BIOREMEDIATION OF THE POLLUTED SOIL WITH CARBAMATE PESTICIDES By *Streptomyces violaceusniger* or/ and *Azospirillum brasilense*

ABSTRACT

This research was carried out to study the persistence rate and decomposition of carbofuran and temik nematicides in autoclaved and non-autoclaved soil inoculated with *S. violaceusniger* or /and *A. brasilense*. Results indicated that the application of either carbofuran or temik to soil led to a decrease in total microbial counts, actinomycetes counts and dehydrogenase activity. In contrast, the above mentioned parameters were increased in soil amended with pesticides and inoculated with either *S. violaceusniger* or *A. brasilense* as well as mixture of them. Inoculation of soil with the above mentioned strains combined with insecticides application accelerated the dissipation rate and transformation of both carbofuran and temik specially when the mixture of the two strains was used. Disappearance rate of carbofuran and temik and their decomposition were more rapid in non-autoclaved soil than that in autoclaved one. Carbofuran and temik rapidly disappeared from the soil inoculated with the mixture of *A. brasilense* and *S. violaceusniger* compared with the inoculated soil with each strain individually. Also, results showed that carbofuran and temik rapidly degraded in soil inoculated with *S.violaceusniger* than that inoculated with *A. brasilense*. The main compounds produced from carbofuran degradation were carbofuran–phenol; 3-keto carbofuran; 3-hydroxy-carbofuran and other unknown compounds. Whereas, temik mainly degraded to temik sulfone, temik sulfoxide and other unknown compounds.

INTRODUCTION

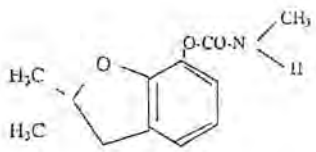
Chemical protection of plants is based on the toxic effect of various organic and inorganic compounds (pesticides) on harmful organisms. Pesticides are distinguished by their high universality which can be used to control most of pests diseases and weeds on all agricultural crops and various lands. Carbofuran (furadan) and temik (aldicarb) are highly effective systemic and contact insecticides and nematocides which are extensively used to control insect pests of maize, rice, tomato, banana and other agricultural crops.There are many microorganisms capable of degrading pesticides .**Charanay & Fournier (1994), Kale *et al* (1996); Barra *et al* (1999), Karpouzas & Walker (2000), Trabue *et al* (2001)** and **Megharaj *et al* (2003)** revealed that various soil microorganisms are responsible for the rapid degradation of pesticides when they were used as soil application.**Duquenne *et al* (1996), Soudamini *et al* (1997) and Das & Mukherjee (2000)** found significant increase in the rate of carbofuran and similar compounds degradation in soil inoculated with either a liquid cell suspension of bacteria or granular formulation compared to uninoculated soil. Moreover, **Talebi & Walker (1993), Kale *et al* (1996)** and **Barra *et al* (1999)** revealed that degradation of carbamate pesticides (carbofuran and temik) was slower in autoclaved soil than that in non-autoclaved one. These results support the hypothesis that microbial community plays an important role in pesticides degradation. Regarding the insecticides and nematicides effect on soil microorganisms, results of previous researches are greatly varied. Some of them showed stimulative effects **(Edwards *et al*,1992; Das *et al*, 1995; Kennedy, 1999** and **Das & Mukherjee, 2000)** whereas, the others showed inhibitive effect **(Sundarababu, 1993; Andrea *et al*, 2000; Omar & Abdel-Sater, 2001** and **Singh *et al*, (2002)** who found that bacteria, fungi and actinomycetes populations significantly decreased with using pesticides at higher levels. Thereby, this research aimed to study the efficiency of Streptomyces violaceusniger and Azospirillum brasilense on carbofuran and temik degradation in non-autoclaved and autoclaved soil.

Zaghloul, R. A [1]; Ehsan A. Hanafy[1]; A. Gh. Rahal [2] and Lobna A. Moussa [2]
1-Agric. Botany Dept. Fac. Agric. Moshtohor, Zagazig Univ. Egypt.
2-Agric. Microbiol. Dept. Res. Soils, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt

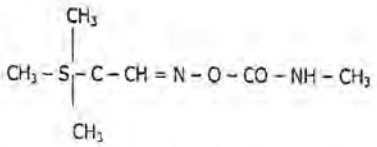
MATERIALS AND METHODS

Pesticides.

- **Carbofuran (furadan):** 2,3 dihdro-2,2 dimethyl-7benzofuranyl N-methylcarbamate.
- **Temik (aldicarb):** 2 methyl 2 (methylthio) propionaldhyde-o-(methyl carbamoyl) oxime..



Carbofuran



Temik

Microorganisms.

Streptomyces violaceusniger *Azospirillum brasilense*

Experimental procedure.

To obtain a general understanding about the role of *S. violaceusniger* and *A. brasilense* strains, which were isolated and identified in our previous study, on the fate of carbofuran and temik in sterilized and non-sterilized soil, a study was conducted using sandy clay loam soil. The soil was air dried, sieved through 2 mm and divided into two parts, one of them was sterilized by autoclaving at 121°C for 1hr for three successive days and the other part was left without sterilization. The soil whether sterilized or non-sterilized was divided into two categories one of them was mixed with the granular carbofuran to give a final concentration of the recommended rate (30 kg/feddan) and the other was mixed with granular temik at recommended rate (17 kg/feddan) then, moisture content was adjusted to 60% of water holding capacity with distilled water. Each treatment was divided into four portions, the first portion was left without inoculation (control), the second was inoculated with 10 ml/kg soil of *A. brasilense* culture (1ml contains about 344 x 10^7 viable cells), the third was inoculated with 10 ml/kg soil of *S. violaceusniger* culture (1ml contains about 130x10^5 spore) and the fourth was inoculated with the mixture of them (1:1).A control treatment was left with neither inoculation nor insecticide addition. The soil portions were well mixed and distributed in 200 ml wide mouthed bottles (100 g soil of each), capped with aluminum foil and incubated at ambient room temperature. After 7, 15, 21 and 30 days of incubation, soil samples were taken for the following assessments:

a)Total counts of bacteria and actinomycetes . b)Dehydrogenase activity.

c) Determination of (carbofuran and temik) amounts and their degraded products.

