



EFFECT OF PESTICIDE TYPE AND IT'S CONCENTRATION ON THE NUMBERS OF NITRIFICATION BACTERIA IN INOCULATED SOIL WITH BIOFERTILIZERS

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Abstract

The research conducted at Basra University_College of Agriculture_Department of Soil Science and Water Resources for year 2014-2015 to study the effect of adding three different chemical insecticide (Vabacosidin 50%), fungi (carbendazim 20%) and herbicide (ground-up) with the recommended concentrations and the double to Abu al_Khasib soil treated with the nitrogen fixing fertilizer *Azotobacter chroococcum* and phosphate dissolving *Bacillus polymyxa* and their mixture on the numbers of nitrifying bacteria for soil samples taken from the pot weekly for a period of 49 days “from the start of the treatment with the pesticides with the use of a treatment without inoculation and without adding chemical pesticides as a comparison. The results showed that the addition of chemical pesticides at the recommended concentration and the double concentration negatively affected” the number of *Nitrobacter* bacteria. And *Nitrosomonas* in the soil, where the inhibition ratio reached (46.94%, 16.47%) for *Nitrobacter* and (45.81%, 14.07%) for *Nitrosomonas* bacteria at the recommended concentrations and the double concentrations measured by the comparison treatment respectively. Add the herbicide at the recommended and double concentration to the soil, as it led to the disappearance of *Nitrobacter* bacteria from the soil after 49 days of treatment with the pesticide. The number of *Nitrosomonas* bacteria increased when treated with the insecticide at the recommended concentration, as the number of bacteria reached 4.50×10^6 CUF g soil for a period of 35 days The number of *Nitrobacter* bacteria in the soil treated with insecticide and fungicide at the recommended concentration did not significantly differ from the comparison treatment for different periods of time.

Key word: Nitrification bacteria, pesticide type, pesticide concentration

Introduction

The nitrification process has an important role in the nitrogen cycle in nature, as it converts the ammonia resulting from the mineralization of nitrogenous organic materials into nitrates and thus plants uptake some of their nitrogen needs, Nitrification is the process of oxidation of ammonia NH_3 and its 13 and this reaction takes place in two stages. The first stage Ammonia is oxidized by oxygen to nitrite under normal conditions of pressure and temperature, liberating energy of 235KJ mol under aerobic conditions and the genera of bacteria that perform the first process are called *Nitrosomonas*, *Nitrosovibrio*, *Nitrosospira*; *Nitrococcus*.

The second stage :- Nitrite is oxidized with an oxygen molecule producing nitrates and this reaction produces energy of 76KJ under normal conditions of pressure and

temperature, which catalyzes this reaction Nitritoxidase and all groups of these bacteria interact in aerobic conditions and belong to it *Nitrobacter*; *Nitrospina*; *Nitrococcus*.

Modern agriculture depends on the use of chemical insect, fungal and herb control materials that are added to the soil with the aim of eliminating pests and increasing productivity (Al- Adel and AbdMawlod, 1979). The microorganisms are usually used in environmental toxicology tests to assess the chemical and biological properties of the soil (Doran and Zeis, 2000). The interest in studying chemical pesticides in the soil comes as a result of the potential for their negative effects in inhibiting the activities of micro-organisms of economic importance in the transformation of nutrients in the soil such as phosphorous and nitrogen. Pesticides were the effect on

soil microorganism through toxic effect or sub-effects that lead to modifications in the behavior of microorganisms or a change in metabolic activity in addition to their entry into the food chain by transmission through plants to consuming animals (Edward, 1973). Several studies indicated that adding chemical pesticides to the soil may have an inhibitory or encouraging effect on biochemical processes, as the organisms responsible for the nitrification process are more sensitive to pesticides than others, depending on the type of pesticide and the concentration with the active substance (Paul, 2007).

In view of the “use of pesticides of different types and concentrations and their potential impact on soil and water pollution, the study aimed to use nitrification bacteria as a bio indicator to detect the extent of soil pollution with chemical pesticides.

Materials and Methods

Samples were collected from the soil of Abu Al-Khasib (Sibilat) in Basra Governorate, at a depth of (030) cm and it was taken into account that the area was not treated with pesticides for at least the last ten years. the soil were air-dried, grind and pass through 4mm sieve it was kept at 4°C. Soil physical, chemical and biological properties were estimated according to Black *et al.*, (1965a, b) table 1.

