EFFICIENCY OF SOME ISOLATED SOIL MICROORGANISMS FOR CARBOFURAN PESTICIDE DEGRADATION

ABSTRACT

The aim of this study concerns the use of locally isolated microorganisms in bioremediation the polluted soil with pesticides. Therefore, the study included isolation and identification of some microorganisms from treated soil increasingly by carbofuran and tested for their tolerance and ability to degrade carbofuran which is extensively used under Egyptian Agricultures against many pests. Obtained results could be summarized as follows:

Only two kinds of the isolated microorganisms were able to grow and withstand the toxicity of carbofuran. These microorganisms were identified as *Streptomyces violaceusniger* and *Azospirillum brasilense*. Results also indicated that carbofuran dissipation rate in culture of *A.brasilense* was faster than that in *S.violaceusniger* one, since only 7.5 and 10.5% of the applied carbofuran were detected by GLC in the culture of *A.brasilense* and *S.violaceusniger*, respectively at the end of experiment (21 days). *S.violaceusniger* was more efficient in biodegradation of carbofuran than *A.brasilense* since, six metabolites were detected in *S.violaceusniger* culture by GLC and TLC, three of them were identified as carbofuran phenol, 3-hydroxy carbofuran and 3-keto carbofuran, whereas the other three metabolites could not be identified. *A.brasilense* degraded carbofuran to only three metabolites, two of them were identified as carbofuran phenol and 3-keto carbofuran, while the third one could not be identified. Moreover, carbofuran dissipation rate in cultivated soil with tomato and treated with carbofuran was higher in case of inoculation with the mixture of *S.violaceusniger* and *A.brasilense* than that inoculated with each one individually. Therefore, soil inoculation with either *S.violaceusniger*, *A.brasilense* or a mixture of them is almost important for biodegradation of carbofuran and removing its residues from the polluted soil.

INTRODUCTION

Pesticides are applied in wide-spread for more than 50 years ago either by spraying the plants or directly in or on the soil surface. Recently, the application of pesticides in a granular form is practiced to facilitate the application of pesticides and to give rapid and good control of soil pests or insect stages.

Generally, the use of pesticides has greatly contributed to crop production. Despite of this success, harmful side effect of pesticides using were observed such as toxicity to human and environment pollution **(Racke & Coats, 1990; Cogger *et al*, 1998; Andrea *et al*, 2000** and **Garcia *et al*, 2001).** The accumulation of pesticides in soil leads to ground water pollution and inhibition of beneficial soil microorganisms **(Das *et al*, 1995; Yen *et al*, 1997; Das & Mukherjee, 2000; Omar & Abdel-Sater, 2001** and **Singh *et al*, 2002)**. Now, it is generally accepted that adaptation of soil microorganisms are necessary for the rapid degradation of pesticides in soils **(Levanon, 1993; Soudamini *et al*, 1997; Karpouzas & Walker, 2000; Trabue *et al*, 2001** and **Megharaj *et al*, 2003)**. The bioremoval of pesticides residues from the environment by microorganisms has become an important area of research.

Therefore, the present investigation was carried out to isolate some microorganisms from Egyptian soil able to degrade carbofuran to be used as inocula to remove its residues from the polluted soil.

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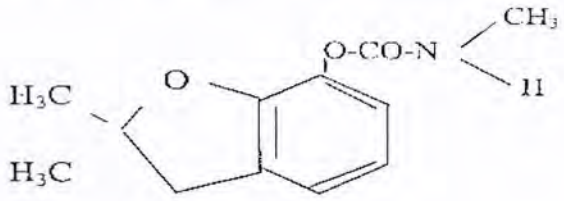
MATERIALS AND METHODS

Carbofuran (furadan): (2,3 dihydro-2,2dimethyl-7benzofuranyl N-methylcarbamate).



Carbofuran

Isolation and identification of microorganisms.

To isolate of microorganisms from the soil able to degrade carbofuran, the method of **Venkateswarlu *et al* (1977)** was used. The microorganisms capable of growing with carbofuran and used it as a sole source of carbon and/or nitrogen were isolated and purified. Mineral salts medium **(Venkateswarlu *et al*, 1977)** was used for isolation process.

The two isolates which confirmed their ability to degrade and utilize carbofuran as a sole source of carbon and nitrogen were purified and subjected to detail morphological and physiological characteristics according to **Bergey's Manual of Determinative Bacteriology (1994)** and were identified as *Streptomyces violaceusniger* and *Azospirillum brasilense.*

Tolerance of the two microorganisms for carbofuran.

The identified strains were tested for their ability to grow in presence of different concentrations of carbofuran namely zero, 50, 100, 150, 200 and 250 ppm.

Inocula preparation.

For preparation of *S.violaceusniger and A.brasilense* inocula, starch nitrate agar medium **(Waksman and Lechevalier, 1961)** and semi-solid malate medium **(Dobereiner, 1978)** were inoculated with *S.violaceusniger and A.brasilense*, respectively then incubated at 33°C and 30°C for 10 and 3 days, respectively.

Biodegradation of carbofuran by isolated and identified strains.

Streptomyces violaceusniger and *A.brasilense* were tested for their ability to degrade carbofuran. Mineral salts solution supplemented with carbofuran at a rate of either 100 or 50 ppm was inoculated with standard inocula from spore suspension of *S.violaceusniger* (1 ml contains about 166 x 10^3 spore/ml) and *A.brasilense* (1 ml contains about $3x10^6$ viable cells), then incubated on a rotary shaker (200 rpm) for 21 days at 33 and 30°C for *S.violaceusniger and A.brasilense,* respectively. Medium supplemented with carbofuran alone without inoculation was used as a control. Samples were taken at zero, 3, 7, 11, 15 and 21 days of incubation to determine the densities of *Streptomyces*, *Azospirillum,* the remained carbofuran and its by products.

Efficiency of the isolated and identified strains to degrade carbofuran in cultivated soil with tomato.

A pot experiment was conducted to study the persistence rate and biodegradation of carbofuran under tomato plants. Erthern pots 25cm in diameter were filled with sandy clay loam soil (about 4 kg/pot) and amended with NPK fertilizers at recommended rates and planted with tomato seedlings (two/pot). The pots were irrigated with tap water every two days, then were divided into two groups (48 pots for each). The first were treated with carbofuran at the recommended rate (1g/pot) at the 15^{th} day of planting and kept for 15 days, then divided into 4 portions for treatment as follows:

a) Twelve pots kept without inoculation (control 1).

b) Twelve pots were inoculated with 15 ml/pot of *S.violaceusniger* culture ($130x10^5$ spores/ml) 7 days-old.

c) Twelve pots were inoculated with 15 ml/pot of *A. brasilense* culture ($344 x10^7$ viable cells/ml)48 hrs-old.

d) Twelve pots were inoculated with 15 ml/pot of both *S.violaceusniger and A.brasilense* cultures.