Inocula preparation.

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

Analysis methods.

Total bacterial count in soil was estimated using soil extract agar medium **(Holm and Jenseon, 1972)**, Whereas, actinomycetes count was estimated using starch nitrate agar medium (**Waksman and Lechevailer 1961),** Dehydrogenase activity was determined according to the method described by **Casida *et al* (1964)**. Carbofuran and temik were extracted and cleaned-up from the soil according the method described by **Racke and Coats (1990)**. Carbofuran and temik as well as their metabolites were determined by GLC, TLC and Gas/Mass spectrometer.

RESULTS AND DISCUSSION

Total microbial densities in non-autoclaved soil treated with either carbofuran or temik and inoculated with the tested microorganisms.

Data in Table (1) showed that total microbial densities were 29 X 10^6 and 32 X 10^6 in soil at the beginning of soil treatment with carbofuran and temik, respectively. Seven days after incubation, microbial densities were increased, such increase may be due to the improvement of the conditions for microbial proliferation such as temperature, moisture and nutrient elements. However, this increase was followed by a decrease in the second week and thereafter it was followed with another increase which continued till the end of the experiment. This fluctuation in the microbial densities may be due to the temperature changes as well as drying and remoistening of soil throughout the experimental period. Total microbial densities were highly suppressed in soil treated with either carbofuran or temik without inoculation along the experimental period. The reduction in microbial counts may be attributed to the toxic effect of carbofuran and temik. This result coincided that obtained by **Jones *et al* (1991), Kennedy *et al* (1999), Andrea *et al* (2000) and Singh *et al* (2002)** who showed that soil microflora might be suppressed by pesticides treatment of soil. On the other hand, **Edwards *et al* (1992)** found that (2 g/kg soil) from carbofuran caused the highest bacterial populations at 7th and 15th day after treatment. Also, it can be observed that the microbial densities were highly increased in soil inoculated with either *A.brasilense* or *A.brasilense* + *S. violaceusniger*. Such increase was higher in case of dually inoculated soil than that inoculated with *A. brasilense* individually. This result may be attributed to the synergistic effect between the introduced inocula and indigenous soil microorganisms in biodegradation of either carbofuran or temik and eliminate their toxicity. **Das *et al* (1995),Kennedy(1999) and Das&Mukherjee(2000)** found similar data.

Actinomycetes densities in soil treated with either carbofuran or temik.

Data in Table (2) showed that actinomycetes densities in soil treated with either carbofuran or temik have similar trend as that obtained in total microbial densities (Table, 1). Actinomycetes densities at the beginning of experiment were 4X10^4 and 6X10^4 in soil for carbofuran and temik, respectively. One week after incubation, actinomycetes densities increased. Such increase may be due to the amelioration in environmental conditions as the suitable moisture content and temperature which were offered under incubation conditions. This increase in actinomycetes populations continued till the 21st day and decreased thereafter.

Table 1. Total microbial densities (counts x 10^6 cfu g^{-1} dry soil) in non- autoclaved soil treated with either carbofuran or temik and inoculated with *Azospirillum brasilense* only and *Streptomyces violaceusniger* + *A.brasilense*.

Days after treatment	Treatments							
	Carbofuran				Temik			
	A	B	C	D	A	B	C	D
7	35.5	12.0	39.2	44.3	34	25.0	36	40
15	30.1	9.00	36.6	42.6	25	19.0	30	42
21	40.4	14.6	42.5	45.0	23	13.0	25	38
30	45.0	13.7	48.4	52.0	30	20.0	26	50

Total microbial densities at the beginning of the experiment were 29X10^6 and 32 X 10^6 for carbofuran and temik, respectively.

A ;Untreated soil. B; Soil treated with either carbofuran or temik.

C; Soil treated with either carbofuran or temik and inoculated with *A. brasilense.*

D; Soil treated with either carbofuran or temik and inoculated with *S .violaceusniger* and *A. brasilense* mixture.

Actinomycetes densities were depressed along the experimental period after treatment with either carbofuran or temik without inoculation, such decrease may be retained to the toxic effect of carbofuran and temik. This result coincided that obtained by **Andrea *et al* (2000) and Omar & Abdel-Sater (2001).**

Table 2 .Actinomycetes densities (counts x 10^4 cfu g^{-1} dry soil) in non- autoclaved soil treated with either carbofuran or temik and inoculated with *S.violaceusniger* only and S. *violaceusniger* + *A.brasilens.*

Days after treatment	Treatments							
	Carbofuran				Temik			
	A	B	C	D	A	B	C	D
7	8.0	4.0	13.7	16.6	14	9.1	20.5	25.3
15	12.3	5.8	17.3	21.0	21	7.8	24.8	29.1
21	23.0	14.0	27.2	29.1	26	6.0	30.3	35.0
30	18.0	11.2	23.9	24.5	20	3.3	29.1	33.8

Actinomycetes densities at the beginning of experiment were 4 X 10^4 and 4.6 X 10^4 for carbofuran and temik ,respectively.

A ;Untreated soil. B; Soil treated with either carbofuran or temik.

C; Soil treated with either carbofuran or temik and inoculated with *S. violaceusniger.*

D; Soil treated with either carbofuran or temik and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

Non-autoclaved soil treated with either carbofuran or temik and inoculated with either *S. violaceusniger* or *S. violaceusniger* + *A. brasilense* mixture showed higher increase in actinomycetes counts when compared with the uninoculated soil. However, such increase is clearly observed in the inoculated soil with the mixture of two strains. This result may be ascribed to the synergistic effect between the endogenous soil microorganisms and the introduced inocula. This result confirmed those obtained by **Kennedy *et al* (1999) and Das & Mukherjee (2000).**

Dehydrogenase activity in non-autoclaved soil treated with either carbofuran or temik and inoculated with the tested microorganisms.