The soil was filled in 5 kg plastic pots and 10 tons ¹of organic matter were added to it, then fertilized with nitrogen, phosphate and potassium fertilizers according to the recommended amount for the barley crop. The barley seeds were surface sterilized “according to Vincent, (1970) and inoculated with *A.chroocum*,

B.polymyxa and their mixtures. Some pots left without inoculation as control. The inoculated and 0¹) After 14 day From germination, the pots were treated with chemical insecticides (Vabcosidine 50%) fungicides (carbenidase 20%) and herbicide (ground-up). at the recommended concentration and double concentration, according to Al-Katrani, (2016) after 7, 21, 35 and 49 days of treatment with chemical pesticides, samples were taken from potted soils and the numbers of nitrifying bacteria were estimated according to Black, (1965).

Results and Discussion

The chemical pesticides negatively affected the **Table 1:** The physical, chemical and biological properties of the study soil.

| Adjective | Units | Value |
|-------------------------------|------------------------|----------------------|
| PH | | 7.4 |
| ECE | dS ⁻¹ | 3.8 |
| CEC | Se mol k ⁻¹ | 45.8 |
| available phosphorous | mg k ⁻¹ | 5.6 |
| Available nitrogen | | 1.78 |
| Total nitrogen | g k ⁻¹ | 0.16 |
| Organic matter | | 5.8 |
| Humidity at field capacity | % | 30.0 |
| Humidity at saturation | | 45.0 |
| Sand | g k ⁻¹ | 79.89 |
| Silt | | 784 |
| Clay | | 76.78 |
| Soil texture | | silty – clay |
| total bacteria | CUF ⁻¹ soil | 8.7*10 ⁷ |
| total fungi | | 4.5*10 ⁵ |
| phosphate-dissolving bacteria | | 6*10 ⁴ |
| Azotobacter bacteria | | 1.15*10 ⁶ |

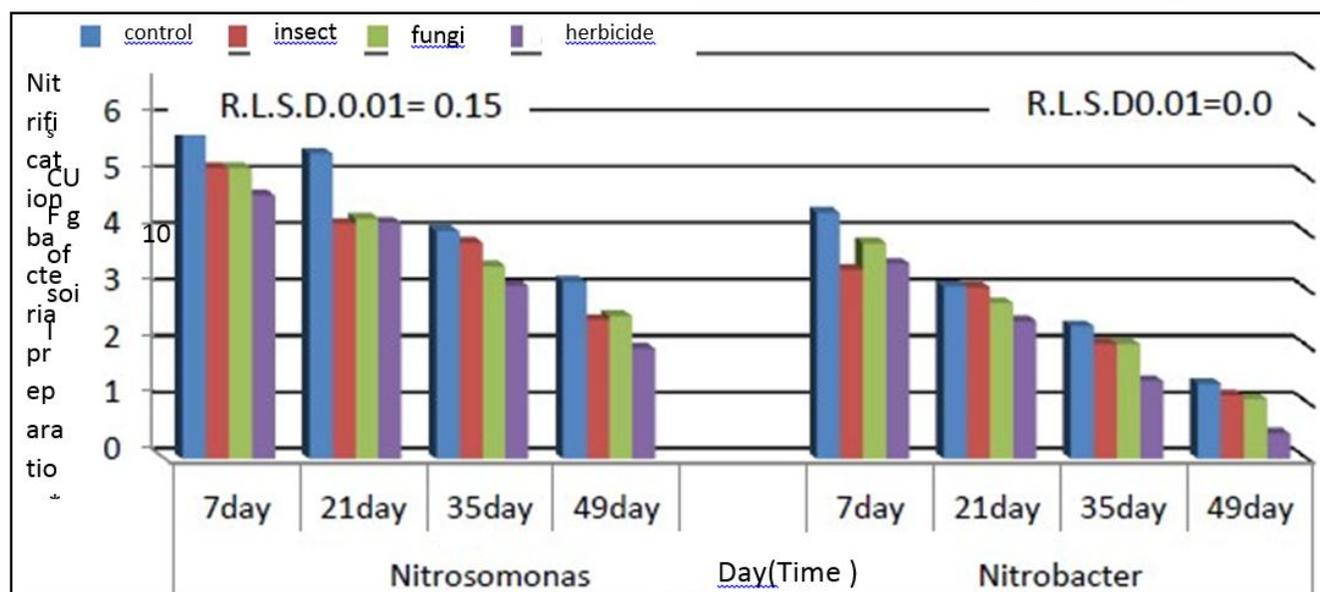


Fig. 1: The effect of the pesticide type on the number of nitrifying bacteria at the different time.

numbers of nitrification bacteria in the soil. The comparison treatment gave the highest rate of *Nitrosomonas* and *Nitrobacter* in the soil, reaching 6.36×10^8 and 4.36×10^8 soil for both bacteria respectively at a period of 7 days which differed significantly from the rest of the treatments over time Fig. 2. Whereas, soil treatment with herbicide gave the lowest numbers of *Nitrobacter* and *Nitrosomonas* bacteria during the time of the experiment, as the inhibition ratio was 30.30% and 26.82% for both bacteria respectively. The sensitivity of *Nitrobacter* and *Nitrosomonas* to the added chemical pesticides also differed during the different time periods, as the *Nitrobacter* bacteria were more sensitive than *Nitrosomonas* to the added chemical pesticides Fig. 1 this due to different in physiological properties of microorganisms. (Chen *et al.*, 2001; Przybulewska and Nowak, 2004).

