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INFLUENCE OF SOME NEMATICIDES ON TOTAL BACTERIAL NUMBERS AND DIAZOTROPHS IN SOIL AMENDED WITH COMPOST

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ABSTRACT: *In an incubation experiment , the effect of three nematicides , namely carbofuran , oxamyl and cadusafas on the total bacterial count and diazotrophs in soil amended with compost was studied. Nematicides were add at two doses, i.e. recommended dose and 3 times of the recommended dose, enriched soil with compost at the levels 0, 1 and 2 %. Control treatments were run parallel. The treatments were moistened constantly to 60% of the water- holding capacity of the soil and incubated at 30+/- 2 °C. Diazotrophs namely, Azotobacter spp., Azospirillum spp. and Bacillus spp. were concerned herein.*

The results obtained revealed that, adding oxamyl and cadusafas at the recommended doses increased the total bacterial numbers in soil, but carbofuran led to decrease them. Increasing nematicides to 3 times of the recommended doses led to more decreases in the total bacterial numbers for the three nematicides, following the order: carbofuran >oxamyl> cadusafas.

Addition of compost at 1% and 2% stimulated the proliferation of bacteria in soil, as appeared in increasing the total bacterial numbers and diazotrophic agents, reaching their highest values after 30 days of incubation.

Incorporation of compost mixed with nematicides diminished the deleterious effects of such agrochemicals and encouraged the bacterial reproduction in soil. The improvement of soil fertility was achieved in soil , confirming the positive effect of organic fertilization.

Key words: *Diazotrophs , Total bacterial numbers, Nematicides, Compos,.*

INTRODUCTION

Pesticides, the synthesized chemicals eradicate various pests, are applied mainly as leaf spray or direct soil treatment. Effect of pesticides on soil microorganisms has been studied and found to differ according to soil properties, growing plant, application mode and dose of the compound and agriculture practices, i. e. manuring, fertilization, and irrigation (Harris, 1988;

Malkomes, 1992). Nematicides are important category of pesticides, that are directly introduces onto the soil to control the funal nematode pests.

The literature indicated discrepancies in regard to the effect of nematicides on soil microorganisms. For instance, carbofuran active ingredient, at 1.62 kg / h^{-1} did not show adverse effect on soil microorganisms (Duah-Yentumi and Johanson, 1986). Das and Mukherjee(2000) found that, application of four insecticides, HCH, phorate, carbofuran and fenvalerate at their field application rates, under laboratory conditions, significantly increased the population of bacteria, actinomycetes and fungi in soil. Similarly, N_2- fixing bacteria was also stimulated with the same insecticides (Das and Mukherjee, 1998).

Application of organic matter or chemical fertilizers to the soil frequently increases the microbial population of soil under temperate climate conditions, organic fertilizers provided a greater increase in microbial populations than chemical fertilizers (Bolton *et al.*, 1985) due to the increase in organic carbon. However, under tropical climate conditions, chemical and organic fertilizers increased microbial populations and microbial carbon and nitrogen (Goyal *et al.*, 1992).

In recent years, many attempts have been undertaken to use various nitrogen – fixing bacteria for increasing the productivity of plants. However, associative nitrogen fixation is defined as nitrogen fixation by free – living diazotrophs under the direct influence of the host plant. In an associative nitrogen- fixing system, plants supply the diazotrophs with organic substrates (exudates, secretions, lysates and sloughed off cells). In turn the microorganisms fix atmospheric nitrogen that is directly or indirectly transferred to the plants. Most of nitrogen – fixing bacteria involved in associated symbiosis are heterotrophic organisms which require a large supply of organic substrates to generate energy needed to fix nitrogen (Mehanni, 1995).

Melero *et al.* (2007) found that addition of two mature composts (vegetal and animal compost)at rates of 30 tm ha-1 over a 4-year period under a dryland system, increased in quantity of total organic carbon and quality (humic acids) of organic matter compared with inorganically fertilized soil. The nutrient content of P and N also showed increases in the plots fertilized by compost. Increases in microbial biomass were more clearly with adding compost.

The present work is an attempt to clear up the influence of three nematicides, usually applied in Egyptian agriculture, namely carbofuran, oxamyl and cadusafas, on total bacterial count and number of some diazotrophs (*Azotobacter spp., Azospirllum spp.* and *Bacillus spp.*) in soil amended with compost.

MATERIALS AND METHODS

Soil:

Samples of agricultural soil were collected from the upper layer (0-20cm) of Nile-alluvium, non –saline from Shibin El-Kom, Menofiya, air-dried and ground to pass a 2-mm sieve. Routine analyses of these soils were carried out following the methods described by Page *et al.* (1982). Data obtained are presented in Table (1). The soil was poor in organic matter and having pH value slightly above neutrality. Texture of the soil was silty clay loam.

Table (1): A analytical data of the experimental soil.

A. Physical Properties

$CaCO_3$ (%)	Organic matter(%)	Particle size distribution(%)				Textural class
		Coarse Sand	Fine Sand	Silt	Clay	
5.2	0.34	14.5	45.3	13.6	25.3	Silty clay loam

B. Chemical Properties

PH*	EC** dSm^{-1}	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^+	Na^+	CO_3^-	HCO_3^-	Cl^-	SO_4^-
7.3	2.4	7.02	10.66	0.41	6.00	-	2.6	7.84	13.65

*In the pH 1: 2.5 soil / water suspension & ** Electrical conductivity in the 1: 5 soil / water extract (w/v).

Compost:

Compost was mixed with the soil at three rates, i.e. 0, 1 & 2%. Chemical analysis of the compost used is presented in Table (2). It contained 60.34% organic matter.

Table (2): Characteristics of the compost used

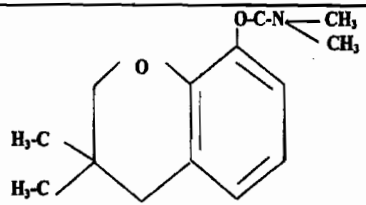
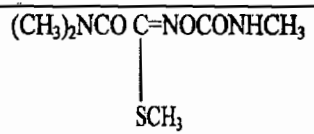
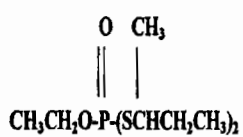
PH	EC. (dS/m^{-1})	Organic C (%)	Organic matter(%)	Total N%	C/N Ratio	Total K (%)	Total P (%)
7.62	4.90	34.99	60.34	1.45	24.1	1.7	1.8

Organic C* = Organic carbon

Nematicides:

Three nematicides having the specifications described in Table (3) were employed in this work. Sub samples of 200 g sifted air dry soil was weighed. Finely pulverized compost was added to each sub sample, at rates of 0, 1 and 2% and entirely mixed, then placed in plastic pots (having dimensions of 15 cm height and 10 cm diameter). The nematicides were added (as colloidal solutions) to the soil at the recommended doses, and three times of the recommended doses. All treatments were moistened with deionized water to 60% of the water- holding capacity of the soil and kept constant along45- day experimental course , by compensating the evaporation losses every three days (by weighing the pots). Each treatment was replicated 12 times , i.e., three for counting the total bacterial numbers, three for enumerating each of the diazotrophs, i.e., *Azotobacter spp.* , *Azospirllum spp.* and *Bacillus spp.* in soil.

