

Microbial, thermal and photodegradation of cadusafos and carbofuran pesticides

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ABSTRACT

Experiments were conducted to investigate the influence of microbial activity, different temperatures, ultraviolet (UV) rays and direct sunlight on the rate of degradation of cadusafos and carbofuran, which are widely used in Egypt as nematicides and insecticides. Microorganisms capable of degrading the two tested pesticides were isolated, using an enrichment technique, and identified as *Pseudomonas sp.*, *Pleurotus sp.* and *Phanerochaete chrysosporium*. *Pseudomonas sp.* was isolated from soil, while *Pleurotus sp.* and *P. chrysosporium* were isolated from mature compost previously treated with the two tested pesticides. Cadusafos half-life values in a liquid mineral medium amended with *Pseudomonas sp.*, *Pleurotus sp.* and *P. chrysosporium* were 29.77, 91.47 and 24.82 days, respectively. On the other hand, carbofuran half-life values in the same medium that amended with the aforementioned microbial strains were 12.7, 16.9 and 17.7 days, consecutively. Thus, *P. chrysosporium* was the most active strain in cadusafos biodegradation and *Pseudomonas sp.* was the most effective strain to degrade carbofuran. Generally, carbofuran was highly biodegradable than cadusafos. Data concerning the role of temperature indicated that increasing half-life values were observed with decreasing the temperature, where the recorded half-life values for cadusafos and carbofuran were 1056.18 & 499.10 hrs at 25 °C; 472.54 & 253.45 hrs at 35 °C and 395.36 & 207 hrs at 45 °C, respectively. As regards UV-rays, results revealed that cadusafos photo-decomposed faster than carbofuran, since their half life values were 7.33 and 16.11 hrs, successively. As respects sunlight, data demonstrated that half-life values of cadusafos and carbofuran were 5.79 and 3.89 hrs, consecutively. It can be observed that sunlight is found to be more effective than UV-rays in

accelerating the photodecomposition of the tested pesticides. Perusal of the early mentioned results clearly showed that degradation rate of the two tested pesticides varied according to their chemical structure, factor causing degradation and time of exposure.

Keywords: Microbial, thermal, photodegradation, cadusafos, carbofuran

INTRODUCTION

The Green Revolution's heavy dependence on pesticides has created different environmental problems. The majority of pesticides after deliberate or inadvertent release enter the soil and water, where they either broken down to simpler forms or remain unaltered for a long time because of their persistent nature, posing a great threat to the ecosystem (Alexander, 1980). Consequently, the efficient disposal of pesticide residues have become of immediate importance in a world faced with increasing pollution. The most promising approach in pesticide disposal is to exploit the catabolic capability of microorganisms.

Microbial degradation of pesticides in the environment is an important route for removal of these compounds. Recent years have witnessed an enormous growth in the controlled practical use of microorganisms to degrade organophosphorus and carbamate pesticides by many investigators (Jauregui *et al.*, 2003; Bano and Musarrat, 2004, Karpouzas *et al.*, 2005 and Singh and Walker, 2006). In Egypt, Pesticides are mainly applied during summer season to control economic pests. During that period, the temperature is high because of the long sunny and uncloudy days. Photodegradation is one of the major transformation processes affecting the fate of pesticides in the environment. Moreover, photodegradation is an abiotic process in the dissipation of pesticides where molecular excitation by absorption of light energy results in various organic reactions (Katagi, 2004) where, the pesticides which having strong absorption in the near ultraviolet (UV) or visible light range can be decomposed directly by sunlight (Keum *et al.*, 2002). It is now well recognized that laboratory studies of the photochemistry of pesticides particularly under sunlight conditions, have a broad significance in relation to their behavior in the environment (Walia *et al.*, 1989). The UV region of sunlight which ranging

from 240-400nm is the primary source of physical means of pesticide degradation (Nandan and Raisuddin, 1992). UV is particularly important in assessing the environmental behaviour of pesticides because when they are sprayed onto plants they form a thin film on the leaves and stems. This film is exposed to sunlight and if the pesticide is UV labile it will quickly breakdown (Shaw and Chadwick, 1998). Several researchers have investigated the degradation of the organophosphorus and carbamate pesticides by temperature (Abd El-Rahman 2005 and Tariq *et al.*, 2006); by ultraviolet rays (Benitez *et al.*, 2002; Hirahara *et al.*, 2003; Zamy *et al.*, 2004 and Eissa, 2005); and by sunlight (Bavcon *et al.*, 2003 and Campbell *et al.*, 2004).

