The Effect of Interference of Acetate and Phosphorous on the Growth of Some Chemical Characteristics and the Inhibition of Types of Pathogenic Bacteria of Ocimum Basilicum Vare Viride

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Abstract
The research included studying the effect of four levels of acetic acid (0, 2, 50, 75 mg. L), which has a symbol (A0 , A1, A2, A3) respectively with four levels of phosphorous (Diphosphate, K2PO4) (0, 3, 6, 8 mg. L) and symbolized by (P0, P1, P2, P3) respectively. And their interactions. In the content of flavonoids, tannins, and phenols in leaves, and measuring the inhibitory activity of basil extract in two types of pathogenic bacteria Staphylococcus aureus, positive for Gram-negative Escherich coli . The results indicated that acetate spraying had a significant superiority in the content of a flavonoids ,tannins and phenols . The spraying at A3 level gave the highest rate of (28.48 , 34.03 and 44.17) mg.gm-1 fresh weight, respectively . Also the A3 level had a significant effect to inhibit positive and negative bacteria, as it got an antagonistic effect of the extract towards bacteria, which reached (23, 24.25) mm For Staphylococcus aureus and E.col Respectively, and the effect of phosphorous spray was significantly superior to the leaves content of flavonoids, thanes and phenols, it reached the highest at the P3 level (29.44, 35.93 and 45.14) mg.gm-1 fresh weight, respectively. Phosphorous spray had a significant effect on inhibiting positive and negative bacteria, and the highest inhibition rate at P3 level was (19.75 and 21) mm for Staphylococcus aureus and E.coli, respectively. The results of the experiment showed that the interaction between the two experimental factors had a significant effect on the leaves content of male traits. The highest effect rate was at the level A and P3 and it reached (22.45, 36.9 and 45.6) mg.gm-1. The level of A3P3 was a significant superiority in inhibiting pathogenic bacteria, as it reached (24, 26) for Staphylococcus aure and E.coli, respectively.
Keywords: inhibition; pathogenic bacteria; chemical characteristics

1. Introduction

The genus Ocimum belongs to the Lamiaceae family, which includes about 200 species. It spreads in different regions, Cassia, West Africa and America (Arya, 2012), and then moved to France, Greece and Hungary through commercial navigation (Encyclopaedia, 2019). The genus Ocimum smells like lemon, rose, licorice, eucalyptus and cloves (Morales, 1997). Basil is characterized by containing medically effective compounds such as glycosides. Alkaloids and terpenes. Basil is used as a medicinal plant to treat head, earache, diarrhea, and cough, and as a folk remedy for indigestion, nausea, gastroenteritis, and to flavor food (Adms& Amer, 2015). It was observed that the methanolic basil leaf extract had a positive effect in relieving pain close to that of aspirin (Choudhury et al., 2010). Both the aqueous and ethanolic extract of all parts of the basil plant showed an increase in the healing of stomach ulcers when applied to experimental mice (Rahman et al., 2010). The use of the extract of the Germanic and alcoholic vegetative parts of the basil plant has a role in retrieval of the memory of mice, and that the effectiveness of these extracts is due to the effectiveness of the antioxidant compounds of Flavonids, Tannis and Terponedes in basil extracts (sarahroodi et al. 2012). It was noted that the volatile oil in the basil plant A role in inhibiting the growth of clinical isolates of bacteria (opalchenova and obrshkova , 2003) Enterococcus Staphylococcus , pseudomonas.

The alcoholic extract and the oil extracted from the leaves and stems are considered to be an inhibitor of food-borne bacteria such as E.coli and staph. Aureus, salmoellea tyohi, and Bacillius cereus (Rahman et al .,2010). The crude methanolic extract of all parts of the plant had an effective effect in inhibiting Mycobacterium tuberculosis H3RV and others. (Siddiqui , 2012).

2. Materials and Methods

1- Method of preparing flavonoids

Leaves were dried for 48 hours, then crushed and weighed 5 grams, then extracted Soxhlet and 300 ml ethanol solvent at (50-55) degrees Celsius for 3-4 hours and then filtered by filter paper. And using the rotary evaporator to concentrate the extract under low pressure at a temperature of 40 degrees, then weigh the extract after the concentration process (2.6 g), and then store it in sealed bottles at 4 degrees Celsius until use. Flavonoids were determined according to the method (Baba & Malik, 2015) in the crude extract by aluminum chloride in

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the presence of rutin. 50 ml of the crude extract was mixed with (1 ml) of methanol and (4 ml) of distilled water, then added to it (0.3) ml of 20% sodium nitrate solution and (0.3 ml) of 20% aluminum chloride, then the mixture was placed in the incubator for (10) minutes, then 2 ml of sodium hydroxide solution was added to the mixture (1molar] and then the volume was completed to 10 ml of distilled water, then the absorbance of the model was recorded at a length of 510 nm, and then the concentration of total flavonoids was calculated in to the titration curve and for rutin. And in units (mg.g-1 dry weight)[1].

