Plant Archives Vol. 18 No. 2, 2018 pp. 1679-1683 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF PESTICIDE RESIDUES CONCENTRATIONS IN THE GROWTH OF SOME SOIL BACTERIA

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Abstract

The aim of the study was to use three types of pesticides (Topic100Ec, Granistar 75 DF Jantastar - 75 DF) with three doses (full dose, half dose and double dose) in the growth and activity of three bacteria (*Bacillus subtilis*, *Azospirillum brasilense*, *Azotobacter chroococcum*). In order to determine the effect of the three bacteria on the degradation of pesticides found in the soil. The bacteria were counted after the survival of these pesticides for four weeks. *Bacillus subtilis* bacteria affected jantastar at twice the recommended dose for four weeks in soil 264.66×10^7 CFU. While, it did not affect granistar at half the recommended dose and was 94×10^7 CFU. Pesticides have a negative effect on the growth of maize plant in terms of the length of the total vegetative and dry weight, which led to the death of the plant. Where the highest length of the vegetative group at the three overlap between granistar and the recommended concentration and *Azotobactor chroococcum* was 11.16 cm. The highest vegetation weight at granistar with the recommended concentration of *B. subtilis* was 1.26 g and the best total root weight was 1.20 g. Non-germination of the seeds with the addition of topic with double concentration recommended for *Bacillus subtilis*.

Key words : *Bacillus subtilis*, *Azospirillum brasilense*, *Azotobacter chroococcum*, TOPIC100Ec, Granistar 75 D.F, Jantastar – 75 D.F.

Introduction

Bioremediation, a good alternative technique used in microorganisms such as bacteria and fungi, as well as plants to break down toxic substances (Biodegradation) and to convert them into less toxic substances through metabolic processes or secretion of enzymes that destroy toxic substances (Vidali, 2001). The effectiveness of microorganisms is directly related to the availability of nutrients and the presence of organic matter in the soil stimulates microbial activity and facilitates the process of biodegradation (Pal *et al*., 2005). The bacteria, one of the most important biological ecosystem components in the soil. They are the most microscopic living organisms in existence and superior in their populations compared to the other living groups of Actinomyces, Fungi, Algae and Protozoa (Atlas, 1997; Alexander, 1977). Many microorganisms in soil produce some substances that help to grow. These materials were first shown in the Rhizosphere area of the plant and their effects were attributed to their production of Indole acetic acid (IAA) (Riviere, 1963). Brown (1975) noted that the growth of wheat plants improved in vitro fertilization with megatium, Azotobacter chroococcium and Azospirillum brasilense. That is due to the production of these organisms for growth organizations such as Auxin, Gibberellin and Cytokines. Kloepper *et al*. (1988) noted that many species of Rhizosphere have the potential to promote plant growth when added to seeds, roots and leaves. These are called plant growth promoters Rhizobacteria (PGPR). These include Acetobacter, Azospirillum, Azotobacter, Bacillus, Entrobacter and Pseudomonas (Bashan *et al*, 1993). The species (*Bacillus subtilis*, *Azospirillum brasilense* and *Azotobacter chroococcum*) were selected for the study of their effect on the degradation of TOPIC100Ec, Granistar 75 D.F. and Jantastar-75 D.F.

Materials and Methods

Azotobacter chroococcum, *Azospirillum brasilense* and *Bacillus subtilis* were obtained from the PhD laboratory at the Plant Protection Department of the Graduate School of Agriculture at the University of Kufa where they were diagnosed at the Biotechnology Center of the Ministry of Science and Technology. It was purified and propagated on the NA medium. After that the dishes and tubes were incubated until use.