Therefore, the present investigation is an attempt to study the degradation of cadusafos and carbofuran insecticides / nematocides using microbial, temperature, ultraviolet rays and direct sunlight methods.

MATERIALS AND METHODS

1. Pesticides and chemicals used: Analytical grade of cadusafos (S,S -di-sec-butyl O- ethyl phosphoro-dithioate) and carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) with purity of 99 % were provided by Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt and used throughout the studies. All other chemicals were of highest purity commercially available

2. Microbial degradation of the tested pesticides:

2.1. Media: M9 - Minimal Medium as mineral salt medium (MSL) and Luria Bertani Medium (LB) were used through this study as described by (Sambrook *et al.*, 1989) as well as Potato Dextrose Agar (PDA) was used also in the present study.

2.2. Isolation of microorganisms: Enrichment cultures of microorganisms capable of degrading of pesticides were established from two different sources as follows: Firstly, samples of soil were collected from El-Bostan region, El-Behira Governorate, Egypt that previously treated with carbofuran and cadusafos. Secondly, mature compost 6 month old (made from rice straw). Samples (100 g) of soil and mature compost were treated with 100 µg a.i/g of

each pesticide and incubated at 30 °C for 20 days. Just, after 20 days, 10 g from either soil or mature compost were suspended in 90 ml sterilized mineral salt medium in 500 ml bottle containing 100 ppm from each pesticide (carbofuran as a sole source of carbon and nitrogen, but cadusafos as a sole source of carbon), incubated at 30 °C and 150 rpm for 20 days. After that 10 ml of cultures were transferred into fresh 90 ml MSL containing same concentration from either the two pesticides. This procedure was repeated four times. Dilution series were prepared after the final time from enrichment culture in glass tube containing 9 ml MSL liquid medium up to $1:10^{-6}$ and then 100 µl of it was spreaded on plates MSA + carbofuran or cadusafos (100 µg a.i./ml) by using drigalisky triangle. The plates were sealed in polyethylene bags and were incubated at 30 °C for 7 days monitored for appearance of colonies. Single colonies growing on these dilution plates were isolated by picking the colonies using sterile needle inoculation and were further purified by the standard spatial streaking for bacterial isolates on complex agar media or using acidic complex medium or addition of ampicilline 800 mg / l to complex medium for fungal isolates (LB for bacteria isolates and PDA for fungal isolates).

The isolated colonies were then tested for their ability to grow in MSL medium containing (100 µg a.i./ml) of carbofuran as a sole source of carbon or nitrogen or cadusafos as a sole source of carbon. One treatment contained the medium and carbofuran or cadusafos and the other contained the medium and the isolate (no carbofuran or cadusafos as control). The cultures were shaken at 150 rpm and 30 °C for 15 days and then checked for an increasing in the intracellular protein for bacterial isolate and mycelial dry weight for fungal isolates. The bacteria cells were digested as described by Belal (2003) and the protein content was determined according to the method described by Lowry *et al.*, (1951) using bovine serum albumin as standard. The mycelial dry weight for fungal isolates was determined as described by Belal (2003).

2.3. Identification: The selected pesticides degrading bacterial isolates were identified as described by Bergy's Manual of Systematic Bacteriology (1984). Also, the selected pesticides degrading fungal isolates identified according to Anonymous (2003 and 2005).

2.4. Optimization of the cultivation conditions (pH and temperature): 30 ml MSL medium supplemented with 2 % glucose/L for fungal isolates and 1 % for bacterial isolates were used to determine the optimum temperature and pH for the pesticides degrading isolates. MSL medium was inoculated by 1 ml from fungal suspension at 10^4 cfu/ml or bacterial cell suspension at 10^7 cfu/ml, respectively. To determine the optimum pH, experiments were carried out at pH 4, 5, 6, 7 and 8 for fungal isolates and at pH 6, 7 and 8 for bacterial isolates and the cultures were incubated on a rotary shaker at 30 °C and 150 rpm for 7 and 3 days for fungal and bacterial isolates, respectively. Also, to determine the optimum temperature MSL medium at pH 7 was incubated at 20, 30 and 40 °C and 150 rpm for 7 and 3 days for fungal and bacterial isolates, respectively. The growth was determined as mycelial dry weight of biomass (g) after 7 days as described by Belal (2003). It was determined as intracellular protein content (μ g/ml) for bacterial isolates after 3 days where the bacterial cells were digested as described by Belal (2003).