Dehydrogenase activity (DHA) was measured as a criterion of respiration rate and microbial activity in soil. Data in Table (3) emphasized that the soil treated with either carbofuran or temik and uninoculated showed lower enzyme activity. It is worthy to mention that soil treated with temik showed remarkably decrease in DHA activity compared to soil treated with carbofuran. This result can be attributed to either the inhibitive effect of temik on soil microflora or to its direct effect on enzyme activity. This result is in agreement with that obtained by **Palaniappan and Balasubramanian (1985)** who found that temik application at 10 ppm inhibited DHA. On the other hand, soil inoculated with the mixture of *S. violaceusniger* and *A. brasilense* gave the highest enzyme activity, followed in a descending order by soil inoculated with *S. violaceusniger* > soil inoculated with *A. brasilense* . The increase in DHA activity as a result of soil inoculation may be due to the synergistic effect between the native soil microorganisms and the introduced ones. Generally, obtained results are concurrently with those previously obtained from the total microbial and actinomycetes densities in soil with different treatments.

Persistence rate of carbofuran and temik in soil .

Persistence data of carbofuran and temik are presented as detectable percentage amounts of the initial concentration and given in Table (4).Data showed that 72 and 76.6 % of the added carbofuran and temik were disappeared throughout 30 days in non-autoclaved and uninoculated soil, respectively. This result coincided with that obtained by **Soudamimi *et al* (1997)** who noticed that carbofuran degrading microorganisms breakdown carbofuran up to 5000 ppm in about 1 to 90 days. The disappearance rate of carbofuran and temik in non-autoclaved and uninoculated soil was faster than that in autoclaved and uninoculated one. These results reveal the importance of native soil microorganisms in pesticides degradation. This result agreed with that obtained by **Getzin & Shanks (1990)** and **Barra *et al* (1999)** who found that degradation of carbofuran was

slow in autoclaved soil and the half-life of carbofuran exceeded 16 weeks in autoclaved soil. Also, **Arunachalam and Lakshamanan (1990)** found that about 75% of the added carbofuran was recovered as residues after 60 days in sterilized and non-flooded soil, whereas about 75% of the added carbofuran was metabolized in non-sterilized and non-flooded one. The dissipation percentage of carbofuran and temik was accelerated in soil inoculated with either *S. violaceusniger* or *A. brasilense* as well as that inoculated with a mixture of them specially in non-autoclaved soil.

Table 3. Dehydrogenase activity in non-autoclaved soil treated with either carbofuran or temik and inoculated with *S. violaceusniger* , *A. brasilense* or a mixture of them (determined as ug TPF g^{1} dry soil).

Days after treatment	Treatments				
	A	B	C	D	E
	Carbofuran				
7	18.2	15.0	24.6	26.0	27.3
15	20.0	13.2	22.6	20.1	23.1
21	26.3	9.50	30.1	22.2	32.0
30	30.8	10.4	35.5	28.6	38.0
	Temik				
7	8.6	3.9	9.90	9.10	12.0
15	11.2	2.8	13.3	12.0	15.7
21	13.0	5.3	14.9	12.9	16.9
30	8.80	5.0	12.0	13.0	12.9

DHA values at the beginning of experiment were 8.5 and 3.2 TPF g^{-1} dry soil day^{-1} for carbofuran and temik respectively.

A ;Untreated soil. B; Soil treated with either carbofuran or temik.

C; Soil treated with either carbofuran or temik and inoculated with *S. violaceusniger*.

D; Soil treated with either carbofuran or temik and inoculated with *A. brasilense*.

E; Soil treated with either carbofuran or temik and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

Since the detectable percentages of carbofuran in non-autoclaved and autoclaved soils after 30 days of inoculation with *S. violaceusniger* were 4 and 6 %, respectively. But, they were 3.2 and 5.2 % in non-autoclaved and autoclaved soil inoculated with *A. brasilense*, respectively. While, detectable percentage of temik in non-autoclaved and autoclaved soil after 30 days of inoculation with *S. violaceusniger* were 6.8 and 11.5 % ,respectively. But, they were 17.4 and 31.1 % in non-autoclaved and autoclaved soil inoculated with *A. brasilense*, respectively. These results revealed that both microorganisms are capable of hydrolyzing the carbamate linkage and may be use the resulting methyl amine as a C and N source.

This result is in harmony with that reported by **Duquenne *et al* (1996), Das & Mukherjee (2000) and Trabue *et al* (2001)** Who found a significant increase in the rate of carbofuran and temik degradation occurred in inoculated soil compared to uninoculated one. **Charanay and Fournier(1994)** also announced that the enhancement degradation of carbofuran corresponded to growth of microorganisms able to its use as a sole source of carbon and nitrogen. Inoculation of soil with the mixture of *S. violaceusniger* and *A. brasilense* exhibited highly dissipation percentage for the two pesticides whether in non-autoclaved or autoclaved soil when compared with the soil inoculated with each microorganism individually. This increase in dissipation percentage with the mixture of both microorganisms may be due to these microorganisms synergistically interacted to improve the degradation of pesticides. Similar results were obtained by **Soudamini *et al* (1997)** and **karpouzas & Walker (2000)** who showed that bacterial cultures isolated from the enrichment culture, two cultures enhanced carbofuran degradation resulting in more than 98% loss of the applied carbofuran in 30 days. The other cultures enhanced the degradation up to 70% within the same period. The mixture of cultures when synergistically interacted enhancing the degradation of carbofuran residues to 96% in 10 days.

Comparing the percentage of carbofuran and temik persistence in non-autoclaved and autoclaved soil, it could be concluded that *S. violaceusniger* and *A. brasilense* are essential microorganisms in acceleration of these pesticides biodegradation in the soil and there is other organisms able to metabolize these pesticides. This was true, since the decomposition percentage was higher in non-sterilized soil than that recorded in sterilized one. Also, results showed that temik decomposition in uninoculated soil was faster than that of carbofuran, since 69.3% and 12.4% of the incorporated amount of temik were undetectable in non-autoclaved and autoclaved soil, respectively. Whereas, the undetectable amounts of carbofuran were 64 and 9.8 % in corresponding soils after the same period of treatment (21 days). This result revealed that temik nematicide is more degradable by native soil microorganisms than carbofuran.

Table 4. persistence rate of carbofuran and temik in non-autoclaved and autoclaved soil .

