Effect of Type and Concentration of Organic Acids on Soil Bacteria Growth

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Abstract

The study was carried out at the University of Kufa - College of Agriculture for the season 2023-2022 to study the effect of the type and concentration of organic acids on the growth of soil bacteria, the first experiment includes the cultivation of bacteria on optional, broth, four types of organic acids were used (Citric acid, Acetic acid, Oxalic acid, Propionic acid) in concentrations (0,25.50.75.100.150) mM factor included four types of bacteria (*Pseudomonas fluorescens*, *Acinetobacter calcoaceticus*, *Bacillus subtilis*, *Enterobacter cloacae*). During this experiment, the minimum inhibitory concentration of these organic acids was determined, the second experiment included planting wheat seeds of a local variety by 4-2seeds per pot and used two types of organic acids (Acetic acid, Oxalic acid) and two types of pathogenic bacteria and biological resistance (*Enterobacter cloacae* and *Bacillus subtilis*). The results of the laboratory experiment showed a significant effect of organic acids in reducing the growth of bacteria, the addition of organic acids to the soil had a significant effect on the values of (Ec, PH) in the soil as oxalic acid obtained the highest rate of 6.370 dc m^{-1} At a concentration of 50 mM, oxalic acid obtained the highest rate of soil reaction score of 6.300 at a concentration of 75.50mM. The results indicate the significant effect of organic acids in inhibiting the total bacteria population in the soil, as oxalic acid obtained the highest rate of 106×114.3 $g\text{-}^{1}$ dry soil at 50mM concentration.

Keywords: Organic Acids , *Enterobacter cloacae* ، *Bacillus Subtilis*

Introduction

Triticum aestivum wheat is one of the important cereal crops in Iraq, and belongs to the Poaceae family of grasses, which is one of the most important strategic crops in the world, as it comes mainly in terms of cultivated area and production, as the global productivity reached 3.48megaha $^{1-}$[1]. In Iraq, it amounted to 2.74mg ha^{1-} [2]. Bacteria *stand out Enterobacter cloacae* being a plant pathogen is a bacteria negative for the dye Cram optionally anaerobic, non-spore and mucous [3], *Bacillus subtilis* is a model for study compared to its counterparts positive for the dye of Cram, due to the possession of physiological, genetic and anatomical characteristics that enable it to survive so it is used in biological resistance, a *Bacillus* bacteria positive for the dye of Cram, growing at temperatures 25-35 It can grow at higher temperatures [4], compulsory aerobic or optional anaerobic [5]. Organic acids are antibiotics used in the food industry for the purpose of inhibiting the growth of important pathogens that compete with plants to absorb nutrients because these acids have a toxic effect on the metabolism of harmful bacteria and fungi [6].

Materials and methods

An experiment was carried out according to the CRD design with two factors, the first factor is different from organic acids (Citric acid, Acetic acid, Oxalic acid, Propionic acid) at concentrations of mM (0,25,50,75,100,150) and the second factor included types of bacteria (*Pseudomonas fluorescensas*, *Acinetobacter calcoaceticus*, *Bacillus*). *Subtilis*, *Enterobacter cloacae*) bacteria were cultured and obtained from the department of Entomology, Faculty of Agriculture, Kufa University. cultured on broth nutrient to study the type and concentration of organic acids in bacterial growth and their effect During this experiment, the minimum inhibitory concentration of organic acids is determined by measuring CFU after 48 hours of growth and density of bacteria and knowing the effect of acids on the pH medium. The second experiment was carried out according to the design (CRD) with two factors, the first two types of organic acids (Acetic Acid, Oxalic acid) are the most inhibited based on the first experiment for the purpose of studying the presence of pathogenic bacteria by adding concentrations of organic acids mM (0, 25, 50 75). The second factor included combinations between organic acids with pathogenic bacteria (*Enterobacter cloacae*) and once with *Bacillus* bioresistant bacteria with where 10 ml / pot was added, and four levels of organic acids (Acetic Acid, Oxalic acid) (0,25,50,75) mM. Spelt seeds were cultivated, a local variety, by 2-4 seeds per pot. These soils are incubated to a temperature 30 °C for 3 months , during which the CFU is measured after 3 months, as well as the degree of interaction and electrical conductivity.

Statistical analysis

The laboratory experiment was completely randomized, whereas the field experiment was a factorial experiment with a completely randomized block design. The means were compared using the least significant difference at a 5% level of significance.

Results and Discussion

Effect of different concentrations of organic acids (Citric, Acetic, Oxalic, Propionic) on the growth of bacteria (*Enterobacter*, *Acinetobacter*, *Bacillus* *Pseudomonas*) on the nutrient medium after different periods of incubation.