2.5. Biodegradation of the two pesticides in MSL liquid medium by the selected isolates: Selected isolates were cultured onto MSA + carbofuran or + cadasufos for 7 days and then the growing colonies were washed with 3ml sterilized MSL liquid medium. The cell suspension (10^7 cfu/ml for bacterial isolates and 10^4 cfu/ml for fungal isolates) was then used to inoculate 100 ml MSL liquid medium containing (100 μ g a.i/ml) from either carbofuran or cadasufos. The cultures were incubated at 30 °C and 150 rpm for 0, 5, 10, 15 and 20 days. The % degradation and the half-live of each pesticide were determined as described afterward. Control flasks of equal volume of liquid mineral medium and pesticide without any microbial population were run in parallel at all intervals to assess abiotic losses. The growth representing in intracellular protein content for bacterial isolate and mycelial dry weight was determined in each treatment as mentioned above.

2.6. Analytical procedure: A known volume of a liquid mineral medium (80 ml), for each time interval, was transferred into 500 ml separatory funnel and partitioned successively three times with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20 %). The combined extracts for each pesticide were filtered through a pad of cotton and anhydrous sodium sulfate then evaporated to dryness using a rotary evaporator at 30 °C. Residue was

quantitatively transferred to standard glass stopper test tube with ethyl acetate as a solvent, then the solvent was evaporated just to dryness and the residue became ready for chromatographic determination. The residue half-life value (RL_{50}) for each tested pesticide was calculated using the equation of Moye *et al.*, (1987).

3. Thermal and photodegradation of the tested pesticides: Aliquots of the tested pesticides representing 1 ml ethyl acetate containing 100 µg a.i. each were spread as uniformly as possible on the surface of uncovered petri-dishes (5 cm i.d). The ethyl acetate solvent was left to dry at room temperature and then the resulted deposits were divided and subjected to different environmental factors.

3.1. Impact of temperature: Treated petri-dishes were exposed to 25, 35 and 45 °C for 0, 24, 48, 96, 144, 192 and 240 hrs inside a dark electric oven provided with temperature regulating system.

3.2. Impact of UV-rays: Treated petri-dishes were exposed to short wave length of an ultraviolet lamp (254 nm) at a distance of 12cm for 0, 2, 4, 6, 8, 10 and 12 hrs.

3.3. Impact of sunlight: Treated petri-dishes were exposed to direct sunlight for 0, 2, 4, 6, 8, 10 and 12 hrs. Dominating temperature was ranged between 32 and 38 °C.

Residues of the tested pesticides which were remained on exposed surface were quantitatively transferred to standard glass stopper test tubes with ethyl acetate and the solvent was evaporated to dryness and the residues were ready for determination by gas liquid chromatography for cadusafos and high performance liquid chromatography for carbofuran.

4. Chromatographic determination:

4.1. Gas chromatographic (GC) determination for cadusafos: A Hewlett-Packard, USA serial 6890 gas chromatograph equipped with Flame Photometric Detector (FPD) operated in the phosphorus mode (526 nm filter) was used for determination of cadusafos residues under the following

conditions: Column: PAS-1701, 25m length x 0.32mm i.d. x 0.52µm film thickness. Temperature (°C): Detector: 250, Injector: 245, Column: 185. Gases flow (ml / min.): Nitrogen carrier gas: 2.7, Hydrogen: 75, Air: 100. Retention time for cadusafos under these conditions was 2.1min.

4.2. High pressure liquid chromatography (HPLC) determination for carbofuran: Agilent 1100 HPLC equipped with Diode-Array Detector provided with Nucleosil 100-5 C 18 (250 mm x 4.6mm x 5µm) column. Carbofuran was eluted using a mobile phase of acetonitrile + water (65 : 35 v/v) at a flow rate of 1ml min⁻¹ and its concentration was measured by UV absorbance at 220nm. The retention time of carbofuran was 2.7 min.

RESULTS AND DISCUSSION

1-Microbial degradation of the tested pesticides:

1.1. Isolation of the pesticides – degrading isolates: Two different sources were used to isolate the pesticides – degrading isolates in the present study. The first source was soil samples which were collected from El-Bostan region, El-Behira Governorate, Egypt that previously treated with carbofuran or cadusafos. The second source was mature compost 6 months old (made from rice straw). By using enrichment techniques, a total of 15 morphologically different microorganisms capable of degrading of pesticides were isolated from the both described microbial sources (Tables 1 and 2). A preliminary classification based on the morphology of the isolates revealed that the pesticide– degrading organisms belong to the group of bacteria as well as to the group of fungi. Ten bacterial isolates of 15 were isolated from soil samples. Nine of 10 bacterial isolates were gram-negative, motile, rods and were oxidase positive. One of 10 bacterial isolates was gram positive, motile, rods, and spore former. Our results are in agreement with previous findings reported by (Karpouzas *et al.*, 2005) who found that enrichment culture technique led to the isolation of two bacterial strains, which able to degrade cadusafos rapidly in liquid cultures. The application of pesticides promotes the evolution of microorganisms that are capable of degrading these xenobiotic compounds in the soil (Chaudhry and Ali, 1988). On the other hand, 5 fungal isolates of 15 were isolated from mature compost (Table 2). Obviously, fungi play an outstanding role in degrading of the two pesticides in compost, since the