Days after treatment	Non-autoclaved soil inoculated with							
	Uninoculated		*S.violaceusniger*		*A.brasilense*		*S.violaceusniger + A.brasilense*	
	Carbofuran	Temik	Carbofuran	Temik	Carbofuran	Temik	Carbofuran	Temik
0	100	100	100	100	100	100	100	100
7	72.0	69.5	41.3	58.8	32.6	80.0	28.9	53.7
15	50.9	46.1	19.0	39.9	15.8	42.7	13.2	35.2
21	36.0	30.7	8.60	18.7	5.90	32.7	4.80	14.7
30	28.0	23.4	4.00	6.80	3.20	17.4	1.30	6.70
	Autoclaved soil inoculated with							
	Uninoculated		*S.violaceusniger*		*A.brasilense*		*S.violaceusniger + A.brasilense*	
	Carbofuran	Temik	Carbofuran	Temik	Carbofuran	Temik	Carbofuran	Temik
0	100	100	100	100	100	100	100	100
7	98.6	93.7	77.2	70.4	70.1	85.0	77.0	65.1
15	95.8	88.9	38.8	58.5	51.4	65.6	35.9	54.8
21	90.2	87.6	28.8	39.2	20.3	54.3	13.4	32.9
30	86.6	85.7	6.00	11.5	5.20	31.1	2.10	22.4

However, inoculation of non-autoclaved and autoclaved soil with the two tested microorganisms enhanced the dissipation rate of temik. For example, the detectable amounts of temik in non-autoclaved and autoclaved soil inoculated with *S. violaceusniger* were 18.7 % and 39.2%, respectively. Whereas, they were 32.7% and 54.3% in the soil inoculated with *A. brasilense* at the 21st day of inoculation.From the above mentioned results, it is clear that the application of *S. violaceusniger* inoculum to the soil accelerated decomposition of temik more than *A. brasilense* inoculum. This result is in agreement with those obtained by **Chaudhry & Ali (1988), Charanay & Fournier (1994), Karpouzas & Walker (2000), Trabue et al (2001)** and **Megharaj *et al* (2003)** who noticed that various soil microorganisms are responsible for the rapid degradation of pesticides when they were used as soil application.

Analysis of technical carbofuran ; temik and their 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, respectively to be as reference to compare these values with those obtained from samples analysis. The limited data are presented in Table (5)

Biodegradation of carbofuran and temik .

Periodical determination of produced products from carbofuran degradation in autoclaved and non-autoclaved soil inoculated with either *S. violaceusniger*, *A. brasilense* or a mixture of them are given in Figs (1,2,3 and 4). Data analysis of non-autoclaved soil extracts revealed that, uninoculated soil showed three compounds which were detected by GLC having retention times (Rt) values 2.3, 2.5 and 0.85 minutes. The second compound could not be identified while, the first and third ones were identified as 3-keto-carbofuran and carbofuran phenol. On the other hand, TLC analysis showed four compounds having retention flow (Rf) values 0.26, 0.48, 0.51 and 0.88. The first and the third compounds could not be identified, whereas the second and the fourth ones were identified as 3-hydroxy–carbofuran and carbofuran phenol. Extract analysis of soil inoculated with *A. brasilense* by GLC showed only one compound having Rt value 2.5 minutes which could not be identified, whereas its analysis by TLC detected one compound having Rf value 0.88 and identified as carbofuran phenol. However, GLC analysis of soil extracts inoculated with *S. violaceusniger* showed three compounds having Rt values 0.85, 1.5 and 2.5 minutes. The first two compounds were identified as carbofuran phenol and 3-OH –carbofuran. Whereas, the last one could not be identified.

Similar results for the same samples were obtained by TLC analysis, since three compounds declared on plates having Rf values 0.26, 0.48 and 0.88. The first compound was not identified, whereas the remainder two were identified as 3-OH-carbofuran and carbofuran phenol. At last, GLC analysis of soil extracts inoculated with the mixture of *S. violaceusniger* and *A. brasilense* showed three compounds having Rt values 1.26, 1.6 and 2.5 minutes. Only the second one was identified as 3-OH-carbofuran, whereas the first and third ones could not be identified. The same extract of samples when analyzed by TLC showed also three compounds having Rf values 0.26, 0.33 and 0.88. The first compound was unknown and the other two compounds were identified as 3-keto – carbofuran and carbofuran phenol. 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,5) and the corresponding compounds were recorded.

Results of GLC and TLC analyses of autoclaved soil extracts revealed a degree of similarity as well as difference in the nature of produced compounds by tested strains at different intervals. One compound was detected by GLC in extracts of autoclaved and inoculated soil with either *A. brasilense* or *S. violaceusniger* as well as the mixture of them. This compound having Rt 0.86 minute and could be identified as carbofuran phenol. Another compounds were always detected with small amount in all soil extracts, these compound could not be identified. TLC analysis of soil inoculated with either *S. violaceusniger* or a mixture of *S. violaceusniger* and *A. brasilense* showed two different compounds having Rf values 0.32 and 0.39 specially at the early interval (7 days). The first compound could not be identified, whereas the second one was identified as 3-keto – carbofuran. Results of the present work demonstrated the superiority of *S. violaceusniger* in carbofuran degradation in soil, being it removed about 71.2 and 94.0% of carbofuran after 21 and 30 days,

Table 5. Data analysis of carbofuran, temik and their metabolites.

Carbofuran

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-ket- carbofuran phenol	0.65	1.00	178	$C_{10}H_{10}O_3$

Temik

Compounds	TLC (Rf values)	GLC (Rt min)	M/e	Molecular formula
Temik	0.90	0.99	190	$C_7H_{14}N_2O_2S$
Temik sulfoxide	0.23	0.94	206	$C_7H_{14}N_2O_3S$
Temik sulfone	0.17	1.28	222	$C_7H_{14}N_2O_4S$

respectively in autoclaved soil inoculated with this strain. Similar results were obtained by **Duquenne *et al*** (1996) **and Soudamini *et al* (1997)** Generally, from these results it can be concluded that carbofuran mainly degraded in the soil inoculated with *A. brasilense* and *S. violaceusniger* to carbofuran phenol; 3-OH – carbofuran; 3-keto – carbofuran and other few unidentified compounds. Similar results were obtained by **Venkateswarlu *et al* (1984)** who isolated a bacterium *A. lipoferum* and two actinomycets (*Streptomyces* sp) from flooded alluvial soil capable of carbofuran degradation to carbofuran phenol as a major product and 3-hydroxy carbofuran as a minor product.