Table (3): Pertinent data on the nematicides used (quoted from Tolmin, 2006).

Common name	Trade name	Imperical Formula (chemical composition)	Molecular Structure	Physical form	Recommend dose (per feddan)	Active Ingredient	Nutrient contents (%)
Carbofuran	Furdan	2, 3- dihydro-2, 2-dimethyl benzofuran-7- methyl carbamate		Granules grey	10kg	10%granules	C 11.77 N 7.00
Oxamyl	Vydate	Methyl 2-(dimethyamino)-N-[(methylamino) carbony] oxy]-2- = oxoethanim- Idothioate		Colourless crystals, with a garlic-like odour	2 L	240 g/L	C 38.36 N 19.20
Cadosafos	Rugby	O-ethyl 5,5-bis (1-methyl-propyl) phosphorodithioate		Colourless to yellow , liquid	600ml	200 g/L	C 44.40 P 11.48

* Feddan = 4200 m^2

Experimental :

Soils in the pots, at each incubation interval (0, 7, 15, 30 & 45 days), were wholly exhausted for enumeration of bacteria and the assigned diazotrophs.

The plate count method of Holm and Jenseon (1972) was adopted for enumerating total bacterial numbers , using soil extract agar. Most probable number (MPN) techniques on moified Ashbys media for *Azotobacter spp.* (Hegazi and Niemela, 1976) , N-deficient media for *Azospirllum spp.* (Dobereirner *et al.,* 1976) and growing N_2- fixing becilli media (Hino and Wilson, 1958).

RESULTS AND DISSCUTION

Total Bacterial Count in the Soil

Results illustrated in Figs.(1,2, and 3) showed that, the total bacterial counts in the control soil increased with time reaching maximum on the 15 day of incubation, thereafter began to decline. Addition of compost at 1or 2% resulted in marked increases in the total bacterial numbers in soil, reaching the maximum after 30 days, with the highest number of bacterial colonies detected with 2% compost. Increases over the control reached 142.2 and 326.8% by using 1 and 2% compost, respectively. This was actually attributed to the beneficial effects of compost as organic matter, which represents the nutritional and energy sources for the major part of soil microbial population, namely, the chemoorganotrophs, besides its direct and indirect roles in soil conditions for the favour of biological activity (Zebarth *et al.*, 1999).

Incorporation of the nematicides tested, i.e. oxamyl and cadusafas at the recommended doses led to stimulate the counts of bacteria. This may be attributed to utilization of such nematicides and their degraded products by the soil microorganisms to derive energy and some nutrient elements for cellular metabolism, particularly phosphorus and sulpher. This result agrees with those obtained by (Bhuyan *et al.*, 1993; Das and Mukherjee, 2000). On the other side, addition of carbofuran to the soil at the recommended dose or 3 times decreased the counts of bacteria. Any way, the addition of nematicides at the higher rates resulted in more decreasing of the bacterial counts in soil. Carbofuran was the most inhibitor in such concern than oxamyl and cadusafas, in order. Generally, extent of inhibition obtained for the nematicides were 38 > 29 > 13% for carbofuran, oxamyl and cadusafas, respectively. These data are in harmony with the result obtained by (Kennedy *et al.*, 1999; Kalam and mukherjee, 2001; and Zaghloul *et al.*, 2003).

It can be seen that the harmful effect of any of the tested nematicides was limited to the earlier periods following its application. Thereafter, such chemicals stimulated the development of microorganisms (Fletcher and Smith, 1964). This stimulating effect might be due to the possibility that, a pesticide applied several times may produce changes in the composition of microflora complexes by increasing the number of microorganisms resistant to the pesticide or decomposing it (Kaufman and Kearney, 1965). On the other hand, such increases in numbers might be due to suppression of protozoa, (Ilijin, 1962).

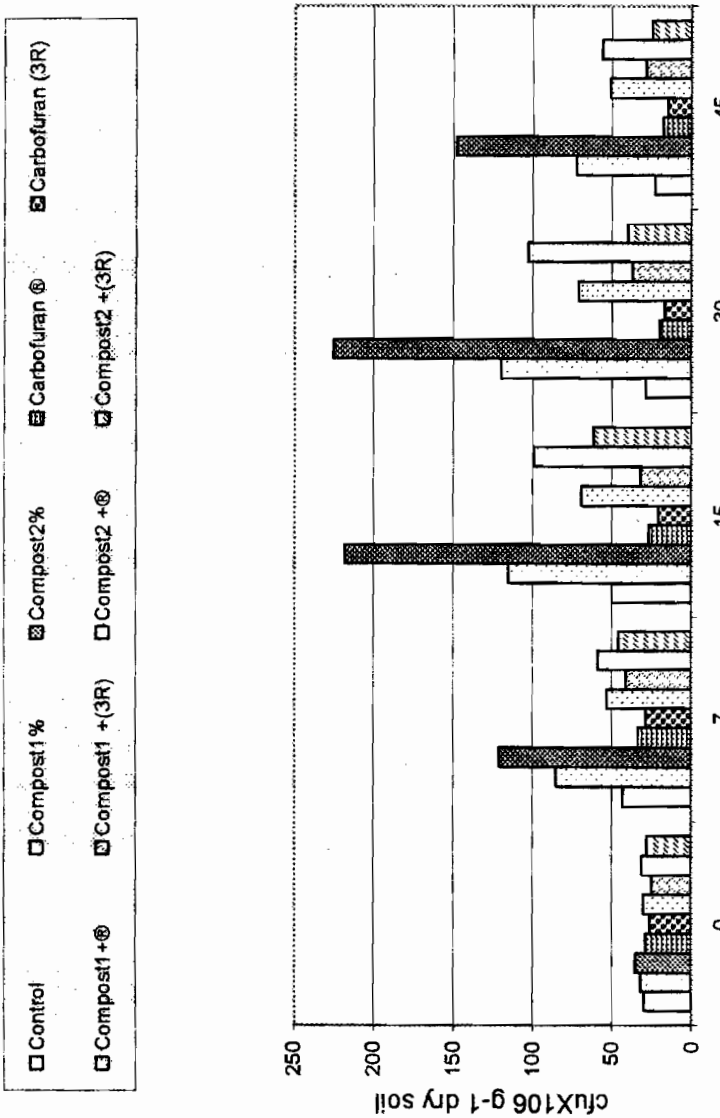


Fig.(1): Effect of different rates of carbofuran and compost on total bacterial count in soil