Bacteria incubated at 30°C and fungi at 25°C. Three pesticides were used to control the wheat bushes (Topic, Granistar and Jantastar) from one of the agricultural offices. These pesticides were prepared to different concentrations. Nutrient Prepared by dissolving 28 g of medium in 1 liter of distilled water according to the manufacturer's instructions (Salucea, Dutch origin) and then sterilized at 121°C and 15 lb/inch^2 for 20 minutes. After the sterilization period was finished, the vials were left to cool. The medium was then poured into the plastic dishes according to the required experiment and kept in the refrigerator at a temperature of 2 m until use. This medium was used to develop the bacteria under study. Nutrient Broth was prepared by dissolving 13 g of medium in 1 liter of distilled water according to the manufacturer's instructions (Salucea, Dutch origin) and then sterilized at 121°C and 15 lb / inch^2 for 20 min. After sterilization the vials were cooled and then stored in the refrigerator. The soil was sterilized and distributed in a 150 g plastic bag with 5 mL of growing bacteria on 48-hour BN medium and 15 ml of pesticides were added in four concentrations (zero, recommended, half recommended, twice recommended) and incubated at 30 percentages. And then calculate the numbers of bacterial colonies after four weeks. Corn seeds were grown in the same polluted soil with 10 seeds per pot. After a week of growth, seed germination rate was calculated. Four weeks later, the required measurements were taken. Where experimental design and statistical analysis were performed by Completely Randomized Design (C.R.D.) and four replicates. The averages were compared using the least significant difference (L.S.D.) and the probability level (0.05) (SAS, 2012).

Results and Discussion

Table 1 shows that the type of pesticide has a significant effect on bacterial growth. The effect of Granistar in *B. subtilius*, which gave the lowest number of cells was 181.83 × 10^7 cells / ml compared to the highest number of 223.49 × 10^7 cells / ml at Jantastar After 4 weeks compared to 281 × 10^7 cell / ml treatment. The results showed that the bacteria were affected by Jantastar over three weeks but were not affected in the fourth week where resistance appeared in it due to a change in the sequence of the bases of the DNA of the bacterial cells, which may affect the metabolic pathway which leads to an increase or Lack of bacteria and thus the efficiency of atmospheric nitrogen stabilization (El-zawahry, 1976).

The lowest number of bacterial cells with the recommended concentration was 132.66 × 10^7 cells / ml and the highest number was at the recommended half concentration at 185.21 × 10^7 cells / ml after 4 weeks' incubation period. We conclude that increasing the concentration of pesticides reduces the growth of bacteria. The interaction between the type and concentration of the pesticide has a significant effect. Jantastar was given with the recommended half concentration and the highest number of bacterial cells was 264.66 × 10^7 cells / mL medium. The lowest number was for the granistar pesticide when added to the weak concentration of 133.33 × 10^7 cells / mL medium after a period of 4 weeks.

Effect of pesticide residue concentration in *Azospirllum brasilense*

The effect of the granistar pesticide on the growth and preparation of bacterial cells was 221 × 10^7 cells / mL medium after the incubation period of 4 weeks. While, topic gave the best growth of bacterial cells after a 4-week incubation period of 265.16 × 10^7 cells / ml compared with the treatment that did not receive a 335 × 10^7 cell / ml incubator after a 4-week incubation period, as shown in table 2. This is because the rate of pesticide degradation depends on the bacterial isolates used for pollination (Burns, 1995).

The added concentration in double dose was the most significant effect of bacterial cells, which reduced their growth and gave about 197.22 × 10^7 cells / mL medium after a 4-week incubation period. While the recommended concentration gave the best results at 228.33 × 10^7 cells/ ml after a 4-week incubation period. The number of dead cells is more than the number of living cells as a result of nutrient depletion due to the degradation of pesticides and the accumulation of waste, so the center becomes inappropriate and then the growth stops (Al-Sharabi *et al.*, 2004). It is believed that bacteria have been able to convert pesticides into poisonous products of the same species that affect the original compound (Alexander, 1982).