Fig.1 .Thin layer chromatogram of non-autoclaved soil.

Fig. 2 .Thin layer chromatogram of autoclaved soil.

C ; Untreated soil(control) . 1&2; 7 and 30 days after treatment by carbofuran.

3&4; 7 and 30 days after treatment by carbofuran and inoculated with *A. brasilense*.

5&6; 7 and 30 days after treatment by carbofuran and inoculated with *S. violaceusniger*.

7&8; 7 and 30 days after treatment by carbofuran and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

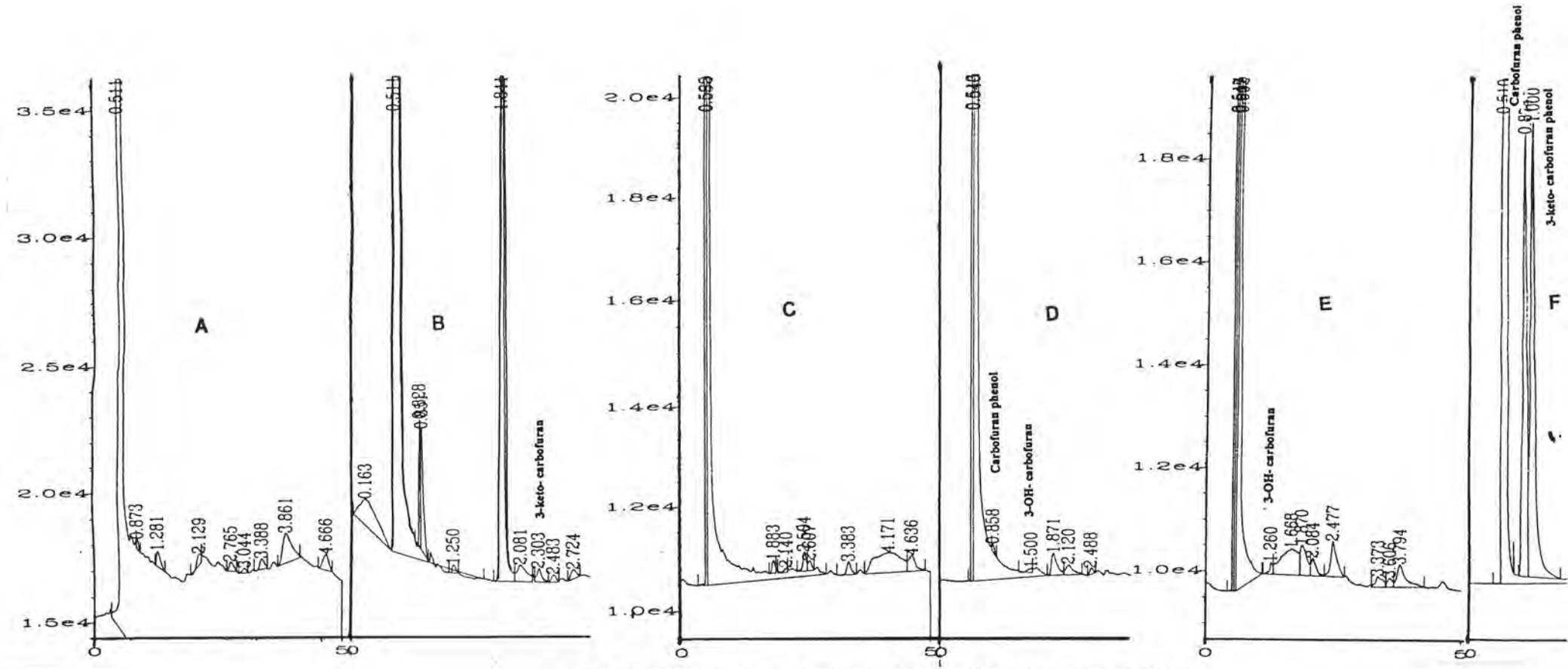


Fig 3. Gas liquid chromatography spectra of non-autoclaved soil extracts at 30 days of:

A ; Untreated soil(control) . B; Soil treated with carbofuran.

C; Soil treated with carbofuran and inoculated with *A. brasilense*.

D; Soil treated with carbofuran and inoculated with *S. violaceusniger*.

E; Soil treated with carbofuran and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

F; Standard carbofuran and its metabolites.

