

In vitro effect of three pesticides on soil bacteria

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Abstract

The results of the effect of three concentrations (1%, 25%, and 50%) and control of each pesticide on the number of bacteria colonies were reported and compared to the control which was 356.9 CFU/gm, with percentage of 100%. The effect of concentrations 1% of Abamectin, Triazophos, and Methomyl on bacteria growth were recorded [234.8 CFU/gm (65.78%), 200.4 CFU/gm (56.1%), and 224.4 CFU/gm (62.8%), respectively].

The effect of concentrations 25% of Abamectin, Triazophos, and Methomyl on bacteria growth were recorded [124.0 CFU/gm (34.7%), 38.2 CFU/gm (10.7%), and 1.9 CFU/gm (0.5%), respectively], While, the bacterial growth at the concentrations [50% of each of three pesticides was 0.7 CFU/gm (0.19%)] recorded for each tested pesticide.

Keywords: Bacterial colonies, Pesticides, Nutrient agar medium

Introduction

Pesticides are widely used against a range of pests infesting agricultural crops. With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct repeated has received more attention.

Different types of pesticides has been characterized in many ways by randomness, resulting in a defect in the components of the ecosystem (soil - plant – microorganisms It would be useful that a pesticide has to be in a degree of stability that is enough to make the intended impact, then disappear. Or, a pesticide quantity is less that it does not affect agricultural crops (22).

The effect of pesticides on microorganisms in soil varies depending on the type of pesticide, the dose used, the period of time the pesticide used and the period after treatment (8), use of Phosphoric insecticides such as Dursban and Cardona led to the revitalization of soil microbes, especially those responsible for the Nitrogen transformations. Other types of Phosphoric insecticide have shown an increase in the number of fungi and a decrease in the number of bacteria, and it was found that the role of microbes in the dismantling of pesticides depends on the type of pesticide and its added Concentration by (28).

In experiments conducted to examine the impact of the Phosphoric insecticide Chloropyrifos, it has been found that its use led to a change in the functions and composition of microorganisms (33). Also, some pesticides led to domination of microbial species and groups at the expense of other groups (21). Despite of this, a number of microorganisms could adapt to different types of pesticides, while others are affected (24, 23, 17).

Pesticides can accumulate in the soil to high enough levels to have adverse effects on the activity of soil microorganisms (42). Most pesticides are soil toxic ingredients affecting different groups of organisms in the soil such as: nematodes, fungi, bacteria, azotobacter, actinomycetes and arthropods (41). Chemical pesticides and soil-additive chemicals affect soil organisms and lead to changes in organisms and, therefore, affect their activity and lead to accumulation of these ingredients in agricultural soils (23; 17). Many genus and species of genera of microorganisms, including phosphate soluble bacteria, are spread in the soil around the rhizosphere near the roots of the plant and are, therefore, the most affected by pesticides (9). Here, we attempt to describe how

pesticide (most commonly used by qat farmers) exposure at different concentrations and its affect bacterial numbers in laboratory.

Materials and Methods

Laboratory experiment

The study has used the method which was adopted (12 , 29 , 39 , 45) Three rates of addition were selected. They were the recommended concentration, the double concentration and the field concentration as follows: 01%, 25% and 50% respectively. The control was taken without treatment for comparison with each concentration.

The tests were carried out in the Microbiology Laboratory at the National Drug Authority in Khormaksar, which belongs to the Ministry of Health - Aden Governorate. In the laboratory, the equipment used in the analysis were provided, as well as all the materials used in the preparation of the bacterial media, where the Nutrient Agar Media was used, which is manufactured by Rapid Biotec Company - Uk. Five soil samples were collected from Corn crop field's city. Soil was collected at a depth of 15 cm and samples were passed through a sieve of 2 mm to remove stones and plant debris. The five samples were mixed together. One gram of samples soil was mixed with 99 ml of sterilized water and mixed by shaking for even distribution of soil in water. The suspense was done by using a sterile pipette. And 1 ml of solution from this test tube was then added to another test tube with 9 ml sterilized water. This gives a dilution of 10^{-1} and in the same pattern dilutions of 10^{-2} , 10^{-3} , 10^{-4} . Using new sterilize pipettes (quantity: 4) we transfer 4 milliliter from the first dilution tube containing 10 ml to 4 petri dishes by 4 replicates per dilution. The same method is repeat on the rest of the tubes containing different dilutions.