majority of strains belong to this group. It is known that many genera of fungi play an important role in degradation of most agricultural wastes, pesticides and biodegradable plastic (Bumpus and Aust, 1987 and Belal, 2003). Due to the paucity of growth which was generally observed on MSA + carbofuran or cadusafos, the bacterial isolates were also routinely streaked onto plates of LB for bacterial isolates but the fungal isolates were further purified by using acidic complex medium (PDA) or addition of ampicilline 800 mg/l to complex medium (PDA).

Results in Tables 1 and 2 showed that isolates were tested for their growth ability on MSL supplemented with carbofuran as a sole source of carbon and nitrogen or cadusafos as a sole source of carbon. Among 15 isolated isolates, 3 isolates (one bacterial isolate designated as M₁ and two fungal isolates designated as M₂ and M₃) exhibited relative high growth ability on the two pesticides as growth substrate. This indicates that these isolates may be are the highest degradability for the pesticides than the other isolates. The obtained results were compared with the growth of the isolates in MSL (no carbofuran or cadusafos enriched).

Table (1): Growth ability of the isolated bacterial in MSL supplemented with either cadusafos or carbofuran.

Bacterial isolates	Protein concentration (µg/ml)		
	MSL (control)	MSL + carbofuran	MSL + cadusafos
M ₁	35	800	450
B ₄	26	700	398
B ₅	18	690	398
B ₆	21	290	120
B ₇	26	271	118
B ₈	33	240	112
B ₉	23	240	112
B ₁₀	29	201	115
B ₁₁	31	213	118
B ₁₂	29	211	119

Table (2): Growth ability of the isolated fungal in MSL supplemented with either cadusafos or carbofuran.

Fungal isolates	Mycelial dry weight (g)		
	MSL (control)	MSL + carbofuran	MSL + cadusafos
M ₂	0.011	0.18	0.12
M ₃	0.015	0.26	0.22
B ₁₃	0.01	0.12	0.07
B ₁₄	0.01	0.111	0.07
B ₁₅	0.012	0.098	0.06

1.2. Optimization of the cultivation conditions for the pesticides-degrading isolates: Normally, the pH and temperature influence the growth of microorganisms and hence, these factors will influence also the degradation process of the pesticides. Karpouzias and Walker (2000) reported that the degradation of ethoprophos by *Pseudomonas putida* strains epI and II affected by pH and temperature. The question is now, what are the optimal conditions (pH and temperature) for the growth of the pesticides-degrading isolates. To determine the optimal growth conditions, glucose was used as a sole source of carbon instead of the pesticides in MSL liquid medium to abbreviate the incubation time.

- Optimum pH: The influence of pH on biomass yield of the selected isolates is shown in Table 3. Generally, the optimum pH was 7 for all the isolates of either bacteria or fungi. The maximum mycelial dry weight for fungal isolates and intracellular protein content for bacterial isolates were recorded at pH 7. The two fungal isolates grew at quite wide pH from 4 to 7. This variation is very useful to use these isolates in degradation test in different environment at different pH. Therefore, it can expect that these isolates can tolerate the pH change during the degradation process thereby increase the degradation potential for these isolates. However, most of bacterial isolates prefer the neutral pH. This bacterial isolate can grow at range from pH 6-8.

Table (3): Effect of pH on growth of the selected isolates (Table 3A for bacterial isolate, and Table 3B for fungal isolates).

Table 3A			
Isolate	Protein concentration (µg/ml) at different pH values		
	pH ₆	pH ₇	pH ₈
<i>Pseudomonas sp.</i> (M ₁)	650	1200	200

Table 3B					
Isolates	Mycelial dry weight (g) at different pH values				
	pH ₄	pH ₅	pH ₆	pH ₇	pH ₈
<i>Pleurotus sp.</i> (M ₂)	0.11	0.18	0.23	0.28	0.003
<i>P. chrysosporium</i> (M ₃)	0.13	0.17	0.23	0.35	0.004

- **Optimum temperature:** The effect of different temperatures on growth of the fungal and bacterial isolates is shown in Table 4. A temperature of 30 °C appears to be the optimum for growth of either fungal or bacterial isolates. The two isolates M₁ and M₃ exhibited growth at 40 °C but the M₂ did not grow at 40 °C. Therefore, these isolates comprised the bacterial and fungal isolates were used for further studies under the optimum growth conditions with the aim of the determination of the degradation potential for the two tested pesticides after different period time (0, 5, 10, 15 and 20 days).