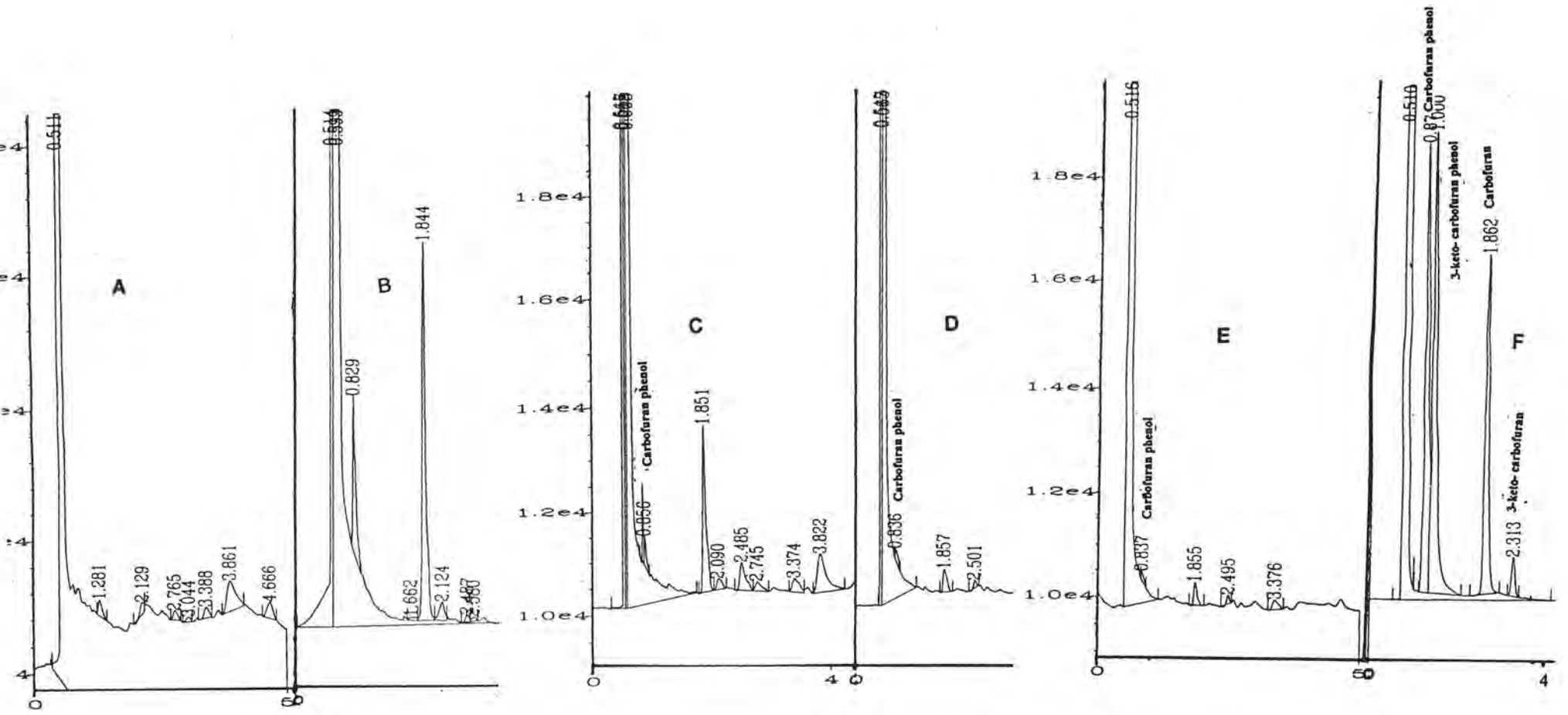


Fig 4. Gas liquid chromatography spectra of autoclaved soil extracts at 30 days of:

A ; Untreated soil(control) . **B; Soil treated with carbofuran.**

C: Soil treated with carbofuran and inoculated with *A. brasilense*.

D; Soil treated with carbofuran and inoculated with *S. violaceusniger*.

E; Soil treated with carbofuran and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

F; Standard carbofuran and its metabolites.

Also, analysis data of GLC and TLC which graphically illustrated in Figs (5, 6, 7 and 8) show periodical determination of produced products of temik. Data analysis of the non-autoclaved soil extracts revealed that in uninoculated soil three compounds were detected by GLC having Rt values 1.3, 1.44 and 3.4 minutes, the first one was identified as temik sulfone whereas the other two compounds could not be identified.

However, the 1st and 3rd compounds were found with negligent amounts while the second one was found with great amount. Analysis by thin layer chromatography showed only one compound having Rf value 0.17 after 7 days of incubation and two compounds having Rf values 0.17 and 0.21 after 30 days of incubation. These compounds were identified as temik sulfone and temik sulfoxide, respectively. Extracts of soil inoculated with *A. brasilense* analyzed by GLC showed six by-products having Rt values 0.92, 1.25, 1.31, 1.44, 1.75 and 1.89 minutes. All compounds appeared with negligible amount except those which had Rt 0.92 and 1.44 minutes appeared with great amounts.

The first compound was identified as temik sulfoxide and the remainders could not be identified, but the analysis of those samples by TLC detected only three by-products having Rf values 0.17, 0.21 and 0.49 , the first one was identified as temik sulfone and the second as temik sulfoxide, whereas the third one could not be identified. Also, GLC analysis of soil inoculated with *S. violaceusniger* showed eight by-products. Only two of them were in legible amounts having Rt values 0.91 and 1.44 minutes and the remainder six compounds were in negligible amounts. Among those metabolites was temik sulfoxide (Rt 0.91minute) which appeared with high quantity and temik sulfone (Rt 1.3 minutes) which was detected with small amount and the other ones were unidentified. TLC analysis of those samples showed only three compounds having Rf values 0.17, 0.21 and 0.49 which were identified as temik sulfone; temik sulfoxide and unknown compound.

Ultimately, GLC analysis of soil inoculated with the mixture of two strains revealed four compounds with legible amounts having Rt values 0.93, 1.24, 1.3 and 1.44 minutes. The first and the third compounds were identified as temik sulfoxide and temik sulfone, whereas the other two compounds could not be identified. Moreover, three other compounds were detected with negligible amounts which were not identified. TLC analysis of these samples revealed only three metabolites having Rf values 0.17, 0.21 and 0.49 which were identified as temik sulfone; temik sulfoxide and unknown compound. Identification of these metabolites was carried out by comparing their Rf and Rt values with those obtained by pure temik and its metabolites which recorded in (Table,5) and the corresponding compounds were recorded.

Results showed that in autoclaved and uninoculated soil, temik was detected by GLC with high peak until the 30th day of incubation, moreover three small peaks represent negligible amounts having Rt values 1.29, 1.49 and 1.94 minutes. The second one was identified as temik sulfone and the other two could not be identified. TLC of these samples showed only two spots having Rf values 0.89 and 0.18. These spots referred to temik and temik sulfone compounds, respectively.

Extract analysis of autoclaved and inoculated soil with *A. brasilense* by GLC showed two compounds having Rt values 1.3 and 1.43 minutes. The first could be identified as temik sulfone and was found in negligable amount, whereas the second was in legible amount but could not be identified. Analysis of those samples by TLC showed also two compounds having Rf values 0.18 and 0.27 which could be identified as temik sulfone and temik sulfoxide, respectively.

GLC analysis of autoclaved and inoculated soil with *S. violaceusniger* showed three compounds having Rt values 1.22, 1.29 and 1.44 minutes. The second one could be identified as temik sulfone, whereas the first and the third ones could not be identified. While, GLC analysis of autoclaved soil inoculated with a mixture of *A. brasilense* and *S. violaceusniger* showed six compounds as metabolites for temik. Two of them only could be identified as temik sulfone and temik sulfoxide while, the four remainder compounds could not be identified.

Also, the TLC analysis of these samples detected only the temik sulfone (Rf 0.18) and temik sulfoxide (Rf 0.27). This may be ascribed to their finding with small amounts or to the applied development system were not convenient for their separation.