The second group were treated with carbofuran at the double recommended rate (2 g/pot) and also divided into 4 portions to be treated as previously described in the first group. Beside that, a group of planted pots was left without any treatment (no carbofuran addition and no inoculation) as control 2. Soil samples were taken at zero, 15 , 30, 45 and 60 days of inoculation with the abovementioned strains for the following assessments:

1-Total bacteria and actinomycetes densities. 2- Soil dehydrogenase activity.

3-Persistence amounts of carbofuran and its by-products.

Analysis methods.

Carbofuran was extracted and cleaned-up from the liquid cultures and soil according to the methods described by **Venkateswarlu *et al* (1977)** and **Racke & Coats (1990)**, respectively. Carbofuran and its metabolites were determined by GLC, TLC and Gas/Mass spectrometer. Densities of streptomyces in liquid culture were determined on starch nitrate agar medium **(Waksman and Lechevalier, 1961)**. Whereas, densities of *Azospirillum* were determined on semi-solid malate medium **(Dobereiner, 1978)** Total bacterial count in soil was determined by using soil extract agar medium according to **Holm and Jenseon (1972)**. While, actinomycetes count in soil was determined by using starch nitrate agar medium according to **Waksman and Lechevalier (1961).** Also, dehydrogenase activity in soil was assayed according to **Casida *et al* (1964)**.

RESULTS AND DISCUSSION

Isolation and identification of carbofuran degrading microorganisms.

Only two isolates of microorganisms were able to grow and withstand the toxicity of carbofuran. These two isolates could identified as *S.violaceusniger* and *A.brasilense*.

Tolerance assessment of *S.violaceusniger and A.brasilense* to carbofuran.

Data given in Table (1) revealed that *S.violaceusniger* normally grows in the liquid culture containing 50ppm carbofuran and could tolerate its concentration up to 100 ppm. When the concentration was increased to 150 or 200 ppm., *S.violaceusniger* could withstand its toxicity.

Whereas, increasing the concentration more than 200 ppm was accompanied with toxic effect. On the other hand, *A.brasilense* is susceptible to carbofuran than *S.violaceusniger*, since it could normally grow in the liquid culture containing 50ppm carbofuran and could withstand toxicity up to 100 ppm but, when the concentration was increased more than 100 ppm it caused toxic effect on the growth.

Table 1. Tolerance of *S.violaceusniger and A.brasilense* to different concentrations of carbofuran.

Concentrations / Strains	Growth in presence of carbofuran					
	0	50	100	150	200	250
S. violaceusniger	3+	3+	2+	+	+	-
A. brasilense	3+	3+	+	-	-	-

Analysis of technical carbofuran and its metabolites.

Thin Layer Chromatography, Gas Liquid Chromatography and Gas/Mass spectrometer were used to determine the values of Rf, Rt and M/e of each compound to be as reference to compare these values with those obtained from samples analysis. The limited data are presented in Table (2) and shown in Figs (1,2 and 3).

Table 2. Data analysis of carbofuran and its metabolites.

Compounds	TLC (Rf values)	GLC (Rt min.)	M/e	Molecular formula
Carbofuran	0.45	1.87	221	$C_{12}H_{15}NO_3$
Carbofuran phenol	0.92	0.87	164	$C_{10}H_{12}O_2$
3-OH- carbofuran	0.48	1.60	237	$C_{12}H_{15}NO_4$
3-Keto- carbofuran	0.38	2.35	235	$C_{12}H_{13}NO_4$
3-Keto carbofuran phenol	0.65	1.00	178	$C_{10}H_{10}O_3$

Fig 1. Thin layer chromatography of carbofuran and its metabolites
1; 3-OH-carbofuran. 4; 3-Keto- carbofuran.
5; carbofuran. 6; 3-keto- carbofuran-phenol.

Periodical changes of microbial densities and carbofuran persistence rate in liquid cultures.

Data in Table (3) show that *S.violaceusniger* densities progressively increased and reached to a high peak at 7th day and then declined until the end of the experiment. This result indicate that *S.violaceusniger* could withstand the toxicity of carbofuran and used it as a sole source of carbon and nitrogen and when its concentration decreased in the medium the densities were declined. Whereas, *A.brasilense* densities progressively decreased until the 11 days of experiment, then increased at 15th day and decreased thereafter. This result indicates that *A.brasilense* is more susceptible to carbofuran than *S.violaceusniger* and this confirms the results previously obtained in the tolerance assessment. After that, *A.brasilense* became adapted to carbofuran and used it as a sole source of carbon and nitrogen. While, at the end of the experiment (21 days) the densities were declined as a result of carbofuran amount decrease.

