

# ***CONTENTS***

	<b>Page</b>
<b>1-INTRODUCTION</b> .....	1
<b>2-REVIEW OF LITERATURE</b> .....	4
2.1. Biological Nitrogen Fixation (BNF)	4
2.2. Environmental Pollution.	6
2.2.1. Definition of Pollution.	6
2.2.2. Sources of Pollution	6
2.2.2.1. Pesticide Pollution of Soils	7
2.2.3. Effect of Pesticides on Total Bacterial Counts in Soil	7
2.2.4. Effect of Pesticides on Diazotrophy Bacteria in Soil	11
2.2.5. Effect of Pesticides on Dehydrogenase Activity in Soil	15
2.2.6. Effect of Pesticides on Nitrogenase Activity in Soil	19
2.2.7. Effect of Pesticides on Phosphatase Activity in Soil	20
2.2.8. Effect of Pesticides on Urease Activity in Soil	22
2.2.9. Relationship Between Organic Matter and Pesticides and their Effect on Microorganisms and Enzymatic Activities in Soil.	24
<b>3- MATERIALS AND METHODS</b> .....	
3.1. Materials	31
3.1.1. Soils	31
3.1.2. Organic matter “ Compost “	31
3.1.3. Nematicides.	31

3.1.4. Bacterial Media.	34
3.1.4.1. Soil extract agar medium.	34
3.1.4.2. Modified Asby's medium for <i>Azotobacter</i> .	34
3.1.4.3. N-deficient medium for <i>Azospirilla</i>	35
3.1.4.4. Growing N <sub>2</sub> -Fixing Bacilli bacteria medium	35
3.2. Methods :	36
3.2.1. Periodic determinations	37
3.2.2. Bacterial determinations	37
3.2.2.1. Total bacterial count.	37
3.2.2.2. <i>Azotobacter</i> count.	37
3.2.2.3. <i>Azospirillum</i> count.	38
3.2.2.4. N <sub>2</sub> -Fixing bacilli count.	38
3.2.3. Enzymatic activities in soils.	38
3.2.3.1. Nitrogenase activity	38
3.2.3.2. Dehydrogenase activity	39
3.2.3.3. Phosphatase Activity	40
3.2.3.4. Urease activity	40
<b>RESULTS AND DISCUSSION</b>	<b>42</b>
4.1. The Effect of Nematicides and Compost on Total bacterial Count in Soil.	42
4.2. Density of diazotrophs	50
4.2.1. <i>Azotobacter</i>	50
4.2.2. <i>Azospirillum</i>	56
4.2.3. <i>Bacillus</i>	62

4.3.The Influence of Nematicides and Compost on Activities Some Soil Enzymes	70
4.3.1 Dehydrogenises activity	70
4.3.2. Nitrogenase activity	77
4.3.3.Phosphatase activity	83
4.3.3.1. Acid phosphatase	83
4.3.3.2. Alkaline phosphatase:	91
4.3.4. Urease activity :	97
<b>SUMMARY</b> .....	104
<b>REFERENCES</b> .....	112
ARABIC SUMMARY .....	

## 5. SUMMARY AND CONCLUSION

Intensive arable farming and semiarid climatological conditions cause a progressive decline in soil organic matter levels and subsequently contribute to degradation of soil quality (Caravaca *et al.* 2002b). The beneficial effects of organic amendments include decreased soil bulk density and increased water-holding capacity, aggregate stability, saturated hydraulic conductivity, water infiltration rate, and biochemical activity (Zebarth *et al.* 1999).

Pesticides, the synthesized chemical compounds used in agriculture to combat or eradicate various pests, are applied mainly as leaf spray or direct soil treatment. Effect of pesticides on soil microbial processes has been studied and found to differ according to soil properties, growing plant, application mode and dose of compound, and agricultural practices (i.e. maturing, fertilization, and irrigation) (Harris, 1988; Malkomes, 1992). Nematicides are important category of pesticides that are directly introduced onto the soil to control the faunal nematode pests.

The objective of the present work was to conducted to "Studies on diazotrophy in soils under chemical pollution conditions."