The addition of the pesticides at the recommended and the double concentration negatively affected the numbers of *Nitrosomonas* and *Nitrobacter* in the soil, as the inhibition ratio reached (46.94%, 16.47%) for *Nitrobacter* and (45.81%, 14.07%) for *Nitrosomonas* at the recommended and the double concentration respectively. The reason is that the chemical pesticides added to both concentrations have a negative effect on the nitrification process, because they inhibit the bio enzymatic activities related to the respectively process and make the bacteria unable to degrade the pesticide and use it as an energy source, as the bacteria It needs the element phosphorus to perform the physiological and biological functions to enter it in carrier-energy compounds.

Table 2 shows that the interaction between inoculation

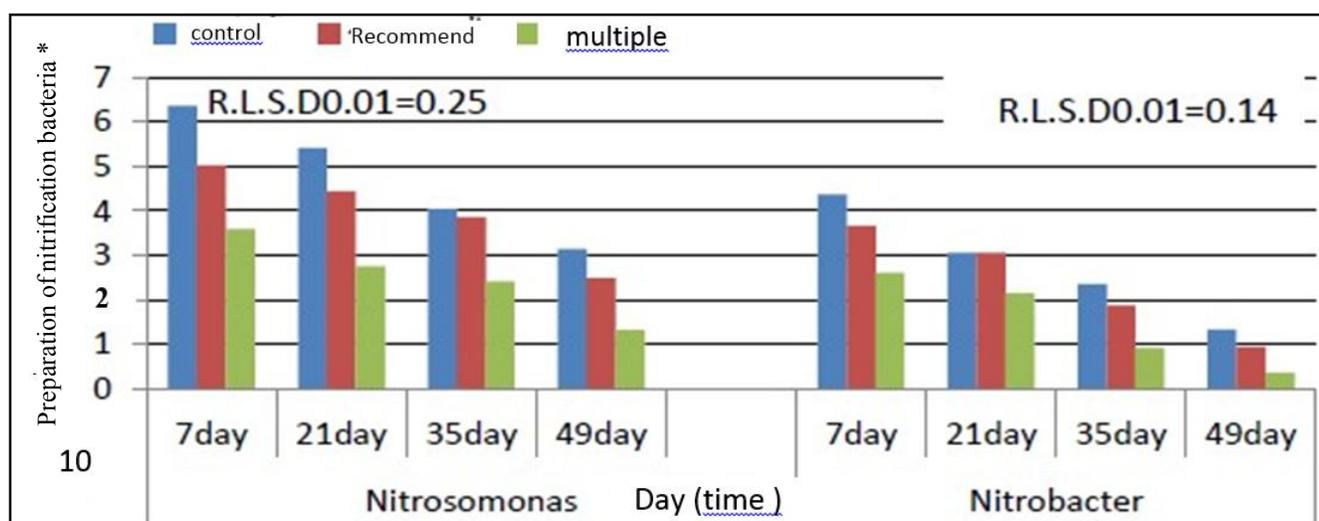


Fig. 2: The effect of the pesticide concentration on the numbers of nitrification bacteria at the different time.

Table 2: The effect of inoculation with some bio-fertilizer and pesticide type on the numbers of nitrification bacteria at different time.