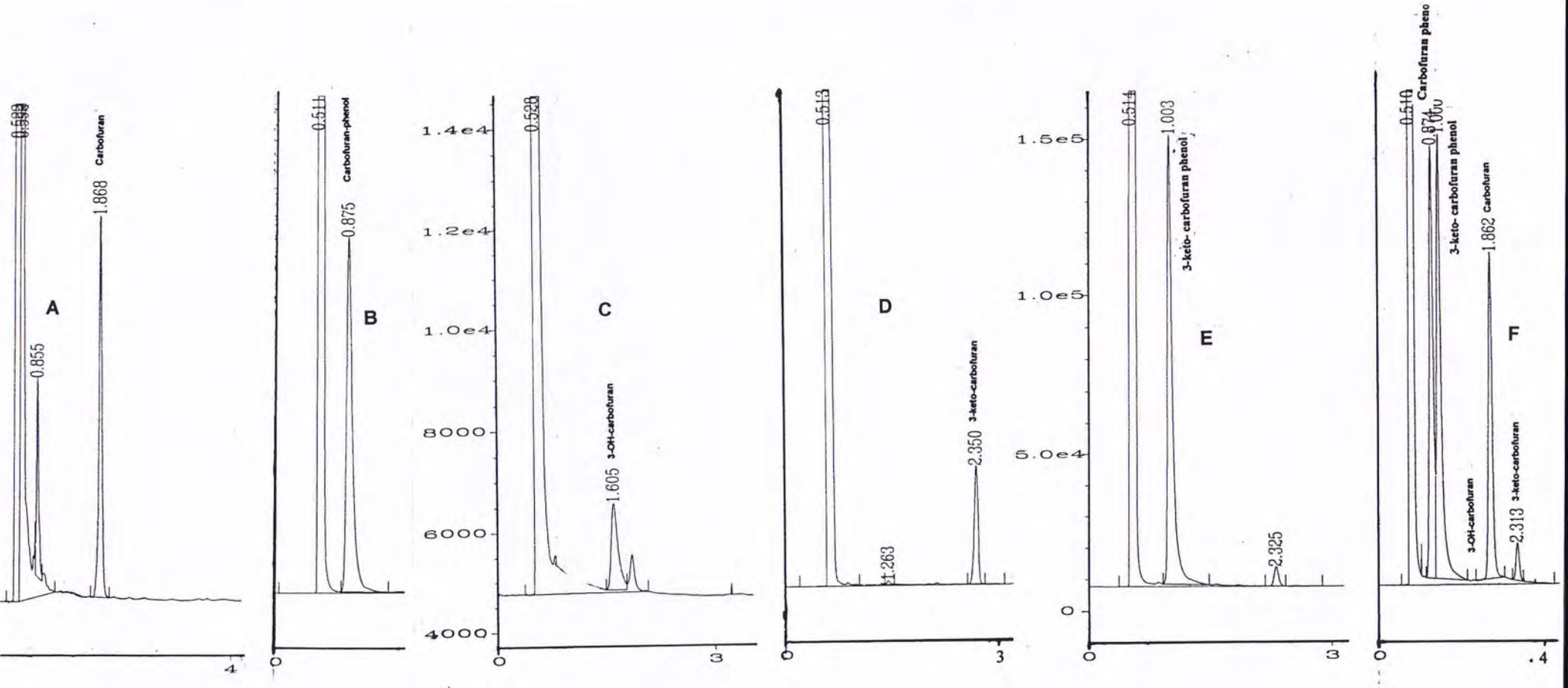


Fig 2. Gas chromatographic spectra of
A) Technical carbofuran B) carbofuran phenol. C) 3-hydroxy carbofuran
D) 3-keto-carbofuran E) 3-keto-carbofuran phenol
F) Mixture of carbofuran and its available metabolites.

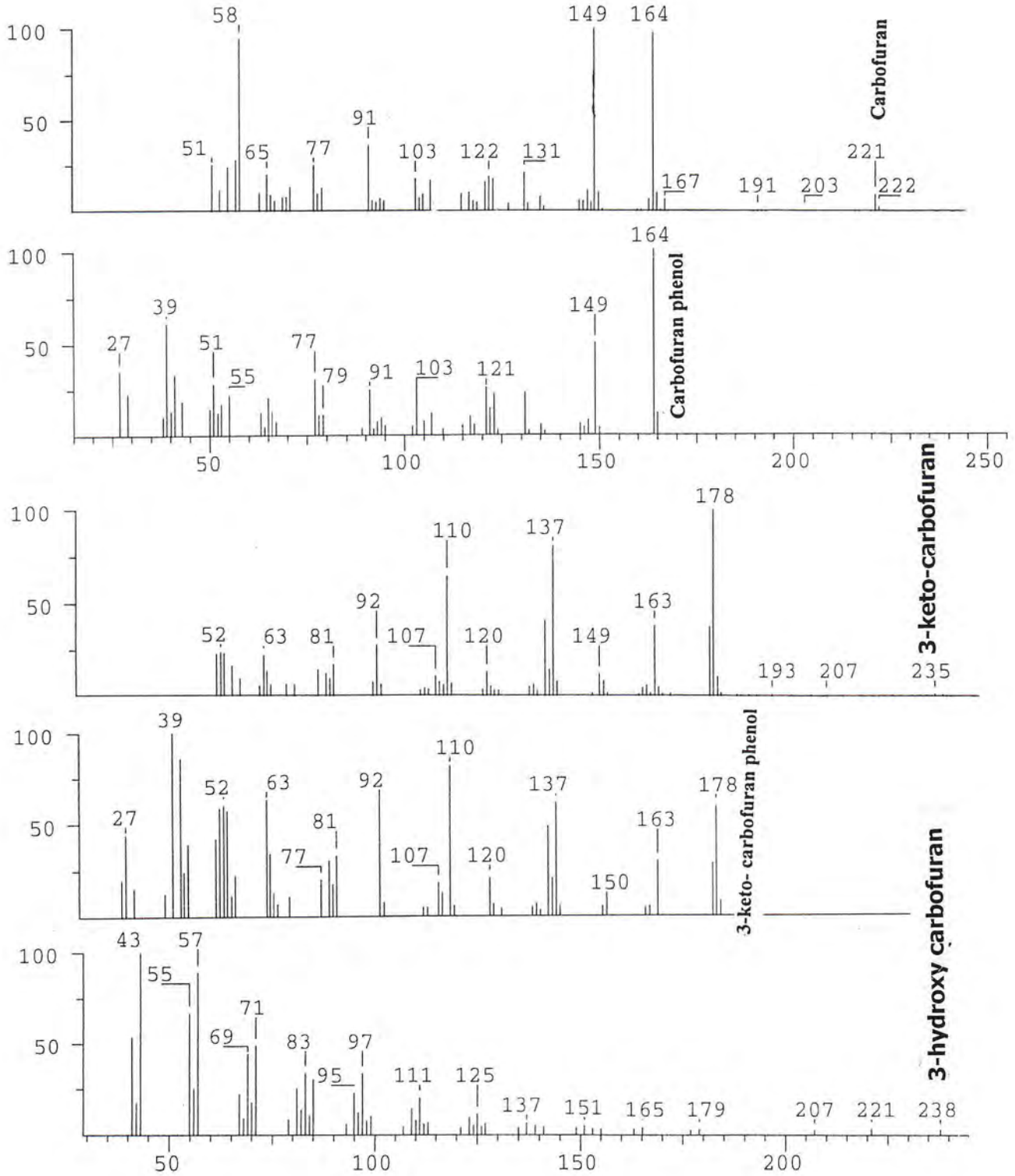


Fig 3. Mass spectrum of carbofuran and its metabolites

Table 3. Microbial densities and persistence rate of carbofuran in uninoculated and inoculated media with *S.violaceusniger and A.brasilense*.

Days after addition	Microbial densities of		Persistence rate of carbofuran (%)			
	S. violaceusniger	*A .brasilense*	*S. violaceusniger*		*A .brasilense*	
	($x10^5$ /ml)	($x10^7$/ml)	Uninoculated medium	Inoculated medium	Uninoculated medium	Inoculated medium
0	16.6	30.0	100	100	100	100
3	17.0	22.1	95.6	69.5	95.1	72.0
7	19.0	6.0	80.4	51.1	85.6	40.8
11	5.20	5.0	75.8	22.5	76.3	22.2
15	2.70	35.0	51.8	18.9	51.2	12.3
21	0.40	14.0	44.2	10.5	42.8	7.50

Data in Table (3) also show that carbofuran amounts decreased with elapsed time in both uninoculated and inoculated media. Dissipation rate of carbofuran in inoculated media was faster than that in uninoculated ones. Since, 24.2, 77.5 and 23.7, 77.8% of the added carbofuran were disappeared from the uninoculated and inoculated media with *S.violaceusniger and A.brasilense*, respectively throughout the first 11 days. At the end of the experiment (21 days), only 44.2, 10.5 and 42.8, 7.5% of the added carbofuran were detected in uninoculated and inoculated media with *S.violaceusniger and A.brasilense,* respectively. These results indicate that the ability of carbofuran to biologically hydrolyze via the effect of microorganisms. These results are in harmony with **Soudamini *et al* (1997), Karpouzas & Walker (2000)** and **Trabue *et al* (2001)** who found that carbamate pesticides were degraded in the media inoculated with either soil suspension or soil microorganisms faster than those in the uninoculated ones.