Nutrient medium was fused to 113 °C then cooled in 45 °C. Pour the food medium into dishes in the insulation chamber. The dishes stirred a circular motion with and counterclockwise to ensure the soil suspension was mixed with the Agar medium. After that, we pour the medium in dishes , these dishes were incubated at 37°C for 48 h. After incubation, Colonies of bacteria were isolated. Four dilutions were made for control on treated with pesticides. Calculation of pesticide concentrations was used in the laboratory and their effect on the growth of bacterial colonies:

The concentration which is recommended by the manufacturer is 100 ml / 100 L water. We take 1 ml of the pesticide and add it to 1 liter of water and then shake it well until the pesticide is mixed with water, then take 4 ml and put 1 ml into each dish containing separate bacteria by 4 replicates for each concentration and we get a 1%. We take 2.5 ml of the pesticide and add it to 1 liter of water then repeat the same aforementioned method and we get a 25% concentration. We take 5 ml of the pesticide and add it to 1 liter of water then repeat the same the same aforementioned method and get a 50% concentration. After that we pour the medium in dishes these dishes were incubated at 37°C for 48 h (10) (27) (14), After incubation, the bacteria grown in the colonies were diagnosed with microscopic examination to determine the morphological characteristics of the developing colonies in the dishes and to record them in pre-prepared tables. The required readings for the number of bacteria grown in the culturing media, The method of enumerating was obtained by a magnifying glass and anatomical microscope, as it was used in various studies, such (2) (26) (4).

Table (1) Effect Number Pesticides Used on (*Catha edulis*) in Study Area on the Bacterial Colonies

| Common Name | Concentrations mL /L. | | | | |
|----------------------|-----------------------|-------------------|------------------|----------------|-------|
| | Control | 1% | 25% | 50% | mcam |
| Abamectin 1.8% EC | 356.9 (100%) | 234.8 (65.78%) | 124.0 (34.7%) | 0.7 (0.19%) | 180.3 |
| Triazophos 40 WSC | 356.9 (100%) | 200.4 (56.1%) | 38.2 (10.7%) | 0.7 (0.19%) | 150.6 |
| Methomyl 90% SP | 356.9 (100%) | 224.4 (62.8%) | 1.9 (0.5%) | 0.7 (0.19%) | 143.2 |
| mcam | 356.9 | 219.9 | 54.7 | 0.7 | |
| L.S.D. 5 % | Pesticides | Concentration | | Overlaps | |
| | 5.84 | 6.74 | | 11.68 | |

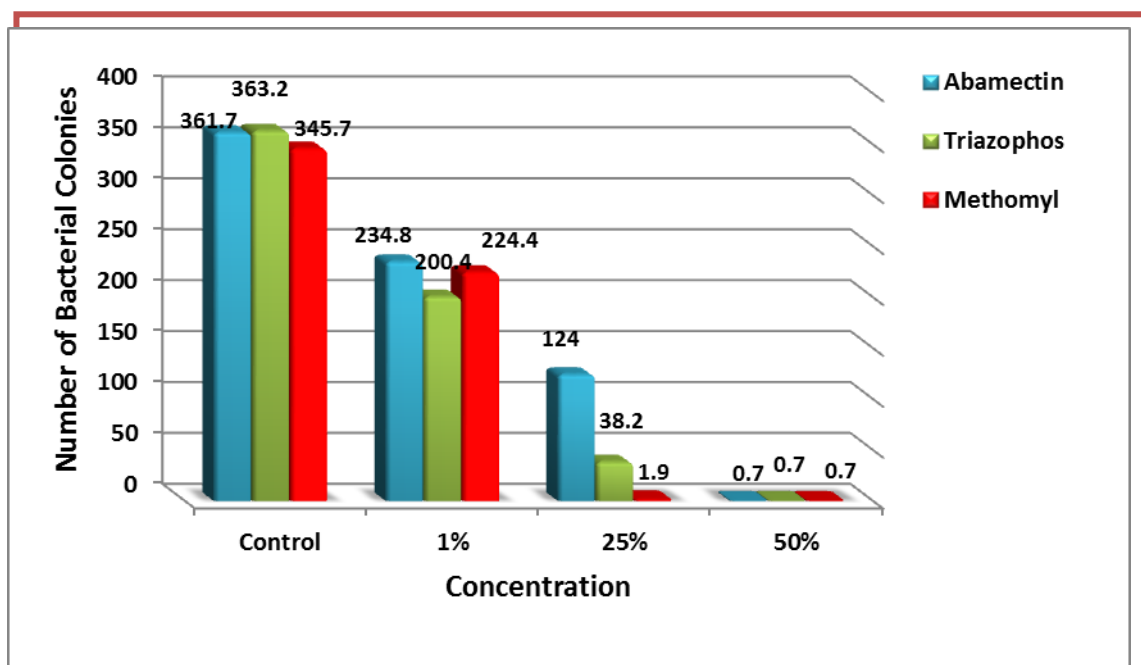


Figure (1):Effect of Some Pesticides Used on Khat Trees in (Al- Husien District, Dhale Governorate) on the Growth of Bacteria

Results and Discussion

Table (1) and Figure (1) show the insecticides used on the Khat trees affect the number of bacterial colonies growing in the medium after the completion of the incubation period "48 hours at 37 ° C". The toxicity of the pesticides varied which was studied on the effect of bacterial colonies growth in comparison with control which was not treated with insecticides. It has been found that the incassating in growth can be described as abundant. The average number of bacteria colonies in all the replicates was average 356.9 CFU/gm with percentage 100%.