Table (4): Effect of temperature on growth of the selected isolates (Table 4A for bacterial isolate, and Table 4B for fungal isolates).

Table 4A			
Isolate	Protein concentration ($\mu\text{g/ml}$) at different temperature		
	20°C	30°C	40°C
<i>Pseudomonas sp.</i> (M ₁)	550	1090	240

Table 4B			
Isolates	Mycelial dry weight (g) at different temperature		
	20°C	30°C	40°C
<i>Pleurotus sp.</i> (M ₂)	0.24	0.3	0
<i>P. chrysosporium</i> (M ₃)	0.24	0.38	0.34

1.3. Biodegradation of the two tested pesticides by the selected isolates:

Results in Table (5) show the difference in the degradation rate of cadusafos by the selected isolates. Cadusafos was degraded faster by M₃ than the other two isolates. The degradation potential by M₂ was lower than the other isolates. Cadusafos half-lives were 29.77, 91.47 and 24.82 days by *Pseudomonas sp.* (M₁), *Pleurotus sp.* (M₂) and *P. chrysosporium* (M₃), respectively. The trend of biodegradability was M₃>M₁>M₂. The growth response of cadusafos degrading isolates (representing in protein concentration of intracellular protein for bacterial isolate and mycelial dry weight for fungal isolates) was increased by increasing the cadusafos degradation percentages. In contrast, cadusafos degradation percentage reached up to 10.2 after 20 days of incubation in control treatment. The obtained results showed that *Phanerochaete chrysosporium* (M₃) play an outstanding role in degradation of cadusafos which may be due to the hydrolytic enzymes which excreted by many microorganisms, particularly fungi and these enzymes are capable of hydrolyzing pesticides (Bollag and Liu, 1990). The different roles of fungi and bacteria in pesticide degradation, as previously described, could be explained as follows: Degradation by fungal enzymes may be due to less specific enzymes, as in the case of the lignin-degrading enzyme system and other hydrolytic enzymes (Bumpus *et al.*, 1985). The ability of the lignin-degrading system of the fungus *P. chrysosporium* to degrade pesticides and xenobiotic chemicals was demonstrated by Ryan and Bumpus, (1989) and Kennedy *et al.*, (1990). Moreover, Masaphy and Levanon (1992) reported that the lignocellulolytic system of *Pleurotus sp.* differs from that of *P. chrysosporium*, and it does not contain the enzyme lignin peroxidase.

Table (5): Biodegradation of cadusafos by the selected microorganisms and their growth response.

Time of incubation (days)	Cadusafos (ug)										
	Control		<i>Pseudomonas sp</i> (M ₁)			<i>Pleurotus sp</i> (M ₂)			<i>P. chrysosporium</i> (M ₃)		
	ug	% Loss	ug	% Loss	Protein conc. (ug/ml)	ug	% Loss	Mycelial dry weight (g)	ug	% Loss	Mycelial dry weight (g)
0	100	0.0	100	0.0	30	100	0.0	0.01	100	0.0	0.025
5	100	0.0	98.7	1.3	100	100	0.0	0.03	95.0	5.0	0.09
10	98.1	1.9	72.9	27.1	250	95.7	4.3	0.07	84.0	16.0	0.14
15	95.2	4.8	63.3	36.7	400	92.6	7.4	0.09	64.5	35.5	0.20
20	89.8	10.2	62.8	37.2	550	85.9	14.1	0.11	57.2	42.8	0.24
RL ₅₀ (days)	128.8		29.77			91.47			24.82		

The degradation patterns of carbofuran and the growth response of *Pseudomonas sp.* (M₁), *Pleurotus sp.* (M₂) and *Phanerochaete chrysosporium* (M₃) isolates in the MSL are shown in Table (6). Degradation of carbofuran was initially slow where 20, 16.9 and 17.9 % were degraded by *Pseudomonas sp.* (M₁), *Pleurotus sp.* (M₂), and *P. chrysosporium* (M₃), 5 days after inoculation, respectively. This slow degradation accompanied by a gradual increase in growth of the microorganisms. About 86.4, 69.9 and 69.3% from carbofuran were degraded by *Pseudomonas sp.* (M₁), *Pleurotus sp.* (M₂) and *P. chrysosporium* (M₃) after 20 days, respectively, with half-lives of 12.7, 16.9 and 17.7 days, respectively. On the other hand, the percent of carbofuran degradation reached to 28.8 at the end of incubation time in control or non-inoculated samples. The trend of biodegradability of carbofuran was M₁>M₂>M₃ indicating that *Pseudomonas sp.* (M₁) play an important role in carbofuran degradation which may be attributed to secretion of carboxylesterase enzyme (Barik *et al.*, 1984). Turco and Konupka (1990) reported that the biodegradation of carbofuran in the soil is a two-step process:

Table (6): Biodegradation of carbofuran by the selected microorganisms and their growth response.