Biodegradation of carbofuran by *S.violaceusniger* and *A.brasilense* in liquid cultures.

Data illustrated in Figs (4,5,6 and 7) revealed that *S.violaceusniger* began to degrade carbofuran after the first three days of incubation. However, the products of carbofuran degradation were more demonstrated after 15 days of incubation. This is probably due to high potential of *S.violaceusniger* strain to degrade carbofuran as time proceeded. The chromatographic analysis of the extracts showed only one metabolite having (Rt 0.87 minute and Rf value 0.92) on GLC and TLC, respectively which appeared at the third day of incubation and still detected up to the end of the experiment. This metabolite could be identified as carbofuran phenol.

After 15 days, five metabolites were detected by GLC having Rt 0.79, 0.87, 1.11, 1.53 and 1.74 minutes, three of them were identified as carbofuran phenol (Rt 0.87), 3-keto carbofuran phenol (Rt 1.1) and 3-OH-carbofuran (Rt 1.53), the other two compounds could not be identified. Those metabolites were detected by GLC until the end of the experiment (21 days) but only two compounds were detected by TLC having Rf values 0.33 and 0.52. This result may be due to their finding with small amounts as far as they could not be detected by TLC. However, GLC analysis is known with its high sensitivity than thin layer chromatography. Identification of these metabolites was carried out by comparing their Rf and Rt values with those obtained by pure carbofuran and its metabolites which recorded in (Table, 2) and the corresponding compounds were recorded.

Generally, it can be concluded that the *S. violaceusniger* strain is able to degrade carbofuran to six compounds, three of them were identified as carbofuran phenol; 3-OH-carbofuran; 3-keto- carbofuran phenol and the three other metabolites were still unidentified. Regarding the chromatographic analysis of *A.brasilense* liquid culture extracts, the chromatograms showed that three metabolites were detected, two of them were identified as carbofuran phenol, 3-keto- carbofuran phenol and the third one remained unidentified. This compound could only detected by GLC at Rt of 0.83 and not detected by TLC.

Fig 4. Thin layer chromatography of liquid culture extract of *Streptomyces violaceusniger* after different intervals from amendment with carbofuran.

Fig 5. Thin layer chromatography of liquid culture extract of *Azospirillum brasilense* after different intervals from amendment with carbofuran.
St; standard carbofuran 1; Zero time 2; 3 days 3; 7 days 4; 11 days 5; 15 days 6; 21 days

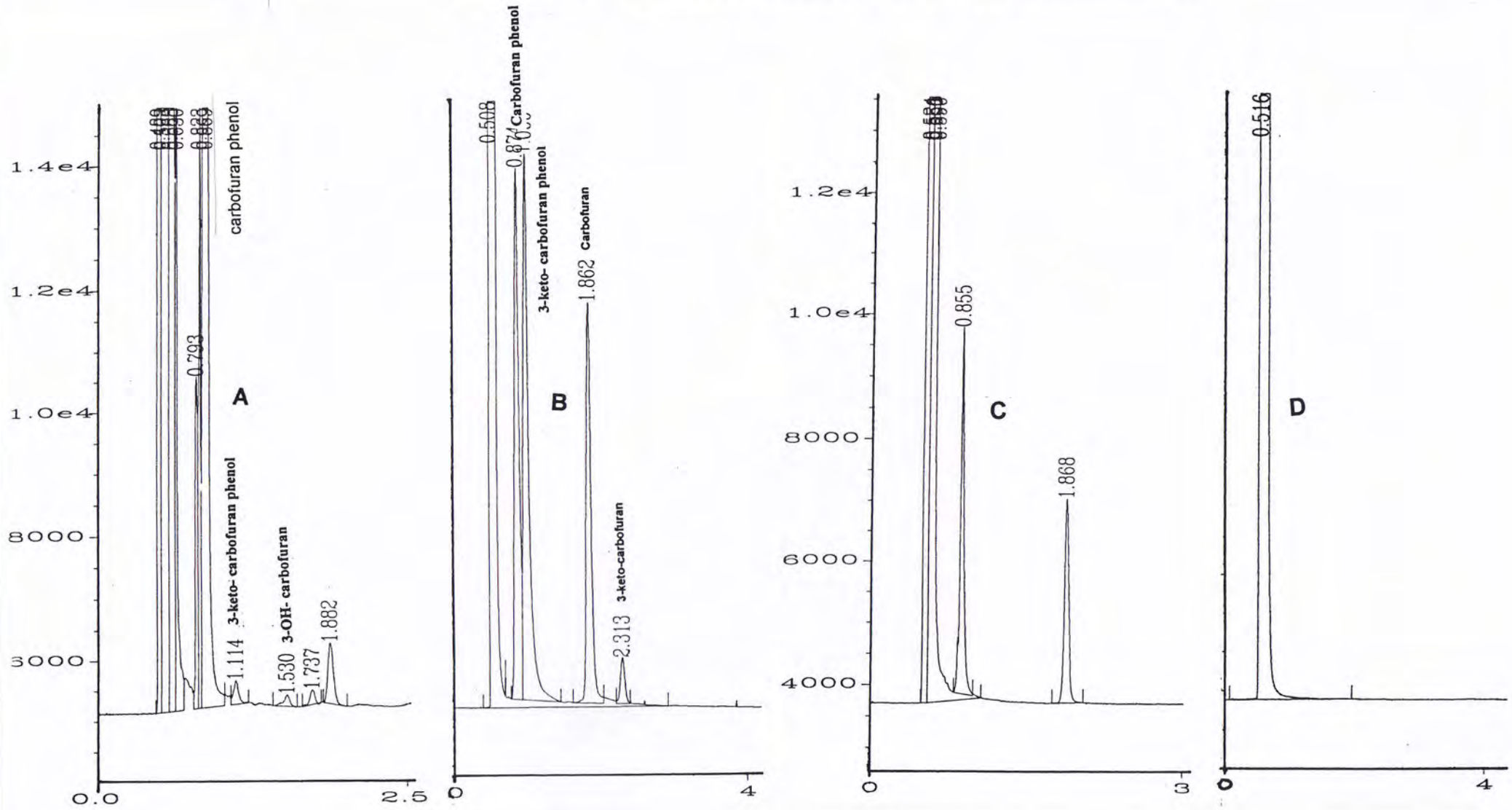


Fig 6. Gas liquid chromatography spectra of
A. Liquid culture extracts of *S. violaceusniger* after 15 days of treatment with carbofuran.
B. Carbofuran and its metabolites. **C. Liquid medium + carbofuran extract .**
D. carbofuran free liquid culture extract.