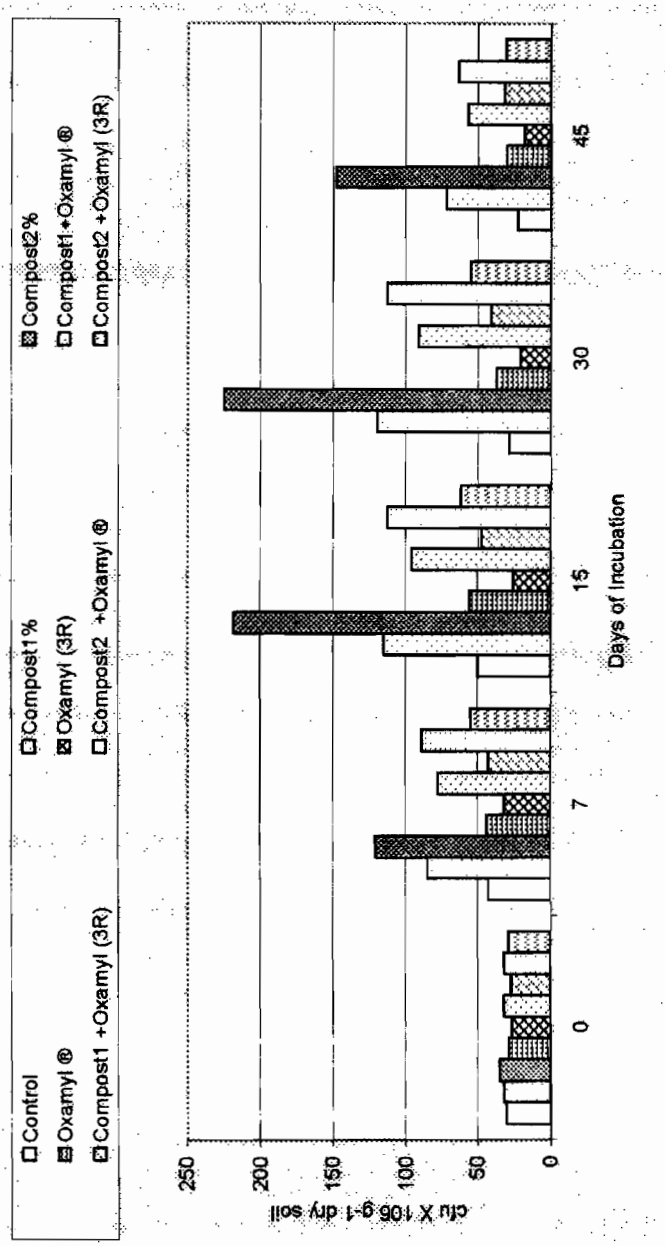


Fig.(2): Effect of different of rate of oxamyl and compost on total bacterial count in soil.

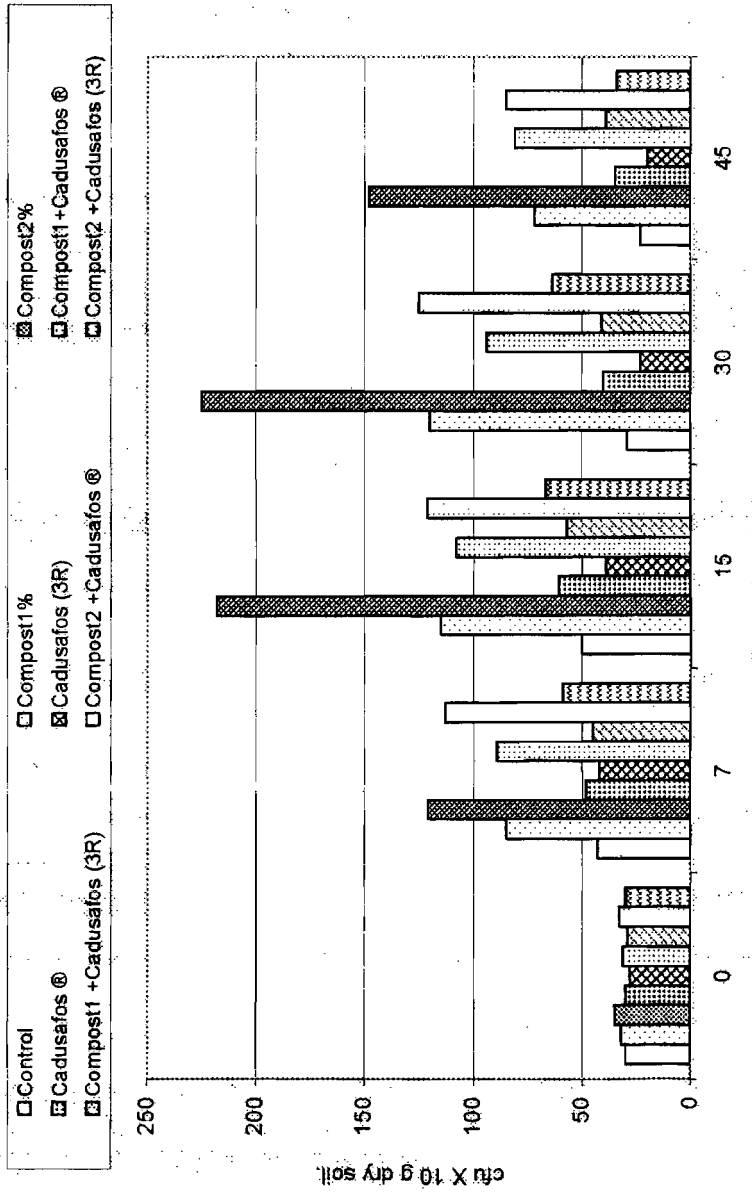


Fig.(3):Effect of different rates ofcadusafos and compost on total bacterial count in soil.

With regard to the influence of the interaction between nematicides and compost on the counts of bacteria in soil, it was obviously noticed that, generally the addition of compost to soil alleviated the deleterious effects and led to stimulate the proliferation of bacteria in soil. This result confirms the findings of earlier workers, i.e. (Bhuyan *et al.*, 1993, El-Shahawy *et al.*, 1999, Das and Mukherjee, 2000 and Lee *et al.*, 2004) who pointed out that different bacteria and *streptomyces* utilized the degraded products of nematicides to derive energy, carbon and other nutrients for their growth. Also, addition organic matter alleviated adverse effects of nematicides.

Counts of Diazotrophs in the Soil

A: *Azotobacter* :

Changes in *Azotobacter* counts with the different soil treatments are drawn in Figs.(4,5 and 6). Data obviously showed that, the addition of 1% compost to the soil increased the counts of *Azotobacter* compared with control when gave 168 % above the control. Also, addition of 2% compost was more pronounced in increasing the counts to record 208 % over the control. It is known that organic matter introduced to soil stimulates soil microbial populations and biological activity. This increase is due to the improvement in environmental conditions such as soil temperature, moisture content and nutrients. Also, compost contained high organic carbon compounds which represent the source of carbon and energy for the heterotrophic microorganisms. These results agree with those obtained by Lee *et al.*,(2004).