Fig.5 .Thin layer chromatogram of non-autoclaved soil.

Fig. 6 .Thin layer chromatogram of autoclaved soil.

C ; Untreated soil(control). 1&2; 7 and 30 days after treatment by temik .

3&4; 7 and 30 days after treatment by temik and inoculated with *A. brasilense*.

5&6; 7 and 30 days after treatment by temik and inoculated with *S. violaceusniger*.

7&8; 7 and 30 days after treatment by temik and inoculated with *S. violaceusniger* and *A. brasilense* mixture

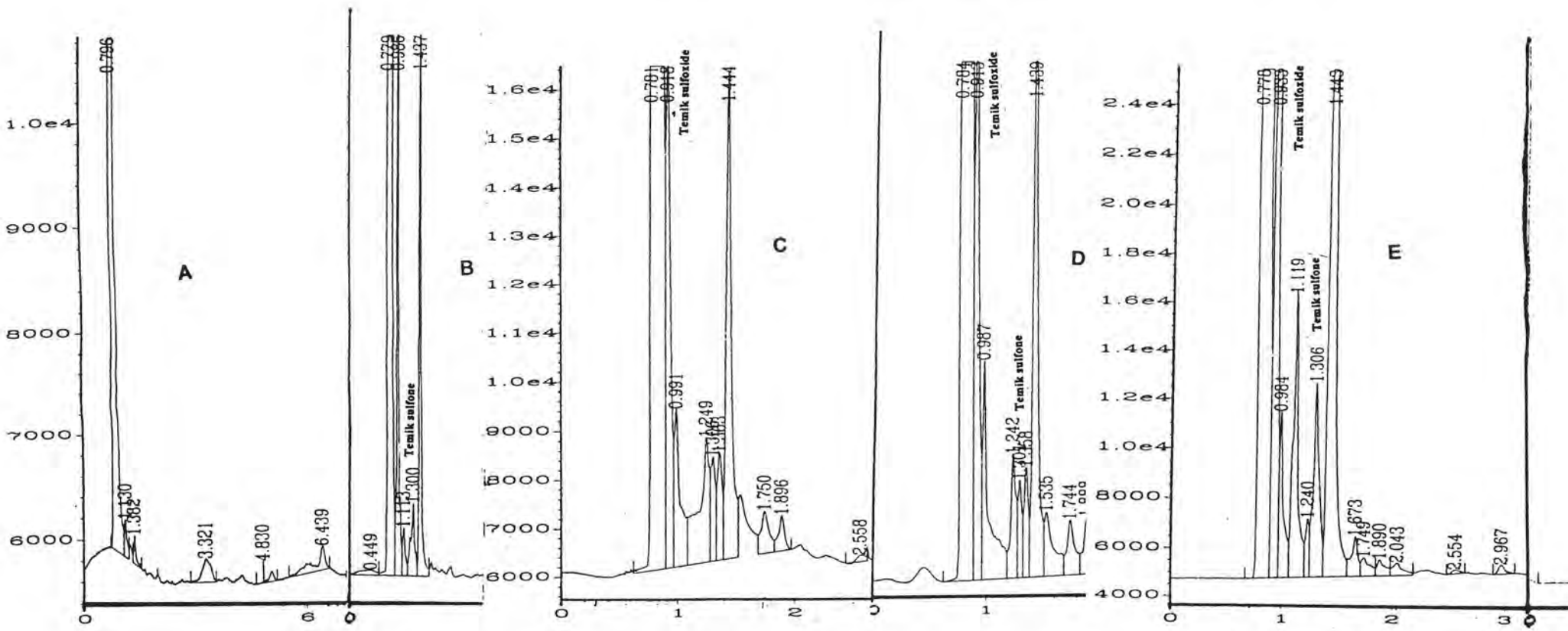


Fig 7. Gas liquid chromatography spectra of non-autoclaved soil extracts at 30 days of:

A ; Untreated soil(control) . **B; Soil treated with temik.**

C; Soil treated with temik and inoculated with *A. brasilense*.

D; Soil treated with temik and inoculated with *S. violaceusniger*.

E; Soil treated with temik and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

F; Standard temik and its metabolites.