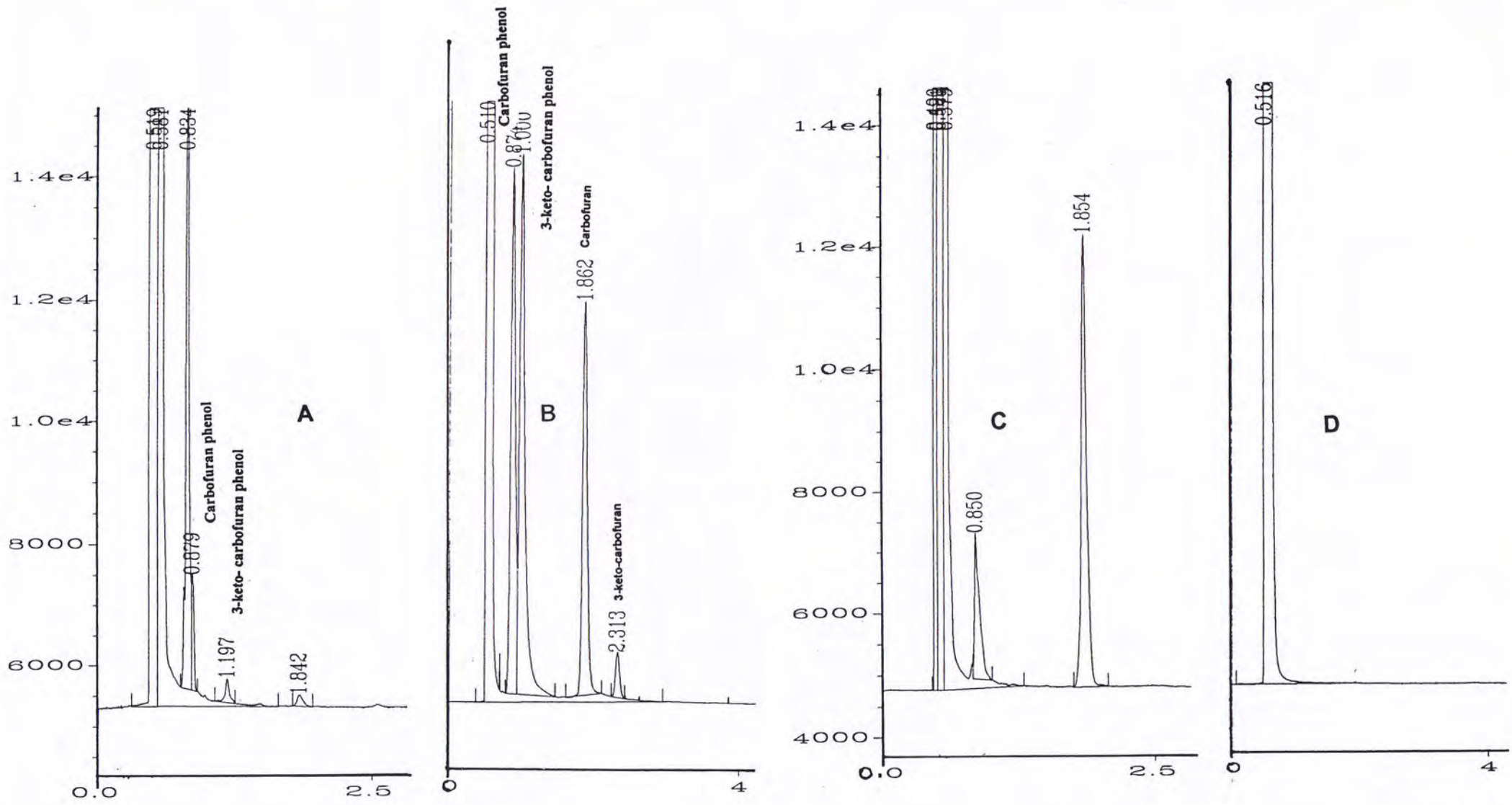


Fig 7. Gas liquid chromatography spectra of

A. Liquid culture extracts of *A brasilense* after 15 days of treatment with carbofuran.

B. Carbofuran and its metabolites.

C. Liquid medium + carbofuran extract .

D. carbofuran free liquid culture extract.

Substantial concentrations of carbofuran phenol product were found in the culture extracts during the first 11 days after treatment and remained as the major constituent during the experimental period. The relative concentration of carbofuran phenol rapidly increased and then slowly decreased with the increasing of incubation time. At the meantime, an increase in the relative concentration of 3-keto-carbofuran phenol and the unknown product was observed. Tentatively, it is suggested that the major pathway of carbofuran phenol transformation is its oxidation to 3- keto-carbofuran phenol. These results are in agreement with the findings of **Saini *et al* (1999)** on the fate of carbofuran in all cell suspension cultures of sugarcane, since they noticed that carbofuran was metabolized to 3-hydroxy carbofuran, carbofuran phenol and 3- hydroxy carbofuran phenol.

Generally, obtained result indicates that the *A.brasilense* strain is able to degrade carbofuran in liquid culture. Similar result was found by **Allan *et al* (1981)** who noticed that two bacterial isolates of *Achromobacter* sp. and *Pseudomonas* sp from soils with continuous using of carbofuran were capable of carbofuran degradation in both pure culture and when added to sterilized soil.

Persistence and biodegradation of carbofuran in cultivated soil with tomato and inoculated with *S.violaceusniger* or/and *A.brasilense.*

To evaluate the efficiency of *S.violaceusniger* or/and *A.brasilense* on carbofuran biodegradation in soil cultivated with tomato, the soil was inoculated with each strains individually as well as with their mixture after 15 days of carbofuran amendment, then the microbial and actinomycetes densities, dehydrogenase activity, carbofuran persistence and its by-products were periodically determined.

Periodical changes of total microbial, actinomycetes densities and dehydrogenase activity.