Incorporation of nematicides at the recommended doses caused slight decreases in the counts of *Azotobacter* in the soil. Decreases in counts, as compared with the control reached 18.11, 76.0 and 26.05 %. On the other hand, using 3 times of the nematicide recommended doses, exhibited more inhibition, and caused high deleterious effect on *Azotobacter* counts in the soil. Decreases in the numbers reached 36.97, 26.89 and 45.37 % with addition carbofuran, oxamyl and cadusafas, respectively. This results coincided with that obtained by (Fayez and Farahat, 1983, Kale, 1989, Aboel Naga,1989 and Santos and Flores,1995), who observed that *Azotobacter spp.* might be suppressed by nematicides treatment of soil.

Incorporation of compost together with the nematicides, generally alleviated the deleterious effects of nematicides specially at higher doses. This result may be due to acceleration of microbial degradation of such toxic substances. (El-Shahawy *et al.*, 1999) reported that amendment of soil with straw alleviated deleterious effects of pesticides, namely furdan, captan and oxamyl.

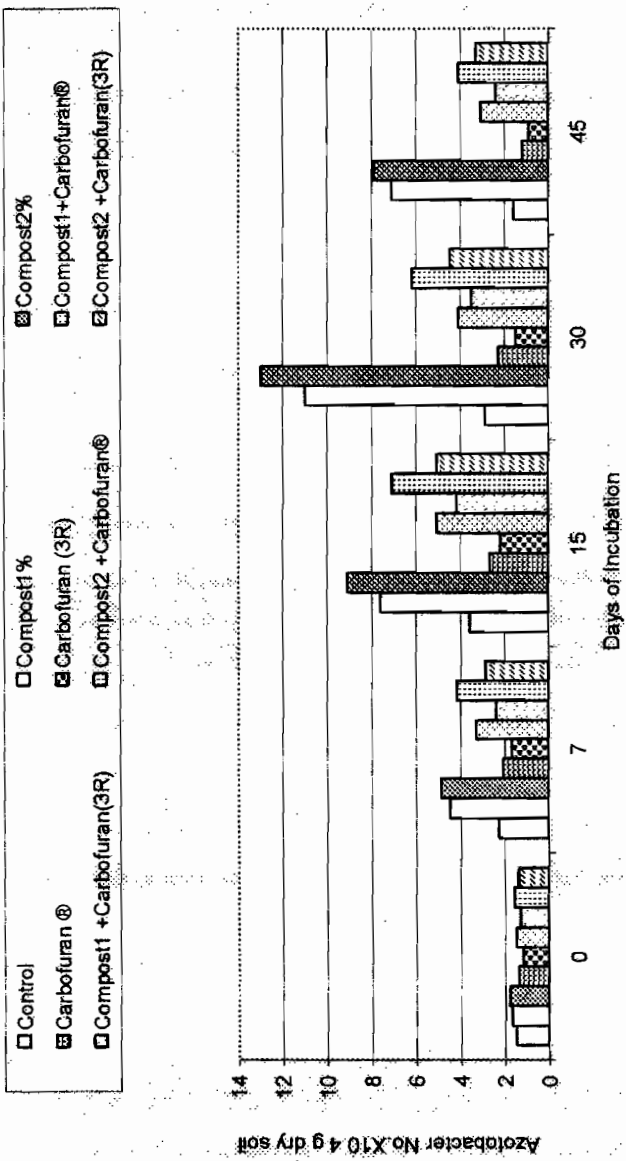


Fig.(4) Effect of different rates of carbofuran and compost on Azotobacter count in soil.

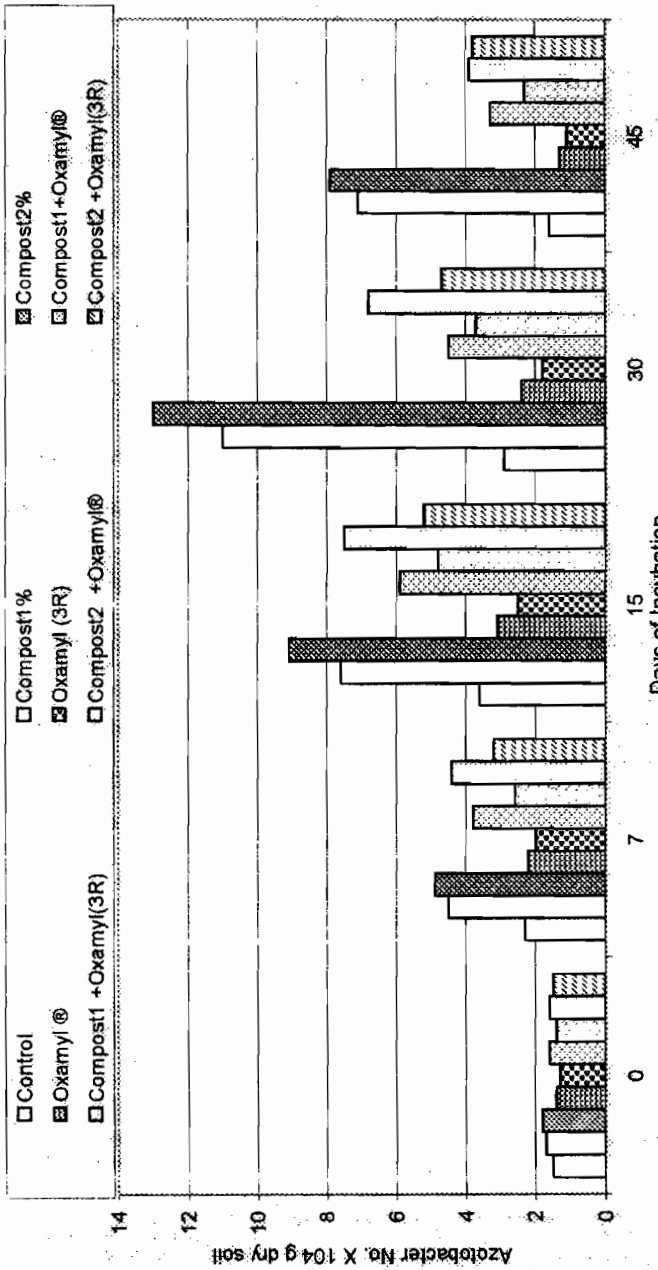


Fig.(5):Effect of different rates of oxamyl and compost on Azotobacter count in soil.