| soil | | | | | | | | | | | | | day |
|---|------------|--------------|--------------------|------------|--------------|----------------------|------------|--------------|------------|------------|--------------|----|-----|
| <i>A.chroococcum</i> + <i>B. polymyxa</i> | | | <i>B. polymyxa</i> | | | <i>A.chroococcum</i> | | | Control | | | | |
| herb-icide | Fung-icide | Insec-ticide | herb-icide | fung-icide | Insec-ticide | herb-icide | Fung-icide | Insec-ticide | herb-icide | fung-icide | Inse-cticide | | |
| 6.89 | 6.91 | 6.54 | 4.18 | 4.52 | 5.19 | 4.27 | 5.48 | 5.14 | 3.33 | 3.68 | 3.72 | 7 | |
| 5.54 | 5.19 | 5.09 | 4.24 | 3.72 | 3.73 | 3.91 | 4.83 | 4.41 | 3.02 | 3.29 | 3.40 | 21 | |
| 4.24 | 4.29 | 4.81 | 3.18 | 3.41 | 3.88 | 3.13 | 3.88 | 4.06 | 1.68 | 2.07 | 2.56 | 35 | |
| 2.93 | 3.43 | 3.27 | 1.93 | 2.41 | 2.32 | 1.92 | 2.80 | 2.68 | 1.00 | 1.47 | 1.58 | 49 | |
| R.L.S.D0.01 = 0.33 | | | | | | | | | | | | | |
| soil | | | | | | | | | | | | | day |
| herb-icide | Fung-icide | Insec-ticide | herb-icide | fung-icide | Insec-ticide | herb-icide | Fung-icide | Insec-ticide | herb-icide | fung-icide | Inse-cticide | | |
| 4.23 | 5.01 | 4.41 | 4.30 | 3.75 | 2.94 | 2.99 | 3.98 | 3.56 | 2.33 | 2.52 | 2.46 | 7 | |
| 3.16 | 3.79 | 4.02 | 2.30 | 2.29 | 2.92 | 2.41 | 2.93 | 3.11 | 1.87 | 2.04 | 2.11 | 21 | |
| 1.31 | 2.63 | 2.67 | 0.96 | 1.51 | 1.70 | 0.94 | 2.49 | 2.14 | 2.32 | 1.47 | 1.59 | 35 | |
| 0.70 | 1.83 | 2.08 | 0.31 | 0.70 | 0.73 | 0.46 | 1.13 | 0.97 | 0.31 | 0.58 | 0.17 | 49 | |
| R.L.S.D0.01B*P=0.13 | | | | | | Time=3.12 | | | | | | | |

Table 3: The effect of inoculation with some bio- fertilizers and the concentration of the pesticide on the numbers of nitrification bacteria at different time.

| <i>Nitrosomonas soil</i> | | | | | | | | | | | | |
|---|------|------|--------------------|------|------|----------------------|------|------|---------|------|------|-----|
| <i>A.chroococcum</i> <i>+B. polymyxa</i> | | | <i>B. polymyxa</i> | | | <i>A.chroococcum</i> | | | Control | | | day |
| C2 | C1 | C0 | C2 | C1 | C0 | C2 | C1 | C0 | C2 | C1 | C0 | |
| 4.62 | 7.19 | 8.53 | 3.99 | 4.50 | 5.40 | 3.38 | 4.71 | 6.80 | 2.32 | 3.71 | 4.70 | 7 |
| 3.41 | 6.24 | 6.17 | 2.54 | 3.98 | 5.17 | 2.87 | 4.56 | 5.73 | 2.17 | 2.98 | 4.57 | 21 |
| 3.06 | 5.09 | 5.20 | 2.59 | 4.04 | 3.83 | 2.25 | 4.29 | 4.53 | 1.70 | 2.03 | 2.57 | 35 |
| 1.82 | 3.71 | 4.10 | 1.39 | 2.31 | 2.97 | 1.22 | 2.58 | 3.60 | 0.81 | 1.34 | 1.90 | 49 |
| R.L.S.D0.01 = 0.23 | | | | | | | | | | | | |
| soil | | | | | | | | | | | | |
| 3.53 | 4.72 | 5.40 | 2.53 | 4.09 | 4.37 | 2.80 | 3.35 | 4.37 | 1.53 | 2.48 | 3.30 | 7 |
| 2.98 | 4.22 | 3.77 | 2.08 | 2.80 | 2.63 | 2.08 | 3.08 | 3.30 | 1.43 | 2.09 | 2.50 | 21 |
| 1.18 | 2.73 | 2.70 | 0.69 | 1.48 | 2.00 | 1.10 | 1.97 | 2.50 | 0.65 | 1.34 | 2.20 | 35 |
| 0.84 | 1.67 | 2.10 | 0.21 | 0.60 | 0.93 | 0.28 | 0.91 | 1.37 | 0.11 | 0.56 | 0.93 | 49 |
| R.L.S.D0.01 B*C=0.11 | | | | | | | | | | | | |