Time of incubation (days)	Carbofuran (ug)										
	Control		<i>Pseudomonas sp.</i> (M ₁)			<i>Pleurotus sp.</i> (M ₂)			<i>P. chrysosporium</i> (M ₃)		
	ug	% Loss	ug	% Loss	Protein conc. (ug/ml)	ug	% Loss	Mycelial dry weight (g)	ug	% Loss	Mycelial dry weight (g)
0	100.0	00.0	100.0	00.0	32	100.0	00.0	0.013	100.0	00.0	0.02
5	96.1	3.9	80.0	20.0	200	83.1	16.9	0.10	82.1	17.9	0.12
10	90.7	9.3	75.0	25.0	480	81.0	19.0	0.14	74.9	25.1	0.19
15	82.3	17.7	26.1	73.9	740	61.7	38.3	0.17	71.2	28.8	0.27
20	71.2	28.8	13.6	86.4	850	30.1	69.9	0.20	30.7	69.3	0.30
RL ₅₀ (days)	40.80		12.70			16.90			17.70		

hydrolysis of the carbonyl side chain followed by the adsorption of the primary metabolite, causing a decrease in ring breakdown. Previous studies by Karpouzias *et al.*, (2000) isolated two ethoprophos-degrading *P. putida* strains, which capable to degrade cadusafos but in a less efficient way compared to the *Flavobacterium* and *Sphingomonas* strains. *Flavobacterium* strains have been

reported to be responsible for the degradation of carbofuran (Chaudhry and Ali, 1988). Several studies have also reported that the isolation of *Sphingomonas* strains which was able to mineralize rapidly carbofuran (Feng and Ogram, 1997). An *Achromobacter sp.* that is capable of hydrolyzing carbofuran has been also reported (Karns *et al.*, 1986).

More potent isolates that degraded carbofuran and cadusafos rapidly were obtained from a soil or mature compost samples to which carbofuran or cadusafos had been applied or exposed for several years or the time an enrichment technique. This indicates that repeated applications or exposure of soil or mature compost to xenobiotic compounds for a long period of time can result in the evolution of microorganisms capable of degrading these compounds rapidly and more extensively. From the described results it can be concluded that carbofuran was degraded faster by these selected isolates than cadusafos which are in coincidence with Smelt *et al.*, (1987) who stated that there is only limited evidence for the enhanced biodegradation of cadusafos in soil. Obviously, the two isolates *Pseudomonas sp.* (M₁) and *Phanerochaete chrysosporium* (M₃) play an important role in the biodegradation of the tested two pesticides.

2. Effect of different temperature degrees on degradation of the tested pesticides:

2.1. Cadusafos pesticide: Data in Table (7) demonstrated that the loss percentages of cadusafos residues were 00.00, 00.00, 1.69, 9.04, 11.62 and

Table (7): Effect of different temperature degrees on degradation of cadusafos

Time of exposure (hrs)	25 °C		35 °C		45°C	
	µg nematocide	% Loss	µg nematocide	% Loss	µg nematocide	% Loss
0	100.00	00.00	100.00	00.00	100.00	00.00
24	100.00	00.00	100.00	00.00	100.00	00.00
48	100.00	00.00	99.70	00.30	99.10	00.90
96	98.31	1.69	90.99	9.01	87.31	12.69
144	90.96	9.04	85.37	14.63	80.22	19.78
192	88.38	11.62	79.56	20.44	75.63	24.37
240	85.43	14.57	70.33	29.67	65.66	34.34
RL ₅₀ (hrs)	1056.18		472.54		395.36	

14.57 % after 24, 48, 96, 144, 192 and 240 hrs of exposure to 25 °C, respectively. The corresponding values when cadusafos was exposed to 35 °C for the aforementioned periods were 00.00, 00.30, 9.01, 14.63, 20.44 and 29.67 %, respectively. On the other hand, the loss percentages were 00.00, 00.90, 12.69, 19.78, 24.37 and 34.34 % after exposure to 45 °C at the above mentioned intervals, consecutively. The half-life values increased with the decreasing of temperatures, where the calculated half-life values of this compound were 1056.18, 472.54 and 395.36 hrs at 25, 35 and 45 °C, respectively.