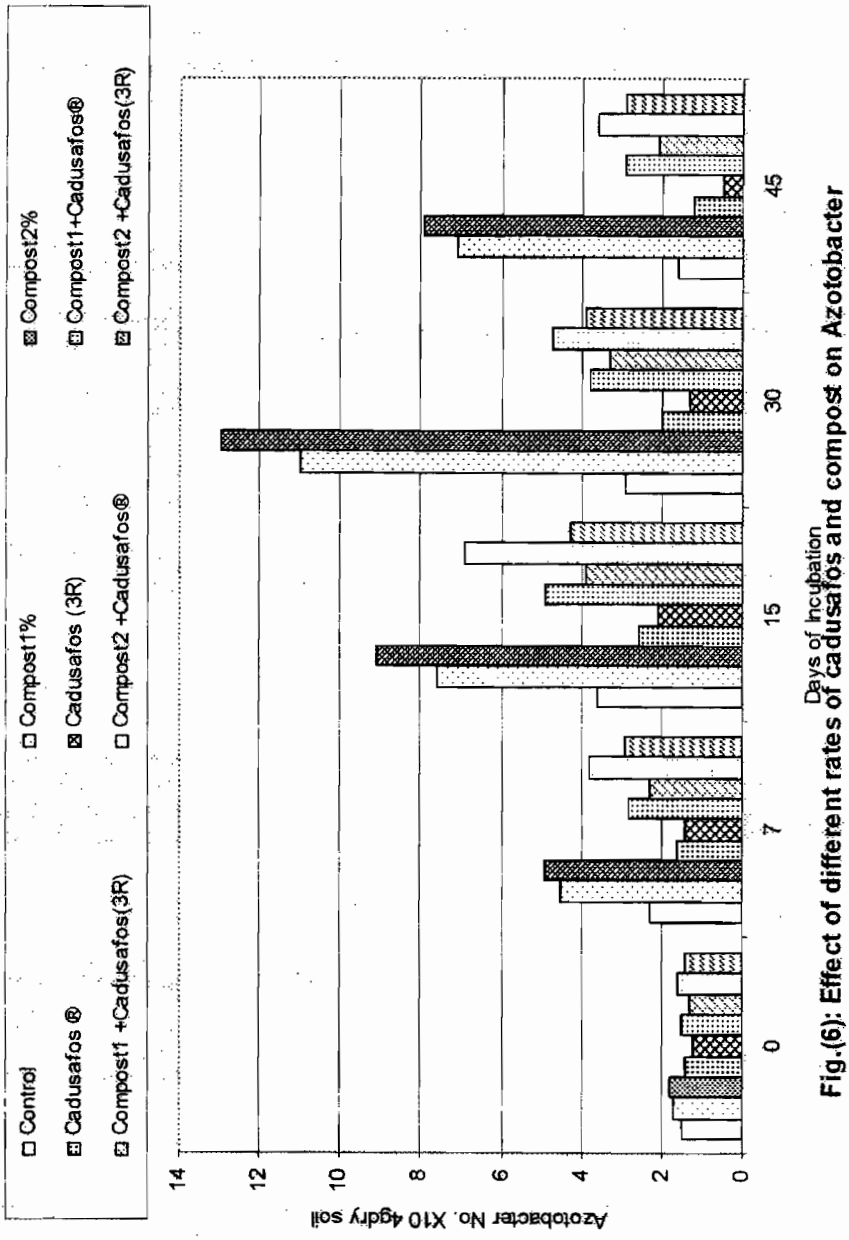


Fig.(6): Effect of different rates of cadusafos and compost on Azotobacter count in soil.

B: *Azspirillum*

Changes in *Azospirillum* counts in the different experimental treatments are revealed in Figs. (7 to 9). Results showed that the used compost its two rates, 1 and 2 % enhanced the counts of *Azospirillum* in the soil. Maximum values were 22X 10^4 and 25X 10^4 cell / g soil at 30 th day of incubation with 1 and 2 % compost, to reach 130 and 179% above the control, respectively.

On the other hand, addition of the nematicides (carbofuran, oxamyl and cadusafas) at their recommended doses reduced *Azospirillum* counts in the treated soil. The reductions were more pronounced upen elevating the nematicides doses. Data showed the following order of inhibition : cadusafas > carbofuran> oxamyl. Gallori, 1991; El-Shahawy *et al.*, 1999 and Revellin, 2001) denoted that *Azospirillum* counts might be suppressed by carbofuran and thiram treatments in soil. On the contrary, Jena *et al.*, 1992and Kanungo *et al*, 1995) found that carbofuran increased the counts of diazotrophs under rice cultivation.

Incorporation of compost at the rate 1 % with the lower doses of nematicides diminished the deleterious effect of the agrochemicals on *Azospirillum* counts in the

soil and caused increases in the counts reaching 60. 06, 96.48 and 46.3 % above the control with carbofuran, oxamyl, and cadusafas, respectively. Addition of the compost with the higher doses of the nematicides lowered the inhibitory effect of cadusafas from -25.2 to -5.7 % as compared with control, and caused slight increases with carbofuran and oxamyl reaching 25.8 and 10.8 %, respectively.

Higher rates of the compost (2 %) applied with both doses of the nematicides diminished the harmful effect of all them and led to more increases in *Azospirillum* counts, when gave increases up to 107, 130, and 75.39 % and 34, 62.9 and 10.86 % for the lower and higher doses of carbofuran, oxamyl and cadusafas, respectively. These results are in harmony with those recorded by El-Shahawy *et al.* (1999) and Lee *et al.* (2004), who found that the addition of organic matter to soil stimulated the proliferation of soil microorganisms and biological activity.

C: *Bacillus :*

Changes of *Bacillus* counts in the different treatments of the present study are shown in Figs.(10 to 12). In the control treatment, the counts increased to5X10^4 cells / g dry soil on the 15 day of incubation, thereafter they began to gradually decline by elapsing the time of investigation .

Incorporation of compost to the soil at the rates of 1 and 2 % increased the counts of *Bacillus* in soil and reached the maximum values, i.e. 18 X 10^4 and 21X 10^4 after 30 days of incubation, representing 173,36 and 233 % above the control for the rates, respectively. Lima *et al.* (1996); Zebarth *et al.* (1999); abdel-wahab *et al.* (2002) and Badawi (2003), came to similar conclusions.