Data presented in Table (4) showed that total microbial densities were affected by low and high concentrations of carbofuran. This effect was more pronounced in case of high concentration since the total microbial densities were lower with the application of double recommended rate than the application of recommended one. Similar trend of results was observed with either uninoculated or inoculated treatments. In various investigation treatments, total microbial densities showed fluctuation throughout the experiment. This fluctuation in the microbial populations may be due to the temperature changes as well as drying and remoistening of soil throughout the experimental period. Soil inoculated with either *S.violaceusniger, A.brasilense* or their mixture increased the total microbial densities compared to uninoculated soil. This was true in case of recommended and double recommended rate of carbofuran application. This increase was more pronounced when the mixture of two microorganisms was used followed by *A.brasilense* and finally with *S.violaceusniger.*

Data also show that actinomycetes densities were generally affected by the application of the nematicide carbofuran. This effect was more pronounced in case of high concentration which caused lower densities. Such decrease in actinomycetes densities may be due to the inhibitive effect of carbofuran. Soil inoculated with either *S.violaceusniger* or *A. brasilense* showed high increase in actinomycetes densities compared to uninoculated one. This trend of results was observed with the application of carbofuran either at recommended rate or double recommended one. Soil inoculated with the mixture of tested microorganisms showed higher increase in the actinomycetes densities than soil inoculated with every microorganism each one strain individually. This indicates that these microorganisms behaved synergistically when they were inoculated together. **Kennedy *et al* (1999), Omar & Abdel-Sater (2001)** and **Singh *et al* (2002)** reported that bacteria, fungi and actinomycetes populations significantly decreased with using pesticides at higher levels.

Table 4. Total microbial, actinomycetes densities and dehydrogenase activity in soil cultivated with tomato and treated with one and double recommended rate of carbofuran and inoculated with *S.violaceusniger* and *A.brasilense* or a mixture of them.

Days after			Total microbial densities X 10^6 cfu g^{-1} dry soil								
Planting	Carbofuran application	Inoculation	Untreated soil	Uninoculated		Soil inoculated with					
						S.violaceusniger		*A.brasilense*		*S.viola. +A .bras*	
				R	2R	R	2R	R	2R	R	2R
30	15	0	80	54	46	94	73.6	92	86	110	100
45	30	15	66.3	44.2	35	60	54	68	56	98.5	92
60	45	30	79.2	65.2	60	84	75	87	69	116	96
75	60	45	55.9	33.8	25	37	31	50	35	47	39
90	75	60	48.3	47.6	32.3	49	45.5	53	50	58.2	51.5
Days after			Actinomycetes densities X 10^5 cfu g^{-1} dry soil dry soil								
Planting	Carbofuran application	Inoculation	Untreated soil	Uninoculated		Soil inoculated with					
						S.violaceusniger		*A.brasilense*		*S.viola. +A .bras*	
				R	2R	R	2R	R	2R	R	2R
30	15	0	50.2	44	25	64.0	52.0	48.8	28.9	58.1	38.0
45	30	15	32.8	19	10	23.0	19.1	20.0	16.0	38.9	34.0
60	45	30	37.3	25	13	28.0	24.0	29.5	20.3	57.0	43.6
75	60	45	33.8	32	20	38.0	25.0	32.0	26.6	36.6	30.8
90	75	60	19.3	8	5.5	22.0	16.0	15.5	12.7	19.6	16.8
Days after			Dehydrogenase activity								
						Soil inoculated with					
Planting	Carbofan application	Inoculatin	Untreated soil	Uninoculated		*S.violaceusniger*		*A.brasilense*		*S.viola. +A .bras*	
				R	2R	R	2R	R	2R	R	2R
30	15	0	33.1	31.0	26.9	46.5	43.9	46.0	42.0	50.0	47.3
45	30	15	30.5	29	25.4	43.1	39.9	45.2	40.1	48.0	46.7
60	45	30	31.8	24.1	22.9	56.3	49.3	56.9	47.2	51.1	49.3
75	60	45	36.9	27.7	19.8	55.5	54.8	55.1	49.8	68.3	63.3
90	75	60	35.4	23.0	21.1	65.3	56.0	54.4	53.4	73.3	67.8

R: Recommended rate. **2R: Double recommended rate.**

Results presented in Table (4) also showed that DHA activity data are concurrently with the results obtained from the total microbial and actinomycetes densities. Since the soil amended with carbofuran at recommended and double recommended rates showed lower DHA activity compared with control (untreated soil). This result can be attributed to the inhibitive effect of carbofuran on soil microbial populations or to its direct effect on DHA activity. On the other hand, soil inoculated with the mixture of *A.brasilense* and *S.violaceusniger* and treated with recommended rate of carbofuran showed the highest values of DHA activity, followed in a descending order by soil inoculated with *S.violaceusniger*, soil inoculated with *A.brasilense.* However, the increase in DHA activity as a result of inoculation was higher in soil treated with recommended rate than that treated with double recommended one. These results are in harmony with **Charnay & Fournier (1994); Andrea *et al* (2000), Omar & Abdel-Sater (2001)** and **Singh *et al* (2002)** who found that the using of pesticides at higher levels decreased the microorganisms proliferation and their enzymatic activity.

Persistence rate of carbofuran in cultivated soil with tomato.

Persistence rate of carbofuran in cultivated soil with tomato plants has been estimated after 15, 30, 45, 60 and 75 days from soil treatment with carbofuran which are corresponding to 0, 15, 30, 45 and 60 days after inoculation. Carbofuran was applied at one and double of the recommended rate.

The persistence rate of carbofuran presented as detectable percentage amounts of the initial concentrations and given in Table (5).

Results showed that considerable amounts of carbofuran were disappeared in case of soil inoculated with the tested microorganisms as well as in uninoculated soil during the first 15 days of inoculation (30 days of carbofuran addition), but the disappearance rate was higher in the inoculated soil than that in uninoculated one. This result reveals that beside chemical hydrolysis of pesticides, soil contains various microorganisms able to degrade carbofuran and other pesticides.

These results are in accordance with **Levanon (1993), Balinove *et al* (1997), Karpouzas & Walker (2000), Trabue *et al* (2001)** and **Megharaj *et al* (2003)**. When carbofuran was applied at double recommended rate in uninoculated soil showed lower disappearance rate than that in its application at recommended one.

Whereas, 0.9 and 2.05% of the added amount were detected as carbofuran at the end of the experiment at recommended and double recommended rate, respectively. Also, inoculation of soil with either *S.violaceusniger, A.brasilense* or a mixture of them accelerated carbofuran biodegradation.

Table 5. Persistence rate of carbofuran in cultivated soil with tomato.