2.2. Carbofuran pesticide: Data in Table (8) clearly revealed that the rate of degradation of carbofuran was influenced by temperature degrees and period of exposure. The loss percentages of carbofuran residues were 00.00, 00.94, 3.19, 15.35, 23.01 and 28.34 % after 24, 48, 96, 144, 192 and 240 hours of exposure at 25 °C, respectively. The corresponding values when carbofuran was exposed to 35 and 45 °C for the same aforementioned periods were (9.41, 12.38, 18.03, 21.54, 34.50 & 48.12 %) and (10.60, 19.66, 22.09, 29.02, 40.69 & 69.54 %), respectively. Therefore, the data showed that increasing in temperature enhanced the degradation of carbofuran. The calculated half-life values of this pesticide were 499.10, 253.45 and 207.00 hrs at 25, 35 and 45 °C, respectively.

Table (8): Effect of different temperature degrees on degradation of carbofuran

Time of exposure (hrs)	25 °C		35 °C		45 °C	
	μg nematocide	% Loss	μg nematocide	% Loss	μg nematocide	% Loss
0	100.00	00.00	100.00	00.00	100.00	00.00
24	100.00	00.00	90.59	9.41	89.40	10.60
48	99.06	00.94	87.62	12.38	80.34	19.66
96	96.81	3.19	81.97	18.03	77.91	22.09
144	84.65	15.35	78.46	21.54	70.98	29.02
192	76.99	23.01	65.5	34.50	59.31	40.69
240	71.66	28.34	51.88	48.12	30.46	69.54
RL ₅₀ (hrs)	499.10		253.45		207.00	

Perusal of the early mentioned data in Tables (7 and 8) obviously showed that the persistence rate of the two tested pesticides was influenced by three factors, i.e. type of the pesticide, temperature and period of exposure. Moreover, such noticeable reductions of the two tested pesticide residues could be due to firstly, volatilization (influenced by the pesticide's volatility or vapor pressure and temperature) and secondly to the chemical degradation (influenced by the molecular make up of the pesticide and by temperature) (Fong *et al.*, 1999). The obtained results are in coincidence with those previously reported by many investigators (Hegazy *et al.*, 2001; Nasr 2001; Soltan 2002; Abd El-Rahman 2005 and Tariq *et al.*, 2006) who confirmed the role of temperature in increasing the degradation of pesticide residues.

In general, cadusafos suffered less loss than carbofuran pesticides when exposed to the different temperature degrees within period of the experiment. So, from the practical point of view in the pest control program, it can be recommended to use cadusafos in area of dominant high temperature during the controlling season and vice versa for carbofuran.

3. Photo degradation of the tested pesticides

3.1. Effect of short ultra-violet rays on degradation of the tested pesticides: Data in Table (9) clearly showed that the rate of degradation of the two tested pesticides exposed as thin film in uncovered petri dishes to irradiation of UV (254 nm) varied according to their chemical structure as well as time of exposure. Cadusafos photo-decomposed faster than that of carbofuran in this respect and more pronounced by the lapse of exposure time. The loss percentages for cadusafos and carbofuran were 24.34 and 0.0 % after two hrs of exposure to UV-rays, respectively. The decomposition percentages of cadusafos increased to 36.79, 40.66, 56.51, 60.39 and 69.75 % after 4, 6, 8, 10 and 12 hrs of exposure to UV-rays, respectively. While these values were 9.30, 29.12, 32.57, 36.76 and 40.33 %, when carbofuran were exposed to UV-rays for the above mentioned periods, respectively. The residue half-life values were 7.33 and 16.11 hrs for cadusafos and carbofuran, respectively.

Generally, it was found that photo degradation is positively correlated with the exposure time. Enhancement of photo degradation by UV-rays may be attributed to the interaction between the pesticide molecule and UV-light

(Santoro *et al.*, 2000). The absorption of electromagnetic radiation by a molecule depends on a correspondence between the radiation energy and the energy of certain molecular transitions. The energy associated with ultraviolet and visible light is sufficient to excite electrons in molecules. The molecules are said to be excited from their ground states to electronically excited states. In many cases, the energies imparted to compounds by such photo-excitation are similar to covalent bond energies and can initiate chemical reactions. The typical high-energy limit available for most photochemical processes is near 143 KCal/mol (598 KJ/mol). That corresponds to a lower wave length limit of about 200 nm for effective transmission of light through air. The energy associated with the wave length 250 nm of the light absorbed is 114.4 KCal/mol, this amount of energy exceeds most bond energies. Therefore, most photochemical reactions are induced with the UV-light. On the other hand, the energy associated with the wavelength 350 nm of the light absorbed is 81.7 KCal/mol. (Pine *et al.*, 1981). These results are in harmony with those previously obtained by Nasr (2001) on carbofuran and ethoprophos; Benitez *et al.*, (2002) on carbofuran; Zamy *et al.*, (2004) on isofenfos and by Eissa (2005) on diazinon, chlorpyrifos-methyl and profenofos.