The interaction between the pesticide type and its concentration was significant. Where the addition of the Topic pesticide to the double dose gave the best number of bacterial cells 265.66 × 10^7 cells / ml medium. While the low-dose carnastar gave the lowest number of bacterial cells at 119 × 10^7 cells / mL medium. The Granistar pesticide has a strong effect on A. brasilense compared to the Topic and Jantastar inhibitors (Usman *et al.*, 2017) demonstrating that some bushicides are hazardous to microorganisms in the soil while others are useful.

Effect of pesticide residue concentration in *Azotobacter chroococcum*

Granistar reduced the growth of *A. chroococcum*. It gave 183.16 × 10^7 cells / mL medium. While the topic pesticide stimulated bacterial growth of 259.08 × 10^7 cells / mL medium. This finding is consistent with Kole *et al.* (1994) that *A. chroococcum* was able to analyze the pesticide by 45% after 10 days and 55% after 20 days (table 3).

The recommended concentration gave a minimum of bacteria of 149.77 × 10^7 cells / mL medium, while the concentration gave the recommended double highest bacteria 211.11 × 10^7 cells / ml medium compared to the type that did not receive a pesticide was 353.33 × 10^7 cells / ml medium and this result contrasts with (Suhail and Fahmi, 2009). The results in table 3 showed that the interaction between the pesticide type and its concentration had a significant effect. The effect of granistar was half-dose on bacteria of 94 × 10^7 cells / ml. The highest dose of the highest dose was 265 × 10^7 cells / ml compared to the non-pesticide type, which reached 353.33 × 10^7 cells/mL medium. It is clear that the granistar pesticide has affected the growth of *A. chroococcum* during the four weeks of growth. The concentration showed the lowest recommended growth rate. This is due to the passage of the bacteria in the Lag phase, which is adapted to the emergency conditions (addition of the pesticide). That a high proportion of the cells of the vaccine cannot grow in the new media that move to die, and after that period gets acclimated in the bacteria as the cells adapt to the new medium, where it takes some time to get accustomed to the new environment and the construction of new enzymes that can benefit from that medium (Sharabi *et al*., 2004). We see an increase in growth as enzymes are fully available (Khafaji, 1987). That the bacteria begin to destroy the compound particles (pesticide mixture) and reduce its toxicity and use as a source of food and energy, resulting in an increase in growth.

The type of pesticide has a significant effect on the length of the vegetative total of the yellow maize tree. The topic gave the lowest rate of plant length of 4 cm, which is significantly different from jantastar and granistar, which did not differ significantly in plant height. The concentration of pesticides significantly affected the length of the vegetative length to give the recommended concentration the lowest rate of 3.9 cm compared to the standard type, which gave the highest rate of 14.5 cm. The use of pesticides in the recommended concentration and half recommended significantly affected the rate of plant height, but there is no significant difference between them. The type of bacteria used did not have any significant effect. We conclude that the triangular interference between the type and concentration of the pesticide and the type of bacteria used have a significant effect on the growth of the yellow maize plant. This is reflected in vegetative length. The topic had a significant effect on vegetative length and reached plant death, especially at the recommended concentration of *Azotobacter* and *Azospirillum*, as well as the recommended concentration using *Azotobacter* and *Bacillus* bacteria. This is evidence of non-resistance of these types of bacteria to this pesticide, which is reflected negatively in plant growth. The growth of maize plant was good when using the recommended concentration of granistar, especially *Azotobacter*, gave a total length of vegetative 11.16 cm, which did not differ significantly when using the same bacteria at the concentration of 0 of the pesticide and the difference jantastar effect in the length of the total vegetative to give the lowest length of the plant at use of the recommended double concentration of 2 cm when using Bacillus bacteria.