The three concentrations 1%, 25% and 50% were tested each pesticides. It was found that the effect of concentration 1% of Abamectin on bacterial growth was 234.8 CFU/gm with percentage

of 65.78%. Also, the bacterial growth at the concentrations 25% and 50% of Abamectin was 124.0 CFU/gm (34.7%) and 0.7 CFU/gm (0.19%), respectively, compared with control.

It was found that the effect of concentration 1% of Triazophos on bacterial growth was 200.4 CFU/gm with percentage of 56.1%. Also, the bacterial growth at the concentrations 25% and 50% of Triazophos was 38.2 CFU/gm (10.7%) and 0.7 CFU/gm (0.19%), respectively, compared with control.

It was found that the effect of concentration 1% of Methomyl on bacterial growth was 224.4 CFU/gm with percentage (62.8%). Also, the bacterial growth at the concentrations 25% and 50% of Methomyl was 1.9 CFU/gm (0.5%) and 0.7 CFU/gm (0.19%), respectively, compared with control.

The addition of insecticides used in the experiment led to a significant reduction in the average number of bacterial colonies in all dilutions used compared to the control which was not treated with pesticides. There was no growth at concentration of 50% for all pesticides tested, Abamectin, Triazophos and Methomyl, due to the double concentration of Pesticides, (field concentration) while at a concentration of 25%, the growth of bacterial colonies was moderate. In the concentration of 1% according to the concentration recommended by the company, the growth was good, as a gradual increase in the number of bacteria was observed with a decrease in the concentration of pesticides, while the number of bacterial colonies decreased when the concentration was high. There was no growth in high concentration at all. The results showed clearly the varied effects appeared in this treatment on the number of bacterial colonies based on the type of pesticide and its concentration and active ingredients and its remaining period in soil.

The study is in line with (31) study that the decreasing of bacterial numbers occurred at high concentrations of Methomyl pesticide where Methomyl is a pesticide with a high survival period in the soil and is highly toxic to animals and humans. This toxicity is increased by the decomposition of this pesticide into other products. This pesticide is Carbamate, which belongs to the same group of Oxamil which leads to kill *E. coli* bacteria.

This study is in consistent with (20) study on the effect of different rates of pesticides (i.e. Abamectin and Benomil) on the growth and the number of aerobic nitrogen-fixing bacteria, *Burkholderia Azotobacter* spp. They pointed out that when Abamectin insecticide was added to the bacterial cells and the concentration was increased to the doubled quantity greater than the recommended quantity led to a reduction in the average number of bacterial cells in all dilutions used respectively and their absence at high concentrations of the pesticide.

(30) Referred, in his study on the effect of Abamectin and Match insecticides on the growth of *A. alternate* fungus, that when added both insecticides to the culture medium, it fixed the diagonal growth of fungus.

(15) Also found that *E. coli* could not decompose Propanil to other compounds in laboratory. (16) indicated that the high concentrations of Oxamil led to a degradation of bacterial numbers to 10.5×10^8 and 11.3×10^8 (WM) g / soil at concentration 1 and 2 ppm respectively.

(35) Indicated that the treatment with Cybercl insecticide at high concentrations (2500 ppm) reduced the diameter of *P. oliganderrum* growth to 6.5 and 6.3 respectively.

The study is in line with (44) that the use of chemicals such as pesticides may have an inhibitory effect of the process of nitrification and thus it may reduce the movement of nitrogen in the soil. This is caused by its impact on soil microbes and reducing its effectiveness as these substances have a toxic effect. The toxic effects of chemicals vary depending on the doses and soil properties, they work to decrease the number of nitrifying bacteria in the soil, which leads to reduce the process of nitrification and diffusion and thus it reduces the amount of nitrogen.

The study is in line with (25) study where he indicated that the reduction of the number of nitrifying bacteria was sensitized to the addition of the Xenobiotics pesticide, which is considered as a chemical, which resulted in inhibited the growth of these bacteria.

This study is in consistent with A (13) study on the effect of Deltamethrin and Chlorpyrophos insecticides on the total number of bacteria of economic importance in the soil. The study displayed that insecticides affected the total number of bacterial groups and the effect of pesticide on the number of bacteria was relevant to the degree of concentration added, where the number of bacteria decreased in the doubled quantity and the quantity prescribed.