Table (9): Effect of short ultra-violet rays (254 nm) on the degradation of cadusafos and carbofuran.

Time of exposure (hrs)	Cadusafos		Carbofuran	
	μg nematicide	% Loss	μg nematicide	% Loss
0	100.00	00.00	100.00	00.00
2	75.66	24.34	100.00	00.00
4	63.21	36.79	90.70	9.30
6	59.34	40.66	70.88	29.12
8	43.49	56.51	67.43	32.57
10	39.61	60.39	63.24	36.76
12	30.25	69.75	59.67	40.33
RL ₅₀ (hrs)	7.33		16.11	

3.2. Effect of sunlight on degradation of the tested pesticides: The photolytic behavior of the tested pesticides as thin film on petri dishes when exposed to natural sunlight was presented in Table (10). The loss percentages for cadusafos and carbofuran were 29.66 and 47.66 %, respectively after two hours of exposure to direct sunlight. The decomposition percentages for carbofuran rapidly increased to 50.95, 66.31, 77.33, 89.49 and 93.66 % after 4, 6, 8, 10 and 12 hrs of exposure to direct sunlight, successively, while the corresponding values of cadusafos were 41.71, 51.21, 63.39, 66.21 and 74.33 %, respectively at the aforementioned intervals of exposure. The calculated half-life values proved the rapid disappearance of carbofuran as compared with cadusafos, i.e. 3.89 and 5.79 hrs, consecutively. Perusal of the data demonstrated that the type of the pesticide and the period of exposure to sunlight play a very critical role in the nonenzymatic degradation process of the tested pesticides. The obtained results are in accordance with the findings of Chukwudebe *et al.*, (1989) on five different phosphorothioate insecticides; Cabras *et al.*, (1998) on diazinon and phosalone; Scholz and Reinhard (1999) on imidacloprid; Bavcon *et al.*, (2003) on diazinon and Campbell *et al.*, (2004) on carbofuran.

Table (10): Effect of sunlight on the degradation of cadusafos and carbofuran.

Time of exposure (hrs)	Cadusafos		Carbofuran	
	μg nematicide	% Loss	μg nematicide	% Loss
0	100.00	00.00	100.00	00.00
2	70.34	29.66	52.34	47.66
4	58.29	41.71	49.05	50.95
6	48.79	51.21	33.69	66.31
8	36.61	63.39	22.67	77.33
10	33.79	66.21	10.51	89.49
12	25.67	74.33	6.34	93.66
RL ₅₀ (hrs)	5.79		3.89	

From the early mentioned results, it can be observed that sunlight was found to be more effective than UV-rays in accelerating the photodecomposition of the tested pesticides. This may be due to thermal evaporation and light intensity considerations. An extraordinary wide variety of reactions of organic compounds are known to occur under the influence of visible and ultraviolet light (Roberts and Caserio, 1967). Under sunlight conditions, residues of the pesticides are rapidly degraded even under low light intensity conditions and it can be expected that degradation rates would be greater with greater exposure to light, greater angle of incidence of the sun, low latitudes and longer periods of day light (Scholz and Reinhard, 1999). The radiation energy of the sunlight might absorb by a pesticide molecule principally at a given wave length. The energy might increase the transitional, rotational, vibrational or electronic energy of the molecule. If enough energy was absorbed to interact with the electrons of the molecules, an electronically excited molecule would result. Energy might disappear or changed from the molecule in a number of ways, one of which is chemical reaction (Plimmer, 1970).

It can be concluded that degradation rate of the two tested pesticides varied according to their chemical structure, pH, temperature and time of exposure. Furthermore, photodegradation through sunlight was the most effective method in degradation of the tested pesticides. On the other hand, cadusafos and carbofuran are fairly mobile pesticides whose residues have been detected in subsoil and finally reached to underground water (Papadopoulou, 1994). These habitats are rich in microorganisms and microbial activity occurs even in those niches which are inaccessible to sunlight. Further studies are required to investigate the ability of the efficient isolates to degrade these tested pesticides residues when introduced back into the highly competitive soil environment.

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