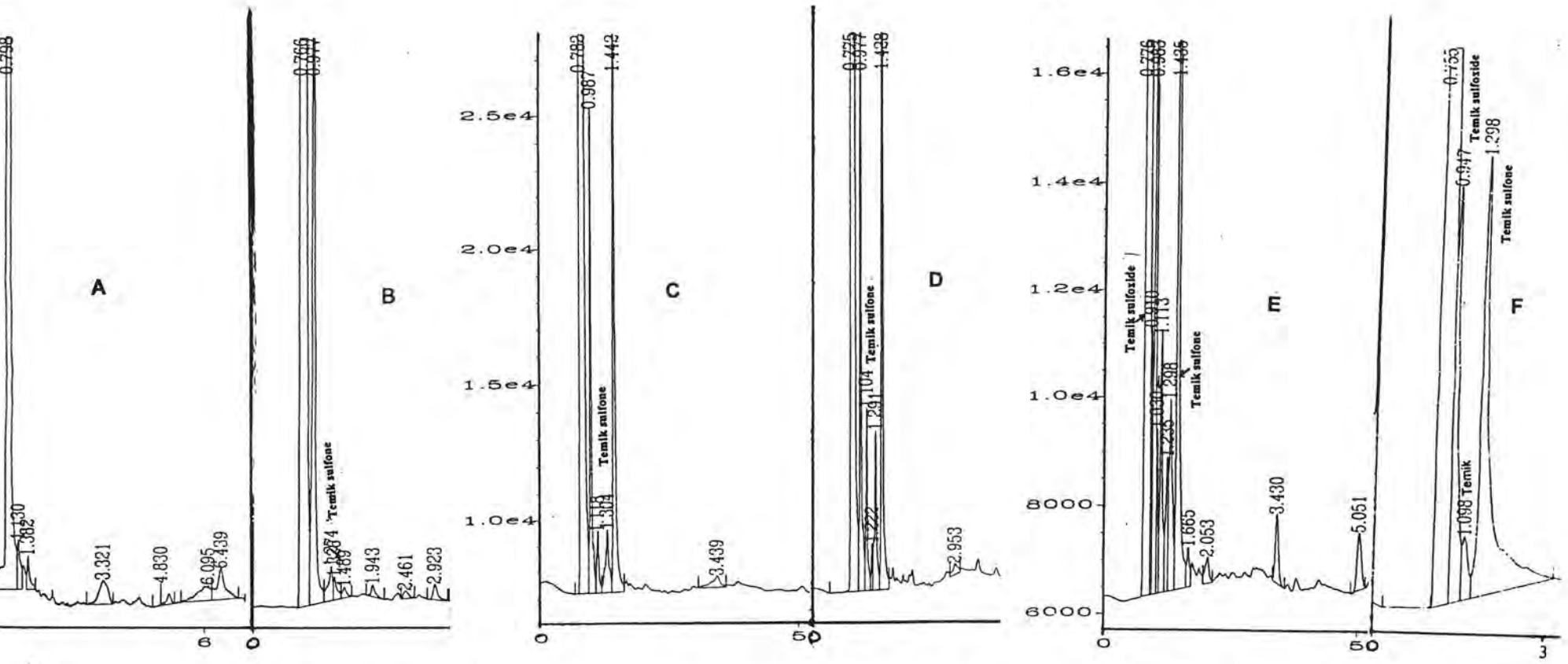


Fig 8. Gas liquid chromatography spectra of autoclaved soil extracts at 30 days of:

A ; Untreated soil(control) . **B; Soil treated with temik.**

C: Soil treated with temik and inoculated with *A. brasilense*.

D; Soil treated with temik and inoculated with *S. violaceusniger*.

E; Soil treated with temik and inoculated with *S. violaceusniger* and *A. brasilense* mixture.

F; Standard temik and its metabolites.

Summing up, obtained results on biodegradation of temik in autoclaved and non-autoclaved soil and the effect of inoculation with the local isolated strains, it could be conclude that temik was degradable in non-autoclaved soil faster than that in autoclaved one. Moreover soil inoculation with any of the strains enhanced the rate of temik biodegradation as well as that inoculated with the mixture of them. However, most of the transformed compounds were identified as temik sulfone and temik sulfoxide with small unknown compounds.

These results are in agreement with those obtained by **Ou *et al* (1986) and Alberto *et al* (2000)** who concluded that under aerobic conditions, aldicarb rapidly disappeared and aldicarb sulfoxide was rapidly formed. The latter in turn was slowly oxidized to aldicarb sulfone. Also, **Lightfoot (1987)** suggested that microbial oxidation is an important degradation mechanism in soil and he noticed that the breakdown of aldicarb residues to non-carbamates was largely than the result of chemical hydrolysis.

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المعالجة الحيوية للتربة الملوثة بالمبيدات الكربامية باستخدام ميكروبات الإستربتوميسيس فيولاثيوسنيجر والأزوسبيريللام برازيلنس

صمم هذا البحث لدراسة معدل اختفاء مبيدي الكربوفيوران(الفيورادان) و التميك (الألديكارب) من التربة بعد تلقيحها بالميكروبات التى أثبتت كفاءتها فى تكسير تلك المبيدات بالمزارع السائلة لميكروبات *S. violaceusniger* and *A. brasilense* في دراسة سابقة. حيث استخدم لهذا الغرض أرض معقمة وأخرى غير معقمة أضيف إليها كلا المبيدين كلٍ على حدة ثم لقحت بالميكروبات سابقة الذكر وخلطت جيداً وحضنت لمدة ثلاثون يوماً وأخذت منها عينات على فترات لتقدير متبقيات كلٍ من المبيدين ونواتج تحللها .كذلك تم دراسة أثر استخدام هذه المبيدات على الأعداد الكلية لميكروبات التربة ، أعداد الأكتينوميسيتات بها وكذلك على معدل نشاط إنزيم الديهيدروجينيز في التربة الغير معقمة. وقد أوضحت نتائج هذه الدراسة ما يلي:

عند إضافة كلاً من مبيدي الكربوفيوران أو التميك إلى التربة أدى إلى انخفاض أعداد الميكروبات الكلية بها وكذلك أعداد الأكتينوميسيتات ونشاط إنزيم الديهيدروجينيز و لكن تلقيح التربة بميكروبات *S. violaceusniger* or/and *A. brasilense* أدى إلى زيادة الأعداد الكلية لميكروبات التربة وكذلك أعداد الأكتينوميسيتات ونشاط إنزيم الديهيدروجينيز بالرغم من وجود المبيدات المذكورة. كذلك لوحظ أن معدل اختفاء مبيدي التميك والكربوفيوران بالتربة الغير معقمة كان أسرع مما فى التربة المعقمة.

أيضا أوضحت النتائج أن معدل اختفاء كلاً من المبيدين من التربة الملقحة بكلٍ من السلالتين على حدة كان أقل من تلك التى لقحت بمخلوط السلالتين. معدل اختفاء كلاً من المبيدين بالتربة الملقحة بـ *S. violaceusniger* كان أسرع من تلك التى لقحت بـ *A . brasilense* . عند تلقيح التربة بمزارع الميكروبات تحت الدراسة زاد معدل سرعة اختفاء كلاً من المبيدين وخاصة عند التلقيح بمخلوط السلالتين.

من المركبات المتحصل عليها كنواتج لتحلل مبيد الكربوفيوران(الفيورادان) في التربة مركب كربوفيوران فينول ، ٣-هيدروكسى كربوفيوران و٣-كيتوكربوفيوران ومركبات أخرى لم يمكن التعرف عليها. بينما نتج عن تحلل مبيد التميك (الألديكارب) بالتربة مركبات التميك سلفون والتميك سلفوكسيد و مركبات أخرى لم يمكن التعرف عليها.

راشد عبد الفتاح زغلول[١]- إحسان أحمد حنفى[١] - أحمد غنيم رحال[٢]- لبنى عبد العزيز موسى[٢]

١- قسم النبات الزراعي – كلية الزراعة بمشتهر – جامعة الزقازيق – مصر.

٢- قسم بحوث الميكروبيولوجيا الزراعية- معهد بحوث الأراضي والمياه والبيئة- مركز البحوث الزراعية – الجيزة-مصر.