The tables (1and 2) showed the interaction between organic acids and concentrations a significant effect in inhibiting the number of bacteria after 24-48 hours of incubation, where the concentration of 50mm of oxalic acid exceeded to give the highest value of 309.0 $\times 10^6$ Cfu ml^{-1} medium at the concentration of 100mM of the same acid to give the lowest number of bacteria of 22.7 $\times 10^6$ Cfu ml^{-1} Medium after 24 hours , while after 48 hours the concentration of 50Mm of oxalic acid was 618.0 × 10^{6} cfu ml^{-1} medium while the concentration of 100Mm of the same acid was 45.3 × 10^{6} cfu ml^{-1}medium.

Table (1): Effect of Different Concentrations of Organic Acids on the Groth of *Enterobacter* Cfu×10^{6} ml^{-1} medium after 24 hours of incubation in nutrient medium .

Enterobacter						
Acid	Concentrations					Medium acid
	25	50	75	100	150	
Acetic	169.3	1400	132.0	184.0	141.3	153.3
Citric	270.0	261.3	294.7	222.3	140.7	237.8
Oxalic	15.0	309.0	218.7	22.7	53.3	123.7
Propionic	71.7	53.3	67.3	73.0	67.3	66.5
Control	253.3					
LSD	62.37					LSD_{Acid} 27.89
Medium Ultrasonic	131.5	190.9	178.2	125.5	100.7	
LSD	31.18					

Table(2):Effect of Different Concentrations of Organic Acids on *Enterobacter* Bacteria Growth After 48 Hours of Incubation

Enterobacter							
Acid	Concentrations					Medium acid	
	25	50	75	100	150		
Acetic	324.7	280	205	340	235	276.9	
Citric	493.3	515.3	511.7	331.7	231.3	416.7	
Oxalic	26.3	618	437.3	45.3	107	246.8	
Propionic	140	100.7	114	141.3	130.7	125.3	
Control	430.3						
LSD Acid*Concentrate	97.09					LSD Acid	43.42
Medium Ultrasonic	246.1	378.5	317	214.6	176		
LSD Concentration	48.55						

The results in a table (3,4) indicate that the interaction between organic acids and concentrations has a significant effect in inhibiting on the number of bacteria *Acinetobacter* after 24-48 hours of incubation where the concentration of 50mm of acetic acid exceeded to give the highest value of 258.7 $\times 10^{6}$ cfu ml^{-1} medium on the concentration of 25mM of citric acid to give 20.0 $\times 10^{6}$ cfu ml^{-1} medium after 24 hours, while after 48 hours the concentration of 50Mm of acetic acid was 555.0$\times 10^{6}$ cfu ml^{-1} MV, while the concentration of 25Mm of citric acid was 26.0$\times 10^{6}$ cfu ml^{-1} medium.

Table (3): Effect of Different Concentrations of Organic Acids on the Growth of *Acinetobacter* Bacteria after 24 Hours of Incubation

Acinetobacter							
Acid	**Concentrations**					Medium acid	
	25	50	75	100	150		
Acetic	93.3	258.7	82.7	31.3	59.3	105.1	
Citric	20.0	183.3	167.3	150.0	150.0	134.1	
Oxalic	249.3	215.0	102.7	122.7	38.0	145.5	
Propionic	165.3	200.0	146.7	160.0	247.3	183.9	
Control	115.3						
$LSD_{Acid*Concentrate}$	77.37					LSD_{Acid}	34.60
Medium Ultrasonic	132.0	214.2	124.8	116.0	123.7		
$LSD_{Concentration}$	38.69						

Table(4):Effect of Different Concentrations of Organic Acids on the Growth of *Acinetobacter* Bacteria after 48 Hours of Incubation

Acinetobacter							
Acid	**Concentrations**					**Medium acid**	
	25	50	75	100	150		
Acetic	220.0	555,0	205.0	61.0	141.0	236.4	
Citric	26.0	330.3	299.7	276.7	286.7	243.9	
Oxalic	320.3	455.0	193.3	221.3	71.7	252.3	
Propionic	311.7	300.0	248.0	301.7	426.3	317.5	
Control	158.0						
LSD acid * concentration	86.87					LSDالحامض	38.85
Medium Ultrasonic	219.5	410.1	236.5	215.2	231.4		
LSD concentration	43.43						

Table (5,6) While the interaction between organic acids concentrations had a significant effect in inhibiting the number of bacteria after (48-24) hours of incubation, where the concentration exceeded 25mm of acetic acid to give the highest value of

249.3× 10 ^{6} cfu m^{l-1} medium on the concentration of 25mM of propionic acid to give 40.0× 10^{6} cfu ml^{-1} medium after the passage of 24 hours, while after 48 hours the highest concentration of 50mm of acetic acid was 425× 10 ^{6} cfu ml^{-1} medium while the lowest concentration was 50mm of citric acid to give67× 10^{6} cfu ml^{-1} medium .