with bio- fertilizers and chemical pesticides had a significant effect on the numbers of nitrification bacteria in the soil, as the highest number of *Nitrosomonas* was reached when mixed inoculation with *B. polymyxa*, *A. chroococcum* and the addition of the fungicide during the period 7 days at a rate of 6.91×10^6 CUF $6. \times 10^6$ CUF gm soil, as for *Nitrobacter* bacteria, the highest number and 5.01×10^6 soil for the previous treatment, while the lowest rate for *Nitrosomonas* and *Nitrobacteria* was 1.00×10^4 0. $\times 10^4$ CUF¹ Soil with the of adding herbicides and not inoculation treatment (control) after 49 day from the addition. The reason is due to the lack of response to the source of nitrogen and mineral phosphorous that the co-inoculation bacteria add due to the effect of pesticides, meaning that there are different degrees of availability and the difference in the

speed of decomposition. due to the chemical composition of the pesticide and the ratio of carbon to nitrogen in it and the number of bacteria in it, *Nitrobacteris* less than that of *Nitrosomonas* and this indicates the sensitivity of *Nitrobacter* bacteria to the added chemical pesticides.

The interaction with biological fertilizers, the concentration of chemical pesticides and the time period had a significant effect on the numbers of nitrification bacteria, the highest rate was 8.53 an 5.40×10^6 soil due to the effect of the treatment of co-inoculation with bacteria and not adding the chemical pesticide on numbers of *Nitrosomonas* and *Nitrobacter* by respectively. at the 7 day period of treatment with the pesticides table 3, 0. 1 0.11×10^6 soil with the effect of the control at the double concentration of the pesticides on *Nitrosomonas* and *Nitrobacter*, respectively, at the 49-day period of

Table 4: The effect of the interaction between chemical pesticides and the concentration of the pesticide on the reaction of nitrification bacteria at different time.

| <i>Nitrosomonas soil</i> | | | | | | | | | |
|--------------------------------|------|------|-----------|------|------|-------------|------|------|-----|
| Herbicide | | | Fungicide | | | Insecticide | | | day |
| C2 | C1 | C0 | C2 | C1 | C0 | C2 | C1 | C0 | |
| 3.16 | 4.48 | 6.36 | 3.71 | 5.37 | 6.36 | 3.62 | 4.92 | 6.36 | 7 |
| 2.66 | 4.46 | 5.41 | 2.81 | 4.56 | 5.41 | 3.52 | 4.29 | 5.41 | 21 |
| 2.02 | 3.13 | 4.03 | 2.23 | 3.97 | 4.03 | 2.95 | 4.50 | 4.03 | 35 |
| 0.94 | 1.76 | 3.14 | 1.50 | 2.95 | 3.14 | 1.49 | 2.75 | 3.14 | 49 |
| R.L.S.D. 0.01 P*C =0.15 | | | | | | | | | |
| soil | | | | | | | | | |
| 2.64 | 3.39 | 4.36 | 2.75 | 4.33 | 4.36 | 2.41 | 3.26 | 4.36 | 7 |
| 1.85 | 2.40 | 3.05 | 2.18 | 3.06 | 3.05 | 2.39 | 3.68 | 3.05 | 21 |
| 0.00 | 0.70 | 2.35 | 1.28 | 2.48 | 2.35 | 1.26 | 2.47 | 2.35 | 35 |
| 0.00 | 0.00 | 1.33 | 0.49 | 1.35 | 1.33 | 0.59 | 1.32 | 1.33 | 49 |
| R.L.S.D.0.01 P*C = 0.10 | | | | | | | | | |

treatment with the pesticide. Also, the numbers of *Nitrosomonas* and *Nitrobacter* were decreases by an increase in the concentration of pesticides in the inoculated and uninoculation treatments at all the studied periods, due to their high sensitivity to the chemical pesticides added to the soil, as well as the effect of the interaction between chemical pesticides and their concentration significantly in the numbers of nitrification bacteria during the time periods. the highest rate was 6.36, 4.36×10^6 CUF¹ soil for *Nitrosomonas* and *Nitrobacter* bacteria respectively by control not treated with chemical pesticides, while the lowest rate was 0.94 and zero $\times 10^6$ soil when adding herbicide with double concentration for both *Nitrosomonas* and *Nitrobacter*, respectively. After 49 days of treatment, the effect of pesticide on decreasing numbers of nitrifying bacteria took the

following order: Herbicide > insecticide > fungicide.

The treatment with herbicide at the recommended and double concentration reduced the numbers of *Nitrobacter* bacteria to zero at the periods of 35 and 49 days of treatment. This was due to its negative effect on cell size due to the inhibit of the process of building proteins in the cell. (Makawi *et al.*, 1979; Liu *et al.*, 1991). The addition of pesticides to the double concentration significantly inhibited the nitrification process and that the difference in the sensitivity of both bacteria was due to genetic differences (Patrick *et al.*, 1991).

We conclude from the study that *Nitrobacter* bacteria can be used as a bio- indicator of pollution of the soil ecosystem with various chemical pesticides.

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