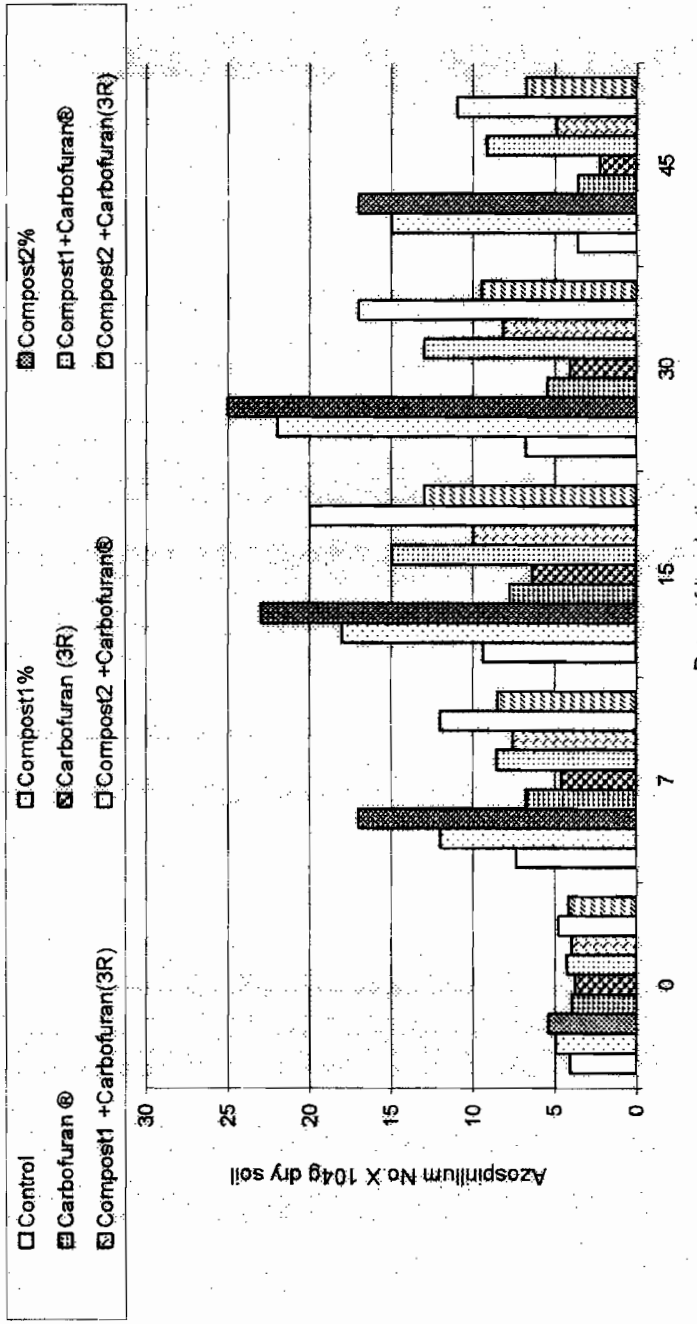


Fig.(7):Effect of different rates of carbofuran and compost on Azospirillum count in soil.

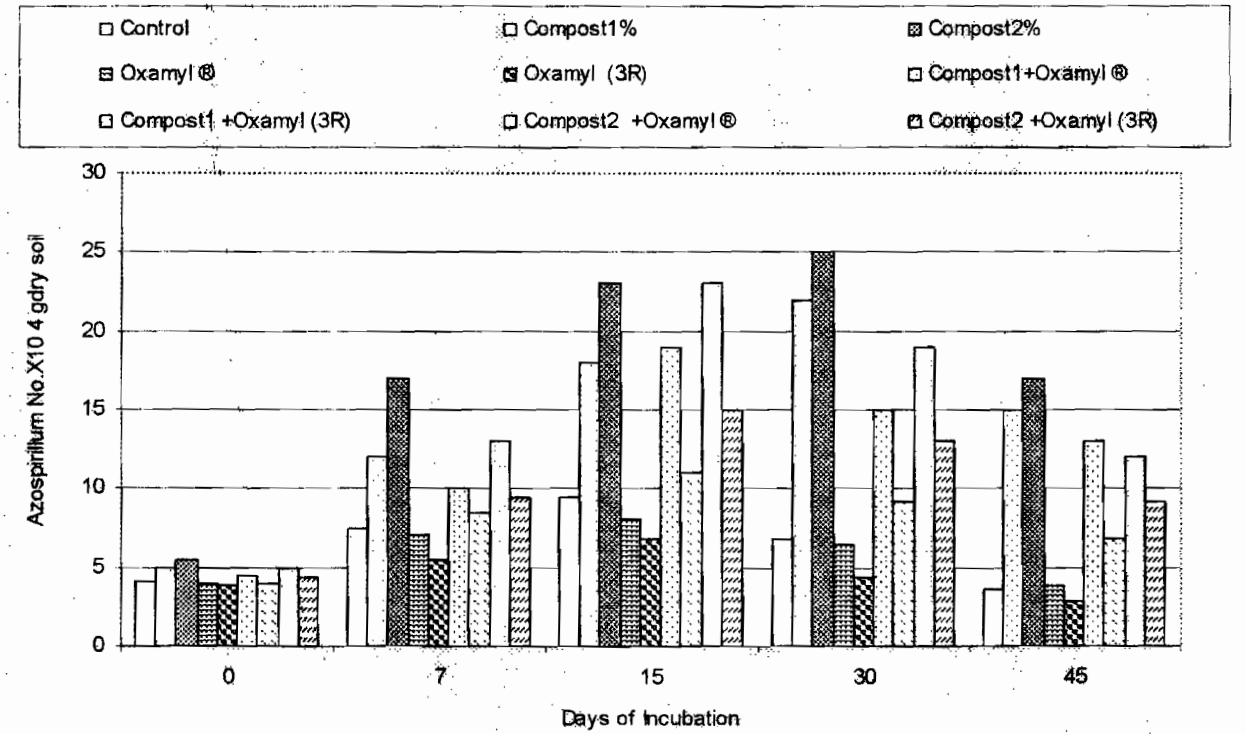


Fig.(8):Effect of different rates of oxamyl and compost on Azospirillum count in soil.

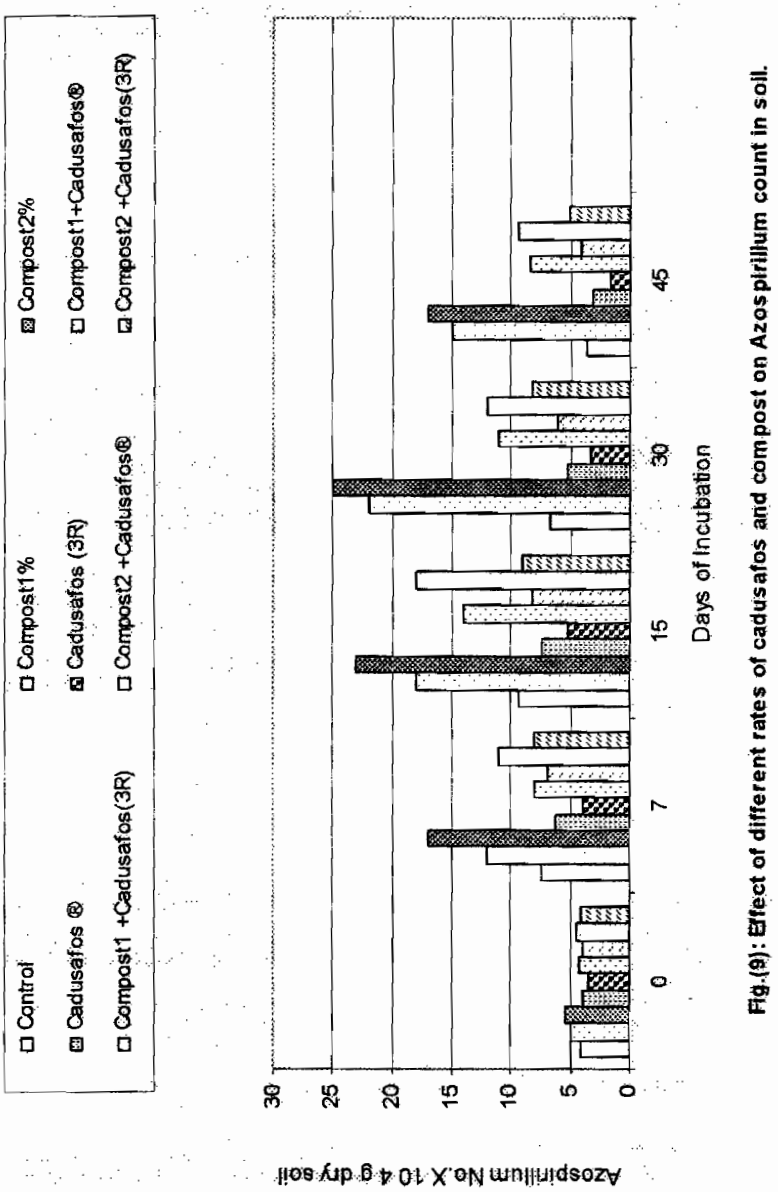


Fig.(9): Effect of different rates of cadusafos and compost on Azospirillum count in soil.