Table (5): Effect of Different Concentrations of Organic Acids on *Bacillus sp* Growth after 24 Hours of Incubation

***Bacillus* Subtilis**							
Acid	**Concentrations**					**Medium acid**	
	25	50	75	100	150		
Acetic	249.3	200	58	123.3	210	168.1	
Citric	52	44	125	112	152	97	
Oxalic	47.3	102	57	145	0	70.3	
Propionic	40	194	95.3	95.3	160	116.9	
Control	86.7						
LSD acid*concentrate	38.19					LSD_{Acid}	17.08
Medium Ultrasonic	97.2	135	83.8	118.9	130.5		
LSD Concentration	19.09						

Table (6): Effect of Different Concentrations of Organic Acids on *Bacillus sp* Bacteria Growth after 48 Hours of Incubation

***Bacillus* Subtilis**							
Acid	**Concentrations**					**Medium acid**	
	25	50	75	100	150		
Acetic	414	425	100	243	389.3	314.3	
Citric	122	67	152.3	220	239.7	160.2	
Oxalic	90.7	183.3	107.3	281	0	132.5	
Propionic	80	361	161.3	141	313.3	211.3	
Control	173.3						
LSD acid*concentrate	72.1					LSD Acid	32.25
Medium Ultrasonic	176.7	259.1	130.2	221.2	235.6		

LSD Concentration	36.05	

Table(7,8) The interaction between organic acids and concentration had a significant effect in inhibiting the number of bacteria after (48-24) hours of incubation, where the concentration exceeded 150mm of oxalic acid to give the highest value of 538.7×10^{6} cfu ml^{-1} medium on the concentration of 100mM of acetic acid to give 0.0×10^{6} cfu m^{-1}medium after 24 hour, whereas after 48 hours the highest concentration was 150mm of acetic acid to give 852×10^{6} cfu ml^{-1} medium while the lowest concentration was 100mm of acetic acid to give3.0×10^{6} cfu ml^{-1} medium

Table (7): Effect of Different Concentrations of Organic Acids on *Pseudomonas* bacteria growth **after 24hours of incubation**

Pseudomonas							
Acid	**Concentrations**					**Medium acid**	
	25	50	75	100	150		
Acetic	51.3	40	221.3	0	492	160.9	
Citric	240	290.3	210.7	232	212.7	237.1	
Oxalic	231.3	106.7	134.7	146	538.7	231.5	
Propionic	214.7	109.3	208	22.7	53.3	121.6	
Control	257.3						
LSD acid*concentrate	77.75					LSD Acid	34.77
Medium Ultrasonic	184.3	136.6	193.7	100.2	324.2		
LSD Concentration	38.88						

Table (8): Effect of Different Concentrations of Organic Acids on *Pseudomonas* Bacteria Growth After 48 Hours of Incubation

Pseudomonas						
Acid	**Concentrations**					**Medium acid**
	25	50	75	100	150	
Acetic	89	157	431	3	852	306
Citric	538	513	312	371	357	418
Oxalic	435	213	269	292	648	372
Propionic	414	201	411	44	105	235
Control	505.3					

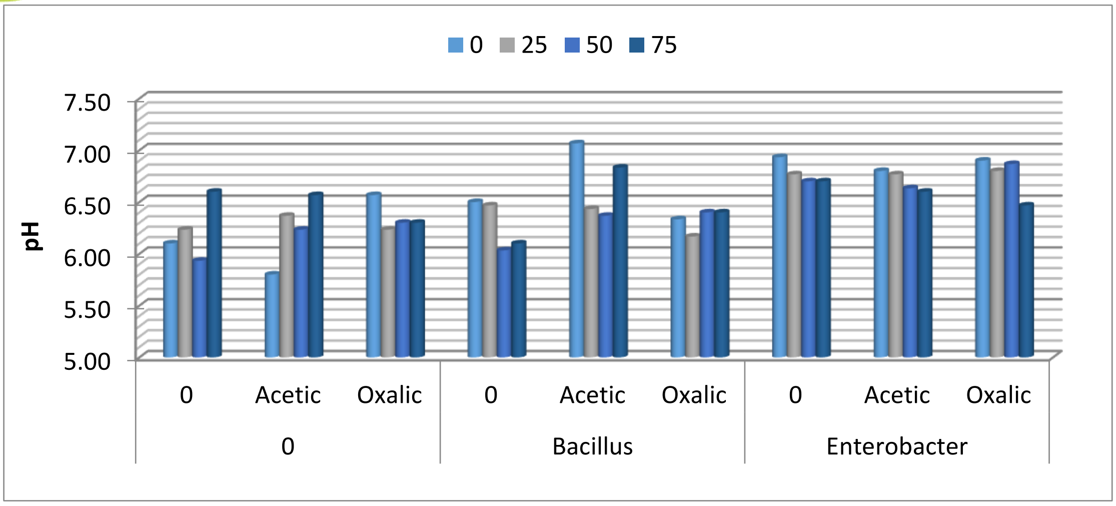
LSD acid*concentrate	163.8					LSD Acid	73.3
Medium Ultrasonic	369	271	356	177	491		
LSD Concentration	81.9						