This study was divided into two parts:

### **Part I.**

It is concerned with effects of nematicides (carbofuran, oxamyl and cadusafos) at recommended and 10 times-recommended dose and compost as organic matter at recommended and double recommended rate on total

bacterial counts and counts of diazotrophs namely *Azotobacter* spp. *Azospirillum* spp and *Bacillus* spp.

## **Part II.**

The effect of the above mentioned factors on the microbial enzymes activities (dehydrogenase, nitrogenase, acid and alkaline phosphatase and urease activity, in soil were determined at intervals for incubation.

**The results obtained are summarized in the following:**

### **5.1.1. Total bacterial count:**

- 1- Addition of compost at recommended rate or double recommended rate increased total microbial count in soil with the time of incubation up to the 30<sup>th</sup> day, thereafter they began decline. Maximum counts were reported on the thirtieth day ( $255 \times 10^6$  and  $120 \times 10^6$  cfu g<sup>-1</sup> dry soil) for double and recommended rate of compost, respectively.
- 2- Addition of compost gave increases 142% and 326% for high and low rate of compost more than that recorded in the control ones. This increase attribute to the beneficial effects of compost as organic matter e.g. source of available macro and micronutrients for microbial growth.
- 3- Addition of oxamyl and cadusafos at recommended dose stimulate the proliferation of total bacterial count but addition of carbofuran showed inhibitory effect and led to decrease of counts, this decrease was 26% compared with control.

- 4- All nematicides at 10-times recommended dose led to decrease of microbial counts in soil. This decrease was 38,29 and 13% for carbofuran, oxamyl and cadusafos, respectively.
- 5- Generally, incorporation of compost mixed with low or high doses of nematicides led to alleviate the adverse effects of nematicides. Addition of compost mixed with nematicides at recommended rates led to increase 57, 102 and 130% for carbofuran, oxamyl and cadusafos, respectively over than those recorded with untreated soil.
- 6- Addition of compost at double recommended rate not only suppressed the inhibitory effect but also increased total microbial counts. This increase may be due to utilization of nematicides as well as their degraded products by the soil microorganisms to derive energy and other nutrient elements for their cellular metabolism.

### **5.1.2. Counts of diazotrophs:**

#### ***Azotobacter* count:**

- 1- Addition of compost at recommended rate (R ) and double recommended rate (2R) led to increase of *Azotobacter* count by 168 and 208% for (R ) and (2R), respectively over than those obtained by untreated soil.
- 2- Concentration of nematicides either recommended dose or 10 times recommended doses of all nematicides showed inhibitory effects on counts of *Azotobacter* spp.. Cadusafos gave a maximum decrease at high dose (10 times recommended dose),

this decrease was 45% relatively to untreated soil. Order among the inhibition effect by nematicides tested was cadusafos > carbofuran > oxamyl.

3- Addition of compost at recommended rate with low dose of nematicides increased *Azotobacter* counts, these increase were 44, 61 and 34% but these increases were 16, 24 and 8% at high dose (10 times recommended doses) for carbofuran, oxamyl and cadusafos, respectively, over than those obtained by control.

4- Generally, addition of compost at double rate increased *Azotobacter* counts either with recommended dose or 10 times-recommended dose of nematicides. These increases were 95, 103 and 73% and 45, 55 and 27% for (2R)+ carbofuran, (2R)+ oxamyl and (2R)+ cadusafos, respectively, over than those obtained with untreated soil.

#### ***Azospirillum* count:**

Results obtained showed the same trend with those previously obtained by *Azotobacter* spp. Addition of compost at recommended rate and double recommended rate increased *Azospirillum* counts. This increase was 130 and 179% over than those obtained by control. Also, addition of nematicides reduced *Azospirillum* count. The reduction was more pronounced with addition of high doses of nematicides, especially with cadusafos followed by carbofuran > oxamyl. The reduction percentages were 12, 6 and 17% and 32, 25 and 43% for carbofuran, oxamyl and cadusafos at low and high dose additions than those obtained by control

Generally, addition of nematicides mixed with compost either at low dose or high dose led to increase of *Azospirillum* counts. These increase ranged between 34 to 107% for carbofuran mixed with compost, 26 to 130% for oxamyl mixed with compost and 11 to 75% for cadusafos mixed with compost.