Days after			Uninoculated soil		Soil inoculated with					
					S.violaceusnr		*A.brasilense*		*S.violaceusnigr + A.brasilense*	
Planting	Carbofurn addition	Inoculation	R	2R	R	2R	R	2R	R	2R
30	15	0	62.2	70.30	60.0	69.8	60.0	69.8	60.0	69.8
45	30	15	24.05	24.8	16.0	18.4	14.2	16.9	10.8	12.6
60	45	30	13.60	16.60	3.0	5.0	5.0	6.6	2.5	3.30
75	60	45	4.80	6.20	0.6	1.2	1.2	2.0	0.0	0.5
90	75	60	0.9	2.05	0.0	0.0	0.0	0.0	0.0	0.0

R = Recommended rate. **2R = Double recommended rate.**

Soil inoculated with the mixture of two strains exhibited higher disappearance rate for carbofuran compared with that inoculated with either *S.violaceusniger* or *A. brasilense* individually. The dissipation rate of carbofuran in the soil inoculated with *S.violaceusniger* was faster than that in soil inoculated with *A.brasilense.* The detectable amounts of carbofuran in soil treated with recommended rate at the 30th day of inoculation with *S.violaceusniger, A.brasilense* and mixture of them were 3.0, 5.0 and 2.5%, respectively; whereas they were 5.0, 6.6 and 3.3% in the soil treated with double recommended rate of carbofuran, respectively. These results indicate that *S.violaceusniger* strain is more degrading carbofuran than *A.brasilense* and when a mixture of them was used, they synergistically affected and enhanced the biodegradation of carbofuran.

Compounds produced from biodegradation of carbofuran.

Carbofuran and its transformed products produced from carbofuran decomposition in soil planted with tomato and inoculated with either *S.violaceusniger* or *A.brasilense* as well as their mixture after 15 days of carbofuran amendment were periodically determined by both TLC and GLC. Analysis data of soil extracts after 30 days of treatment with recommended rate of carbofuran (15 days after inoculation) are shown in Figs (8 & 9).

TLC analysis declared four compounds having Rf values 0.49 ; 0.68; 0.79 and 0.91. Extract of soil inoculated with *S.violaceusniger* showed three metabolites having R_f values 0.68; 0.79 and 0.91, the first and third compounds were identified as 3-keto carbofuran and carbofuran phenol, respectively. Whereas, the remainder compound could not be identified. Soil inoculated with *A.brasilense* showed only two metabolic products having R_f values 0.49 and 0.91 which were identified as 3-hydroxy carbofuran and carbofuran phenol, respectively. Soil inoculated with the mixture of tested microorganisms showed in TLC plates three metabolic compounds having Rf values 0.49; 0.79 and 0.91 which could be identified as 3-hydroxy carbofuran, unknown and carbofuran phenol, respectively. Carbofuran phenol was the only detectable compound with high concentration.

Fig 8. Thin layer chromatography of cultivated soil extract at different intervals after treatment by carbofuran and inoculated with the tested strains.
St ; Carbofuran standard. C; Untreated soil.

1; Soil extract after 30 days of treatment by carbofuran at double recommended rate.
2; Soil extract after 30 days of treatment by carbofuran at recommended rate.
3;Soil extract after 30 days of treatment by carbofuran and inoculated with *S.violaceusniger* .
4; Soil extract after 30 days of treatment by carbofuran and inoculated with *A. brasilense.*
5; Soil extract after 30 days of treatment by carbofuran and inoculated with mixture of two strains.
6;Soil extract after 45 days of treatment by carbofuran and inoculated with *S.violaceusniger*
7; Soil extract after 45 days of treatment by carbofuran and inoculated with *A. brasilense.*
8; Soil extract after 45 days of treatment by carbofuran and inoculated with mixture of two strains.
9; Soil extract after 60 days of treatment by carbofuran and inoculated with mixture of two strains.

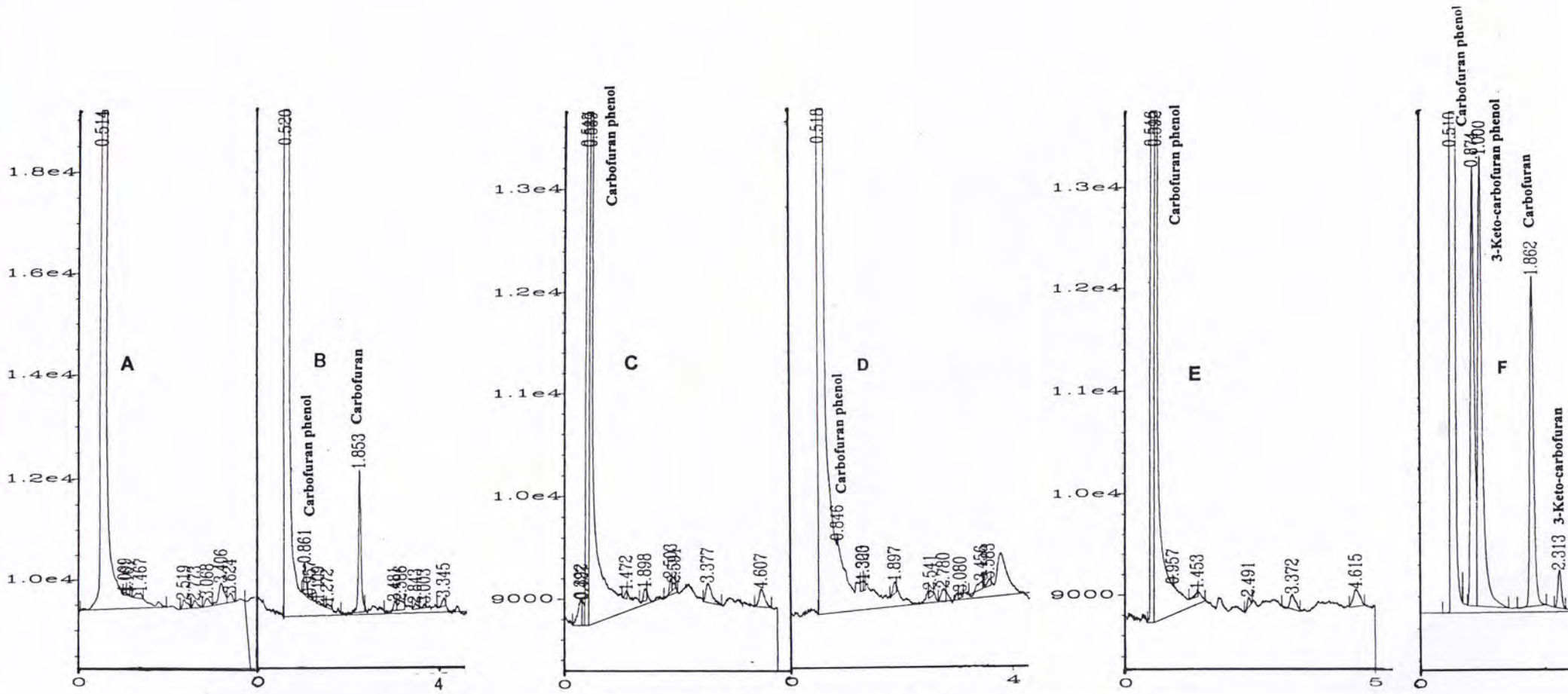


Fig 9. Gas liquid chromatograph of soil extracts after 30 days of soil treatment by recommended rate of carbofuran.