The results in tables (1,2,3,4,5,6,7,8) indicate the significant effect of organic acids on the growth of bacteria of various kinds, as the addition of organic acids (Citric, Acetic, Oxalic, Propionic) led to reducing or inhibiting the growth of bacteria and this is confirmed [7], due to the fact that organic acids tend to enhance the disruption of the proton driving force resulting from microorganisms on the cell surface. This disorder subsequently leads to the creation of an environment Not suitable for the growth of microorganisms [8] .

Second experiment

Effect of Concentrations of Organic Acids and Bacteria on the Degree of Soil Reaction

(Figure 1)The interaction between organic acids and Enterobacter pathogenic bacteria obtained a significant effect on the degree of soil reaction, as oxalic acid obtained the highest rate of 6.867 at a concentration of 50mM while the highest rate of acetic acid at a concentration of 6.767, compared to a comparative treatment that obtained a rate of 6.933 and explains this decrease in the degree of soil interaction when adding microorganisms to the soil (*Enterobacter* and *B.subtilis).* These microorganisms have a role in the secretion of organic acids (oxalic, malic and succinic) in their middle increase the acidity of the soil as it releases during its vital activity carbon dioxide CO_2, which dissolves in water forming carbonic acid, which leads to a reduction in soil pH and then increase the readiness of nutrients and this is consistent with [9]



Figure(1):Effect of concentrations of organic acids and two types of bacteria (pathogenic and bioresistant) on the degree of soil reaction.

Effect of concentrations of organic acids and two types of bacteria (pathogenic and bioresistant Soil conductivity values after planting (EC) dc m^{-1}

(Figure 2)The interaction between organic acids and the pathogen *Enterobacter* bacteria had a significant effect on the electrical conductivity values, as oxalic acid obtained the highest rate of 4.973 dc m^{-1} at a concentration of 75 mM while the highest rate of acetic acid at the same concentration was 4.687 dc m^{-1}, compared with a comparative treatment that obtained a rate of 3.500 dc m^{-1}. This result differs with what was stated [10] as the addition of organic acids greatly affected the electrical conductivity of the soil and the soluble salt content, as the electrical conductivity of the soil and the values of soluble salt decreased significantly. The reason for this decrease is that organic acids are tricarboxyls that can separate a large amount of H+ to neutralize basic ions and use anions to absorb $^{Na+}$ in the soil and thus reduce salinity [11] Also, when organic acids decompose in the soil, they work to practice the process of removing heavy metal and eliminating soil salinity [12].