### ***Bacillus* count:**

The results of *Bacillus* counts were similarly to those previously obtained with *Azotobacter* and *Azospirillum* counts. The high increase (233%) was recorded by addition of double rate of compost, on the other hand, the lowest decrease (-40%) was recorded by addition of high dose of nematicides.

Carbofuran showed more inhibitory effect than cadusafos or oxamyl on *Bacillus* count. This decrease can attributed to inhibitory effect of carbofuran on *bacillus*.

Incorporation compost mixed with nematicides led to increase of *Bacillus* counts. This increase ranged between 12 to 61% for carbofuran mixed with low and high rate of compost, 34 to 127% for oxamyl mixed with low and high rate of compost and 17 to 97% for cadusafos mixed with low and high rate of compost, respectively than those obtained by control.



## 5.2. Soil enzymes:

It is concerned with the effect of nematicides (carbofuran, oxamyl and cadusafos) at recommended and 10-times recommend doses and compost at recommended and double recommended rate on soil enzymes.

### 5.2.1. Dehydrogenase activity:

Dehydrogenase activity (DHA) is important index of the microbial biomass. Addition of compost at recommended or double recommended rate led to increase DHA, this increase was 50.0% and 128.6% for low and high rate of compost over than those obtained by control. While the addition of nematicides caused an inhibitory effect where DHA decreased. This decrease was 21, 8 and 14% and 35, 17 and 25% for carbofuran, oxamyl and cadusafos at low and high dose, respectively compared with control.

Carbofuran gave maximum decrease of DHA (47.4%) followed by oxamyl > cadusafos. On the other hand, the addition of nematicides mixed with low or high rate of compost led to increase DHA. It is cleared that the highest DHA was appeared with using double rate of compost mixed with recommended dose of nematicides when gave 28.1, 39.9 and 50.7% over control. Generally, the results of DHA have the same trend as the results obtained in total microbial counts.

### 5.2.2. Nitrogenase ( $N_2$ -ase) activity:

Addition of compost at recommended rate gave increase in  $N_2$ ase activity 43% compared with control, but double recommended rate of compost decreased  $N_2$ ase activity in soil. But, addition of nematicides at low or high dose reduced  $N_2$ ase activity 21, 8 and 14% and 35, 17 and

25% for carbofuran, oxamyl and cadusafos at low and high dose compared with untreated soil.

All treatments of nematicides either addition singly or mixed with compost gave decrease of  $N_2$ -ase activity except compost (R )+ oxamyl and compost (R )+ cadusafos slightly increased  $N_2$ -ase activity.

### **5.2.3. Phosphatase activity:**

#### **Acid phosphatase activity:**

Addition of compost increased acid phosphatase activity either at recommended dose or double recommended dose, this increase was 34.5 and 44.2% for R compost and 2R compost, respectively above than control.

Addition of nematicides at recommended dose did not affect acid phosphatase activity, but when adding high doses caused a slight inhibition compared with untreated soil.

Addition of nematicides mixed with (R) or (2R) compost led to increase of acid phosphatase activity.

#### **Alkaline phosphatase activity:**

Addition of compost led to increase of alkaline phosphatase activity (24 and 40%) for recommended and double recommended rate of compost, over than control. This enzyme was not affected by using nematicides at recommended dose. Carbofuran had more inhibitory effect on the enzyme at high does than oxamyl or cadusafos. The application of nematicides mixed with compost either at low rate or hight rate led to increase in alkaline phosphatase activity. These increases ranged between

9.1-11.7%, 29.6-27% and 29.1-30.2% for carbofuran + compost, oxamyl + compost and cadusafos + compost.

#### **5.2.4. Urease activity:**

Addition of compost led to increase in urease activity 44 and 77.9% for recommended rate and double recommended rate of compost, respectively, relatively to corresponding control. Also, addition of nematicides at recommended dose gave a slight increase ranged between 1.8 to 3.2%.

Application of 10-times recommended dose of nematicides led to inhibition of urease activity. The inhibitory effects of carbofuran and cadusabos on urease activity were equal 7.6%. While this inhibitory effect was suppressed after adding compost.

Generally, addition of compost either mixed with low or high dose of nematicides led to increase in urease activity.