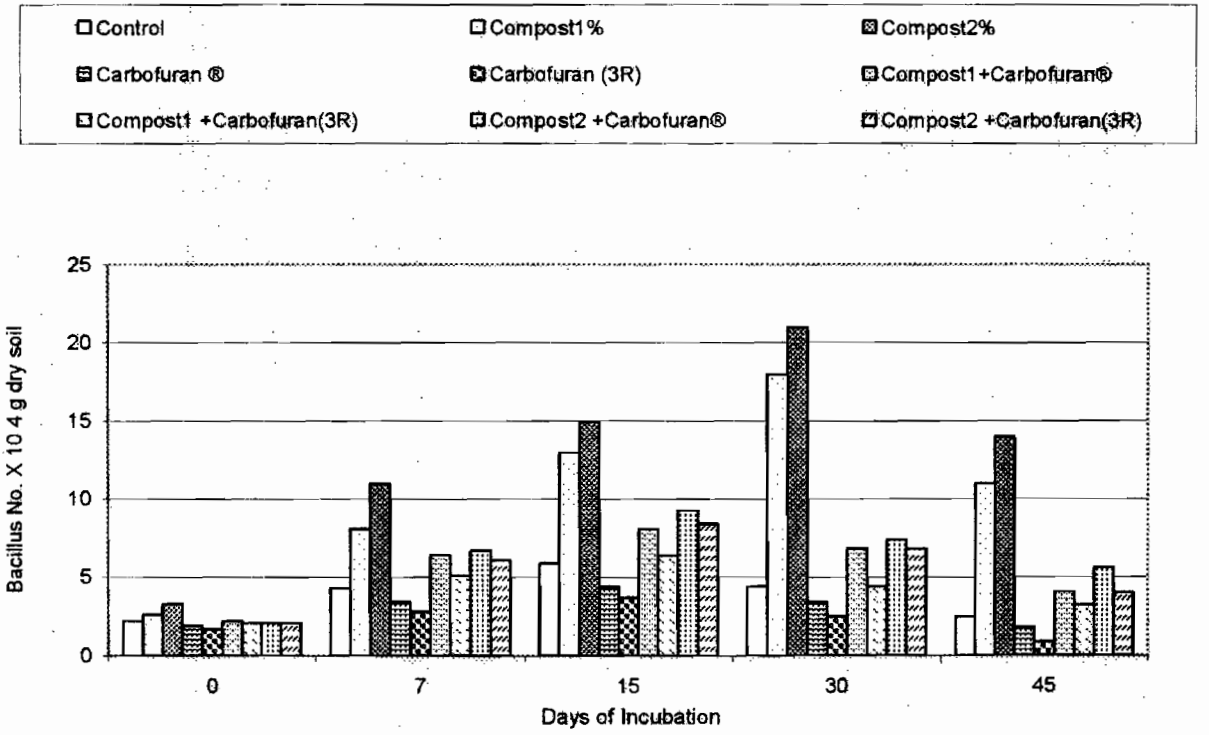


Fig.(10):Effect of different rates of carbofuran and compost on bacillus count in soil.

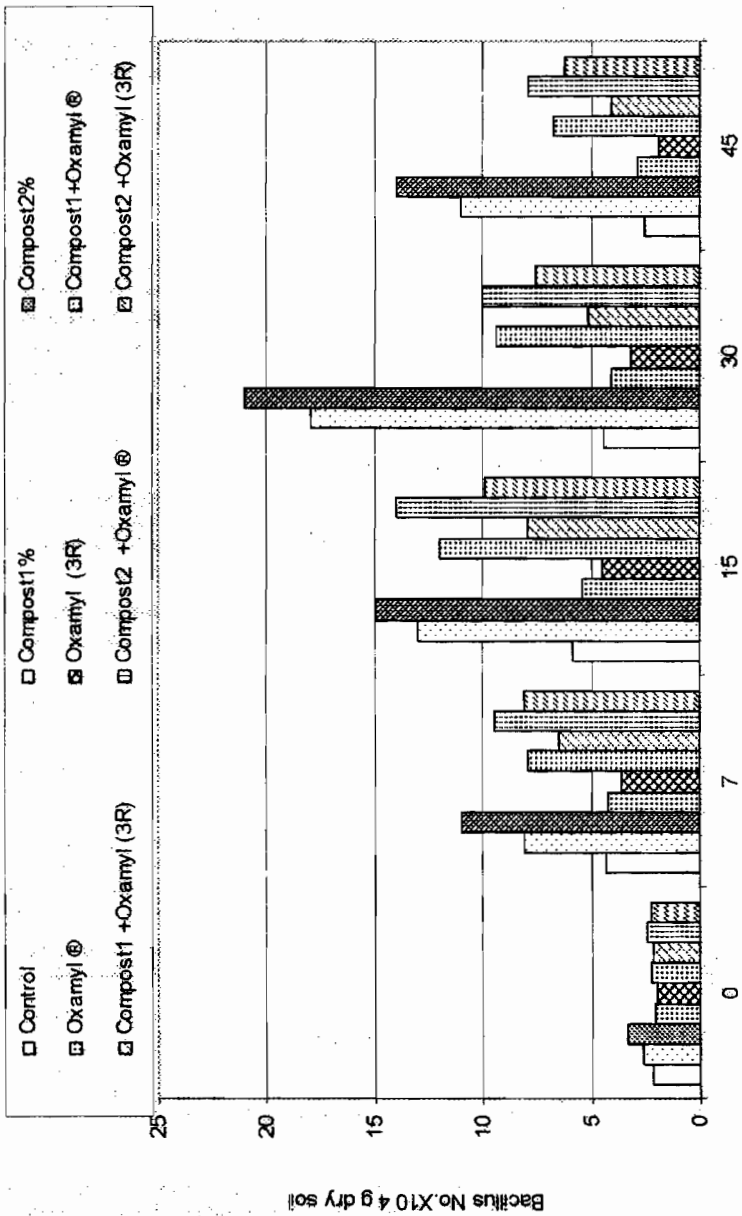


Fig .(11):Effect of different rates of oxamyl and compost on bacillus count in soil.

