1996) 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 (1995) 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 (Kale and Raghu, 1996). Kale et al. (2001) and Tariq et al. (2006) showed that temperature and moisture contents significantly reduced the half-life values of pesticides in soil. Kazemi et al. (2009) found that carbofuran estimated values of the degradation rates were approximately 40 to 49% lower in dry soil than in wet soil.

Carbofuran kinetic disappearance rates (14C free) followed a first kinetic order for the three rates of soil humidity with correlation coefficients of r²=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 (Siddaramappa et al., 1978). In general, these values were within the reported half-life range of 30 to 120 days by Extoxnet (1996).

Graebing and Chib (2004) 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. (2008) 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. (2001) reported in temperate soils half-life values ranging from 66 to 115.5 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.

Effect of soil sterilization on mineralization and degradation

Mineralisation

To confirm weather carbofuran mineralization is a biotic phenomenon or not, mineralization was measured in natural and autoclaved soil conditions. The results presented in Figure 6, showed that the sterilisation inhibited the mineralization process; 20.3% was mineralized in natural soil against only 1.2% in autoclaved soil condition. This is because of 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. (2010).

Dissipation

Dissipation of carbofuran has been studied in autoclaved and non-autoclaved soils under laboratory condition. The results presented in Figure 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 (Figure 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 (Figure 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. (1984).

Our results are in accordance with Miles et al. (1981) who reported that carbofuran was relatively more persistent in sterile soil than in non sterile soil. Arunachalam and Lakshmanan (1990) reported the degradation of only 20% of the initial dose of carbofuran in sterile soil 60 days of incubation. Getzin and Shanks (1990) 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 (1996) 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. (2008) 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 (Bending and Rodriguez-Cruz, 2007). 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 arise a little period after insecticide application.

**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 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 to further judge on potential impacts of carbofuran fate in the environment.

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