This study is in line with (36), which indicated that insecticides reduced the number of nitrogen-fixing bacteria. These insecticides may be non-toxic when they are used at the recommended concentration, but the repeated use of these pesticides for a long time may lead to their accumulation, which is reflected in microorganisms and plant roots.

(3) Also indicated that there was an inhibition of bacterial growth after the Tridemorph fungicide had been added.

This study is also in consistent with (32), which indicated that the growth of bacteria in an environment with a high pesticide content led to stunted growth of bacteria and consequently its death.

(34) Pointed out that the use of the Mankogel fungicide has caused the inhibition of the growth of bacteria (*A. Chroococcum*) as there was no growth in any concentration. There were no numbers of such bacteria at high concentrations of the pesticide.

(36) Reported that insecticides such as Chloropyrifos and Profenfos played a significant role to reduce the numbers of nitrogen fixing bacteria.

(40) Showed that the addition of the Dipterex insecticide to different concentrations led to a decrease in the number of bacteria when increasing the concentration in the medium.

(37) Indicated that the inhibition of bacteria number occurred when the pesticide was added at a concentration that is greater than what was recommended.

(18) indicated that *Azobacter* is a biofertilizer in rhizosphere soil and its existence is determined by several factors, including the type of pesticide and its concentration and the method and time of addition, where it showed the sensitivity of these bacteria to the added pesticides (i.e. fungicides and insecticides) when they were analyzed microbiologically.

(11) Also pointed out that the increase of Ronstar pesticide concentration led to a decrease in bacterial cell numbers.

This study also is in line with (38) that excessive and repeated use of pesticides for a long time has many negative effects, including the emergence of problems of environmental contamination, which reflected negatively on the health of humans, animals and plants and also showed negative effects on the soil microbes through eliminating them

The study also is in line with (28) (6) (5) They pointed out that insecticides affect microbes activity and this effect lasts for a long period of time, where increasing the concentration of the pesticide above the prescribed quantity and doubled quantity led to the reduction of bacteria numbers and inhibition of them. This has been displayed by checking their numbers.

(1) indicated in their study on the effect of the interaction between soil salinity and the Topik herbicide on the numbers of bacteria (i.e. *Nitrosomonas* and *Nitrobacter*) of different tissues soil. The study has shown that there was a significant effect of Topik herbicide on the numbers of bacteria (i.e. *Nitrosomonas* and *Nitrobacter*) during the incubation period by adding 50%, 75% and 100% from the recommended concentration of the herbicide, where the number of bacteria decreased, because the herbicide reaching the soil is considered as a chemical substance affects the microorganisms, which disrupts the natural balance of the ecosystem in the soil.

This study is in line with (43) study on Determination of Minimum Inhibitory Concentration of chemical pesticides on the number of *Alzotobacter* bacteria under different periods of incubation. They pointed out that increasing the concentration of (super tracidine) pesticide from zero to 0.8 ml/ liter led to lack of growth of Bacteria in all dilutions used.

The study is in line with (7) study on the effect of Cypermethrin pesticide on some types of bacteria, where he pointed out that the rates of numbers of bacterial colonies that were found in the

environment naturally decreased when the concentration of the pesticide was increased. This means that the concentration of the pesticide has an adverse effect on the rates of numbers of bacterial colonies growing in the treated medium with the pesticide.

Conclusion

Present investigation was carried out to the effect of six pesticides used on soil in laboratory. This study revealed that the all pesticides used for high concentration have generally led to an inhibitory in the growth of bacteria in the soil and activity increased of bacterial counts with a decrease in the concentration of pesticides. Proving that the inhibitory effect is concentration-dependent.

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فحص تأثير ثلاثة أنواع من المبيدات على بكتيريا التربة

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المخلص

أظهرت نتائج دراسة تأثير ثلاثة تراكيز 1% و 25% و 50% من كل مبيد على نمو البكتيريا وتم مقارنتها مع المجموعة الضابطة التي كان عدد المستعمرات البكتيرية فيه 356.9 لكل واحد جرام من التربة بنسبة 100%. وجد بأن عدد المستعمرات البكتيرية عند تركيز 1% من كل مبيد ابامكتين، ترايزوفوس و ميثوميل 234.8/1 جم (65.78%) و 200.4/1 جم (56.1%) و 224.4/1 جم (62.8%) على التوالي. بينما وجد بأن عدد المستعمرات البكتيرية عند تركيز 25% من كل مبيد ابامكتين، ترايزوفوس و ميثوميل 124.0/1 جم (34.7%) و 38.2/1 جم (10.7%) و 1.9/1 جم (0.5%) على التوالي. بينما كان معدل عدد المستعمرات البكتيرية عند تركيز 50% في كل المبيدات الثلاثة 0.7/1 جم (0.19%).

الكلمات المفتاحية: مستعمرات بكتيرية، مبيدات، بيئة الاجار الغذائية.