Topic had the lowest vegetative weight of 0.516 g, which differed significantly from the other jantastar and granistar pesticides, which did not differ significantly in vegetative weight. The recommended multiplier gave the lowest rate of 0.63 cm compared to the standard that gave the highest rate of 1.36 g. Pesticides of the recommended concentration and half of the recommended significant effect on the weight of the total vegetative but there is no significant difference between them. Vegetative weight is good when using the recommended concentration of granistar, especially in *Azotobacter chroococcum* and *Bacillus subtilis*, where they reached 1.20 g and 1.26 g respectively, which did not differ significantly when using the same bacteria at zero concentration and jantastar effect in total vegetative weight to give the lowest weight when using concentration. The recommended multiplier was 0.73 g at *Bacillus subtilis*. The best dry weight was zero concentration of the pesticide with *Azotobacter chroococcum*, which reached 1.5 g.

Topic gave the lowest mean root weight of 0.45 g, which differed significantly from jantastar and granistar. The effect of the insecticide was significantly lower in the total root weight. The recommended double concentration was 0.58 g, compared to the standard of 1.4 g. The recommended concentration and half of the recommended concentration in the total root weight was significantly different. The most beneficial effect of Bacillus was 0.96 g. It concludes that the triangular

Table 1 : Effect of pesticide residues in numbers of *Bacillus subtilis.*

Pesticide	Pesticide Concentration				Average
	No	Half of the recommended	Recommended	Double recommended	
Topic	281.00	153.66	151.66	105.33	172.91
Jantastar	281.00	264.66	189.00	159.33	223.49
Granistar	281.00	137.33	175.66	133.33	181.83
Average	281.00	185.21	172.10	132.66	192.74
L.S.D(0.05)	Type of pesticide = 9.74		Type of pesticide = 10.59		For Interference = 20.19

Table 2 : Effect of pesticide residues in numbers of *Azospirllum brasilense*.

Pesticide	Pesticide Concentration				Average
	No	Half of the recommended	Recommended	Double recommended	
Topic	335.00	235.00	225.00	265.66	265.16
Jantastar	335.00	210.00	240.00	207.00	248.00
Granistar	335.00	211.00	220.00	119.00	221.25
Average	335.00	218.66	228.33	197.22	244.80
L.S.D(0.05)	Type of pesticide = 7.76		Type of pesticide = 8.90		For Interference =17.84

Table 3 : Effect of pesticide residues in numbers of *Azotobacter chroococcum*.

Pesticide	Pesticide Concentration				Average
	No	Half of the recommended	Recommended	Double recommended	
Topic	353.33	252.33	165.66	265.00	259.08
Jantastar	353.33	154.00	173.33	193.33	218.49
Granistar	353.33	94.00	110.33	175.00	183.16
Average	353.33	166.77	149.77	211.11	220.24
L.S.D(0.05)	Type of pesticide=11.75		Type of pesticide=13.66		For Interference =22.61

interference between the type and concentration of the pesticide and the type of bacteria has a significant effect on the root weight of the maize plant. Topic has a significant effect on the root weight, resulting in plant death, especially at the recommended concentration of *Azotobacter chroococcum*, *Bacillus* and *Azotobacter chroococcum*. This indicates the non-resistance of these types of bacteria to this pesticide as it adversely affected the growth of the plant. Plant growth was good at jantastar, especially at *Azotobacter*. While the highest root weight was 1.93 g, followed by Bacillus at the same concentration of 1.31 g and the lowest rate at the double concentration of Bacillus with 0.56 g. Granistar gave the highest concentration at the recommended concentration of *Bacillus* with 1.20 g, while the lowest concentration was with azospirillum 0.53 g. That pesticide residues have a negative effect on maize and this is consistent with Annonymous (2008) and Haathela *et al.* (1988) and Saleh and Muhammad (2010). The results differ with Ebadi (2007), which has shown no apparent negative effects of residues in the soil. The bacteria were also able to reduce the effect of pesticide residues (Khalid and Khokhar, 2013).

Acknowledgement

The authors acknowledge the financial support of the University of Kufa, Iraq. The author is grateful to Dr. Basim A. Almayahi, Department of Ecology, College of Science, University of Kufa (basimnajaf@yahoo.com) for assisting me throughout conducting the present research.

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