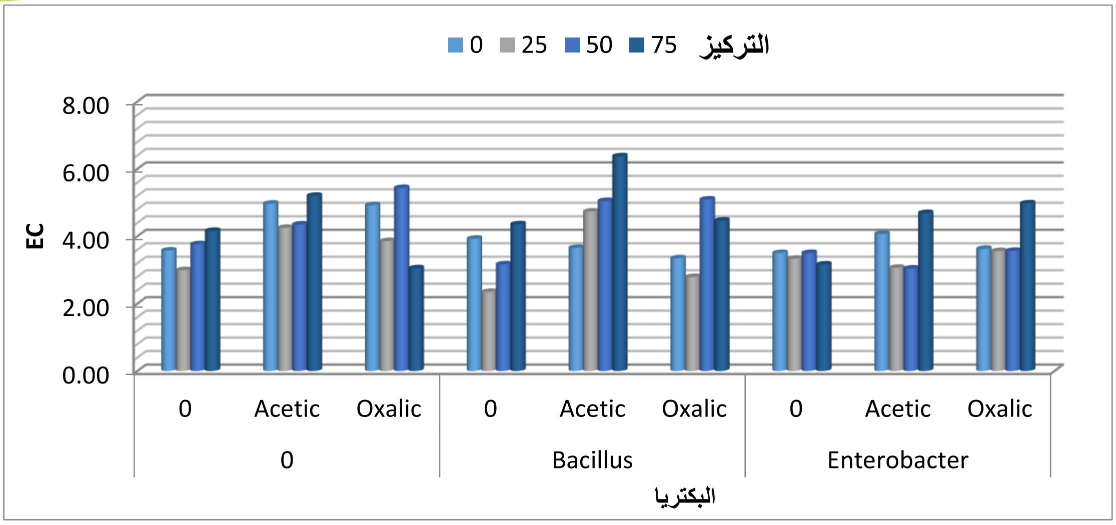


Figure (2):Effect of concentrations of organic acids and two types of bacteria (pathogenic and bioresistant Soil conductivity values after planting (EC) Dc M^{-1}

Effect of Concentrations of Organic Acids and Two Types of Bacteria (Pathogenic and Bioresistant) on Electroconductivity Values on Plant height

(Figure 3)The interaction between organic acids and *Bacillus subtilis* bio-resistant bacteria had a non-significant effect as the concentration of 50 mM of oxalic acid reached 14.00 cm $plant^{-1}$, and acetic acid at 25 mM obtained a highest rate of 15.33, compared to the comparison treatment of 22.33 cm $plant^{-1}$. While the interaction between organic acids and pathogenic *bacteria obtained Enterobacter* had a significant effect on plant height, as oxalic acid obtained the highest rate of 15.00 cm^{-1} at a concentration of 50mM while the highest rate of acetic acid was 17.00 cm^{-1} at a concentration of 75mM, compared with a comparative treatment that obtained an average of 22.33 cm $plant^{-1}$. There was a non-significant increase in plant height when spraying with organic acids and varies with [14] as spraying wheat plants in different concentrations of 100 and 200 mg / liter citric acid and oxalic acid in the sowing dates in the normal date and late date led to a significant increase in stem length. *Bacillus* bacteria are also affected The reason for this is due to the improvement of metabolic processes and encourage the absorption of nutrients as well as the absorption of water and various nutrients from the soil, which reflects positively on the state of plant growth [15] .

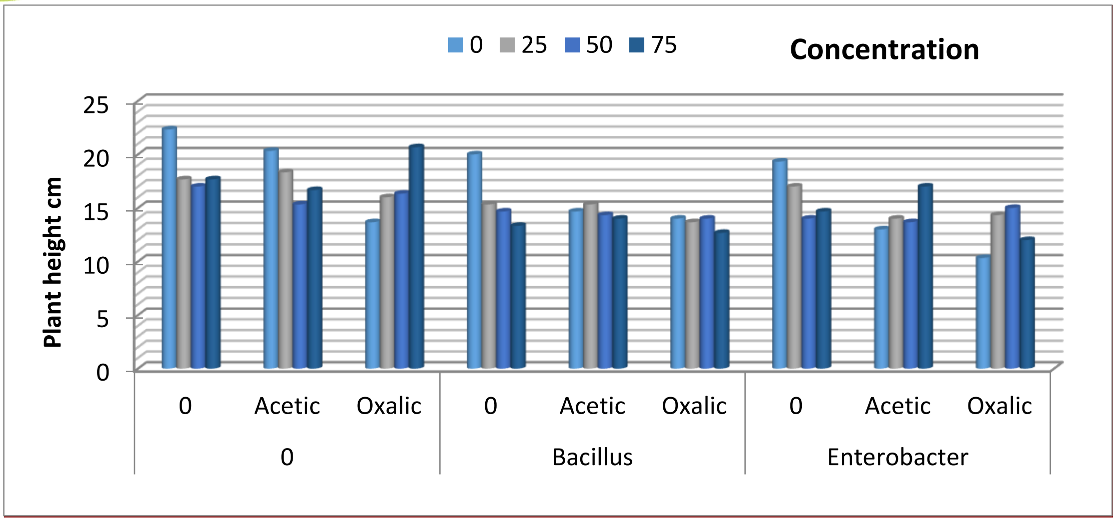


Figure (3): Effect of concentrations of organic acids and two types of bacteria (pathogenic and bioresistant) on plant height

The addition of organic acids such as citric acid had a significant effect in inhibiting the number of bacteria *Enterobacter cloacae*. The possibility of using organic acids with *Bacillus* bacteria, which have significantly affected plant growth and improve soil qualities.

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