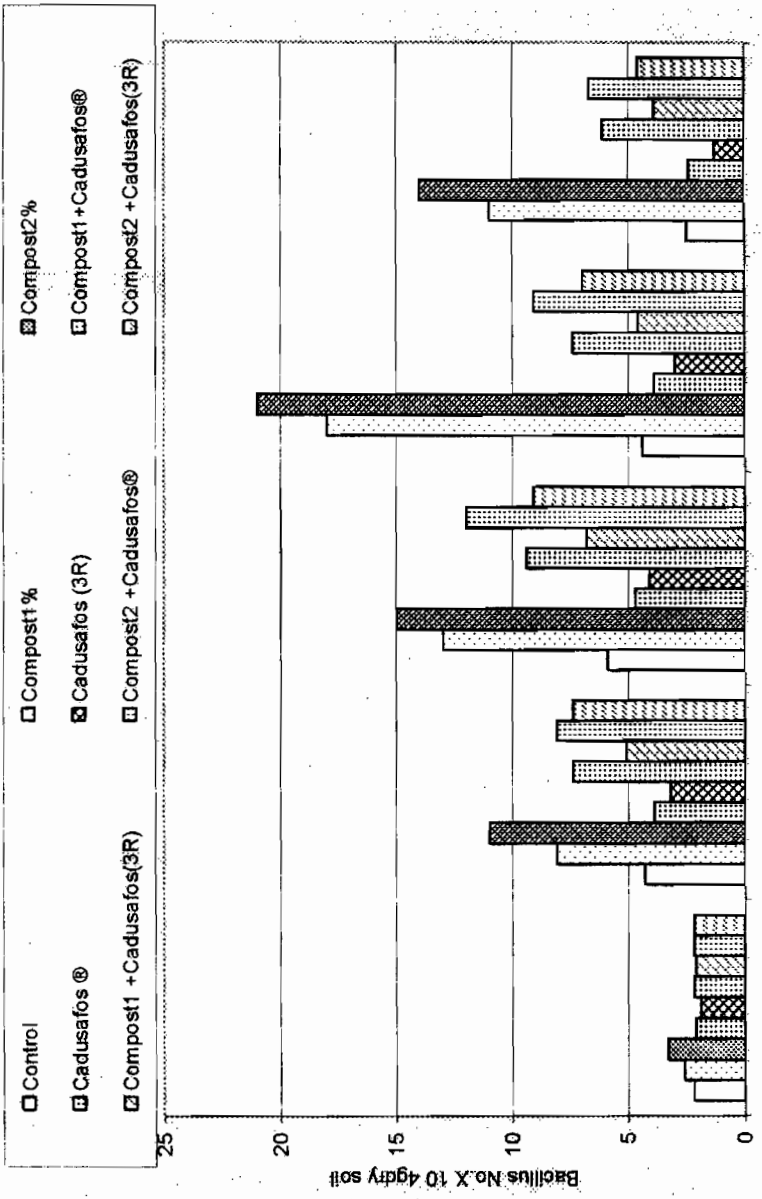


Fig.(12):Effect of different rates of cadusafos and compost on bacillus count in soil.

Introduction of nematicides at the recommended doses caused slight decreases in the counts of *Bacillus* numbers in soil, to make 21.57, 3.1 and 11.9 % below the control. Such decreases were more pronounced by using the higher doses of the nematicides, when reached 38.9, 21.24 and 30% below the control, for carbofuran, oxamyl and cadusafas, respectively. Concerning the incorporation of compost at the rate of 1 % with either dose of the nematicides diminished their deleterious effects and stimulated the *Bacillus* population in the soil, and caused increases reached to 43.26, 98.96 and 68. 39% above the control and 11.57, 33.67 and 16.58% for carbofuran, oxamyl and cadusafas at the lower and higher doses, respectively. Application of compost at the rate 2 % with either dose of the nematicides, diminished their harmful effect and led to more increases of *Bacillus* counts in the soil, to reach 61.08, 126.9 and 97.4% with the lower doses and 42.2, 76.68 and 56.99 % with the higher doses of carbofuran, oxamyl and cadusafas, respectively.

These results are in harmony with those recorded by (Fayez and Farhat , 1983; Jena *et al.*, 1992; el-Shahawy *et al.*, 1999 and Zaghloul *et al.*, 2003) who reported that, the addition of either carbofuran, oxamyl or cadusafos to soil led to a decrease in total microbial count and diazotrophs, i.e. *Azotobacter, Azospirillum and Bacillus.* It was also showed that, the addition of organic matter to soil resulted in alleviating the deleterious effects of pesticides and improvement of soil conditions to microbial proliferation and subsequently accelerates degradation of such agrochemicals by soil microorganisms. This depends on the nature of the pesticide and its rate of application, soil properties, growing plant, and environmental conditions (El-Shahawy *et al.*, 1999).

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تأثير بعض المبيدات النيماتودية مع إضافة الكومبوست على أعداد البكتيريا وبعض مثبتات النيتروجين الجوى فى الأرض

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الملخص العربي

فى تجربة معملية ، تم دراسة تأثير ثلاث مبيدات نيماتوية تستخدم فى الزراعة المصرية وهى : الكربوفيران و الاكساميل و الكادوسافوس فى وجود الكومبوست أو عدم وجود الكومبوست على أعداد بكتيريا الأرض.

حيث تم إضافة المبيدات على مستويين : المعدل الموصى به و ثلاثة أمثال المعدل الموصى به .وكذلك تم استخدام الكومبوست بمعدلين هما ١ و ٢% . وتم ضبط الرطوبة عند ٦٠ % من السعة المائية الكلية للأرض ، مع تعويض الفاقد بالوزن. وقدرت الإعداد البكتيرية الكلية و مثبتات الازوت : الازوتوباكتر ، الازوسبريلليم و الباسلس ،على فترات من التحضين الميزوفيلى للمعاملات .

وأظهرت النتائج المتحصل عليها أن إضافة الاكساميل و الكادوسافوس بالمعدل الموصى به شجع نمو البكتيريا فى الأرض بصفة عامة ،حيث زادت أعدادها ، بينما أدت إضافة الكربوفيران الى تناقص العدد الكلى للبكتيريا وكذلك أدت إضافة الجرعة العالية من المبيدات النيماتودية الثلاثة الى نقص كبير فى العدد الكلى للبكتيريا فى الأرض و كذلك أخذت أعداد البكتيريا المثبتة للنيتروجين نفس الاتجاه . واتبع معدل التثبيط الترتيب التنازلي التالي : الكربوفيران ثم الاكساميل ثم الكادوسافوس.

وأدت إضافة المادة العضوية (الكومبوست) بأى من معدليها إلى زيادة كبيرة فى الأعـداد البكتيرية عامة و مثبتات النيتروجين الجوى فى الأرض بصفة خاصة .

وكان لإضافة الكومبوست مع المبيدات النيماتودية فائدة فى تخفيض الأثر الضار للمبيدات وتنشيط الأعداد البكتيرية و زيادتها فى الأرض، مما يوضح الأثر الايجـابي لإضـافة المـادة العضوية على النشاط الحيوى بالأرض.