A)Untreated soil B)Soil treated with carbofuran C)Soil treated with carbofuran and inoculated with *Strpetomyces violaceusinger*.

D)Soil treated with carbofuran and inoculated with *A. brasilense*

E) Soil treated with carbofuran and inoculated with mixture of two strains.

F)Standard carbofuran and its metabolites.

Obtained results showed that one compound was detected by GLC in extracts of uninoculated soil as well as in the inoculated one with either *S.violaceusniger* or *A.brasilense* or mixed of both strains. This compound had Rt 0.86 minute which was identified as carbofuran phenol. This compound appeared at negligible amount in uninoculated soil extract, but it represents the dominant peak in extracts of soil treated with carbofuran and inoculated with *S.violaceusniger* and that inoculated with the mixture of the tested microorganisms.

Carbofuran was also detected with small peak having Rt 1.89 in all analyzed samples except that in uninoculated soil extracts since, it was detected with high peak. Also, other few peaks were detected in all extracts having various retention times. These results indicate to soil impurities plus unidentified degraded compounds where appeared with negligible amounts.

Obtained results also reveal that soil inoculated with any of the tested microorganisms accelerated the biodegradation of carbofuran in the soil. Inoculation of soil with their mixture gave the best results in biodegradation, since carbofuran was not detectable at the 30th day of soil treatment when inoculated with the mixture of tested microorganisms. From the previous results it can be concluded that biodegradation of carbofuran in cultivated soil was faster than that in non-cultivated one. This result indicates that the presence of other microorganisms in rhizosphere region capable of carbofuran degradation. Similar results were obtained by **Jinhe *et al* (1989), Battu *et al* (2000)** and **Moreno *et al* (2001)** who showed that carbofuran degraded nonenzymatically and by microorganisms under sub-tropical conditions. The degradation occurs by hydroxylation at the benzylic carbon to give 3-hydroxy carbofuran and 3-keto-carbofuran under upland conditions and via hydrolysis to form phenols, i.e. carbofuran phenol, 3-hydroxy carbofuran phenol and 3-keto-carbofuran phenol.

CONCLUSION

Summing up, Egyptian soils are a good source for isolating the microorganisms which are potent in pesticides decomposition. Thereby, two microorganisms were isolated from the tested soil and identified as *S. violaceusniger* and *A. brasilense.* Data on the biodegradation of carbofuran by *S. violaceusniger* and *A. brasilense* revealed that *S. violaceusniger* is able to degrade of carbofuran to six metabolites, three of them were identified as carbofuran phenol , 3-OH-carbofuran and 3-keto-carbofuran phenol and the other three metabolites could not be identified. Whereas, *A. brasilense* is able to degrade of carbofuran to only three metabolites, two of them were identified as carbofuran phenol and 3-keto-carbofuran phenol and the third one could not be identified. Results also show that carbofuran disappearance rate was higher in inoculated soil with *S. violaceusniger* or/ and *A. brasilense* than that in uninoculated one. Soil inoculated with the mixture of the two strains exhibited higher disappearance rate of carbofuran compared to that inoculated with either *S. violaceusniger* or *A. brasilense* individually.

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كفاءة بعض الميكروبات المعزولة من التربة في تحلل مبيد الكربوفيوران

الهدف من هذا البحث هو دراسة إمكانية استخدام الكائنات الحية الدقيقة المعزولة من الأراضى المصرية فى تحليل المبيدات الكربباميتية لإزالة ما يحدث بها من تلوث نتيجة استخدام هذه المبيدات. لذلك تضمنت هذه الدراسة عزل وتعريف بعض الميكروبات من التربة المعاملة بتركيزات متزايدة من مبيد الكربوفيوران واختبار مدى تحملها ومقدرتها على تحلل هذا المبيد والذى يستخدم بكثرة تحت ظروف الزراعة المصرية ضد كثير من الآفات ويمكن تلخيص نتائج هذه الدراسة فيما يلى:

تم عزل و تعريف نوعين من الميكروبات لهما المقدرة على تحمل سمية مبيد الكربوفيوران وأوضحت نتائج التعريف لهذه الميكروبات أن أحدهما هو *Streptomyces violaceusniger* والأخر هو *Azospirillum brasilense* . عند دراسة تحلل مبيد الكربوفيوران في البيئة السائلة بواسطة الميكروبات تحت الدراسة أوضحت النتائج أن سرعة اختفاء مبيد الكربوفيوران كان أعلى فى حالة التلقيح بميكروب *A.brasilense* بالمقارنة بميكروب *S.violaceusniger* حيث أظهرت نتائج التحليل بواسطة جهاز الـ GLC أن ٧,٥ ، ١٠,٥% من كمية الكربوفيوران المضافة لهذه المزارع هى التى تبقت بعد إنتهاء فترة التجربة وذلك فى حالة *A.brasilense* و *S.violaceusniger* على التوالى.

كذلك اوضحت النتائج أن ميكروب *S.violaceusniger* كان أكفأ فى تحلل مبيد الكربوفيوران عن ميكروب *A.brasilense* حيث أظهرت نتائج التحليل بواسطة الـ GLC ، الـ TLC وجود ستة مركبات ناتجة من تحلل مبيد الكربوفيوران بواسطة *S.violaceusniger* أمكن تعريف ثلاثة منهم على أنهم كربوفيوران فينول ، ٣- هيدروكس كربوفيوران و ٣-كيتوكربوفيوران بينما الثلاث مركبات الأخرى لم يمكن تعريفهم. بينما أظهرت نتائج تحلل مبيد الكربوفيوران بواسطة *A.brasilense* وجود ثلاثة مركبات فقط أمكن تعريف اثنين منهم على أنهما كربوفيوران فينول و ٣-كيتوكربوفيوران أما المركب الثالث فلم يمكن تعريفه.

أيضاً ، أوضحت النتائج أن معدل تحلل مبيد الكربوفيوران فى التربة المنزرعة بنباتات الطماطم والمعاملة بمبيد الكربوفيوران والملقحة بميكروب *S.violaceusniger* أو ميكروب *A.brasilense* كان أعلي بالمقارنة بالتربة المعاملة بالمبيد غير الملقحة. أعلى معدل اختفاء لمبيد الكربوفيوران لوحظ عند تلقيح التربة المنزرعة بمخلوط السلالتين تحت الدراسة.

من نتائج هذه الدراسة يتضح أن إجراء تلقيح التربة بواسطة بعض السلالات ذات المقدرة على تحلل المبيدات يمكن أن يساهم فى إزالة بقايا المبيدات وهذا يقلل من تلوث البيئة الذى ينتج من تراكم بقايا هذه المبيدات.

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