2- Determination of the leaves content of tannins

The contents were determined using catechin as a standard compound, as 400 microliters of extract were taken to (3 ml) of vanillin (4%) in Methanol and (5 ml) concentrated hydrochloric acid and after 5 minutes of preservation read the wavelength of 500 nm. Then determine the level of tannin from a curve extrapolation that is done using a catechin solution (Khaabadi et al., 2011)[2]

3- Determination of leaf content of phenols

The total phenolic content of dry extracts was estimated using Folin ciocaltem reagent, where (1 ml) of the sample was mixed with (1 ml) of phenol reagent (Folin Ciocalto). After 5 minutes, (10 ml) of 7% sodium carbonate solution is added. To the mixture add 13 ml of distilled water. Mix the solution. Then kept in the dark for 90 minutes at 23 degrees Celsius. Then read the absorbance at a wavelength of 760 nm according to the method (Khaabadi et al., 2011)

3. 4- Testing the biological activity of basil leaf extract

4-1 Preparation of the extract

The leaves were dried in an electric oven at 60° C for 48 hours and ground. Take 20 gm of the ground powder for each concentration and dissolve it in 100 ml of concentrated ethanol. The solution returns. Then it is placed in a water bath, at 40°C for 3 hours. Filter with a piece of gauze to remove large parts. Then filtered by filter paper,the filtrate was collected in opaque glass dishes until use (Naik et al., 2015).

4 -2 Bioactivity of the extract was tested on bacteria

The activity of basil extract was tested on Gram-positive and Gram-negative bacteria using Wall etching method in solid agar media (Harley & Prescoh, 2002). Dissolve the Nutrient Agar by dissolving 28 g of it in a liter of water, then melting by heating and then sterilizing with an Auto clave device for 30 minutes at a temperature of 121 ° C and a pressure of 1.5 atmospheres, then the mixture is cooled and poured into sterilized dishes and left to solidify. Cotton swab It is completely planned to ensure even distribution of bacteria on the medium Small swabs with a diameter of 9 ml number 5 in each plate using a corkborer and 0.5 ml of each extract was made according to the required concentrations The plates were placed in the oven at 37 degrees for 24 hours and then measured The diameters of the inhibition zones in millimeters and using the listed ruler[3]

4. Results

1- The content of the leaves in flavonoids (mg.gm-1) It is shown in the table (1) that the spraying of acetate and phosphorous and their interactions had a significant effect on the content of flavonoids, the leaves were flavonoids, and the highest rate of acetate spraying was at the A3 level, which amounted to (28.48 mg.gm-1) in the lowest rate was at level A0 and reached (21.79 mg.gm-1). From the table we note that phosphorous spray had a significant superiority when spraying it on basil plant in the content of flavonoids in leaves. The rate was higher at level P3 and it reached (29.44mg.gm-1) compared to the lower rate at the Po level of (18.34 mg. gm -1) .Also the interaction between the two experimental factors had a significant effect, as it reached the highest rate for treatment A1 P3, which amounted to (29.89 mg.gm-1) compared to the control treatment (the comparison) which amounted to (14.88 mg, gm-1)

Table (1) effect of acetate and phosphorous spraying on the leaves content of flavonoids (mg/gm fresh weight.

Average effect of phosphorus (mg. L^{-1})	Acetate concentration (mg. L^{-1})				Acetate concentration (mg. L^{-1}) Phosphorus (mg. L^{-1})
	A_3	A_2	A_1	A_0	
18.34 d	27.8 e	15.75 l	14.92 m	14.88 m	P_0
22.47 c	28.24 d	21.97 jk	21.26 j	19.52 k	P_1
27.03 b	28.5 da	26.64 g	28.92 i	24.08 h	P_2
29.44a	29.4 b	29.8 a	29.89 a	28.68 d	P_3
	28.48 a	23.54 b	23.74 c	21.79 l	Average effect of acetate (mg. L^{-1})

Averages with similar letters that do not different from each other within the main factors or their interactions according to Duncan's polynomial test 0.05.

2- The content of tannins in the leaves

Through Table (2), acetate spraying had a significant effect on the tannin content of leaves, and the highest rate was at the A3 leves and it reached (34.03 mg.gm-1), and the lowest rate for acetate effect at level A0 was (27.34 mg.gm). Phosphorous had a significant effect, and the highest rate at the P3 level was (35.93 mg.g) compared to the lowest rate at the

PO level, which amounted to (27.34 mg.gm-1), and the interaction between the two experimental factors had a significant effect, the highest rate was (36.9 mg.gm-1) . The lowest rate when treating the coupling was d 19.18 mg.gm-1.

3- The content of leaves from phenols

It can be seen from Table No. 2) that acetate spraying had a significant effect on the leaves content of phenols , the highest average value at level A3 amounting to (44.17 mg.gm-1), while the lowest rate was at level A 0, it amounted to (38.07 mg.gm-1). We also note from the table that phosphorous was significantly affected by the content of phenols in the leaves, so the highest rate was at the P3 level (45.14 mg.gm-1), while the lowest rate was at the P0 level, which amounted to (38.46 mg.gm). We note from the table that the interaction of the two experimental factors had a significant effect, as the highest rate was at the level A3P3 and it amounted to (45.6 mg.gm-1) and the lowest rate was when the comparison treatment was (34.17 mg.gm-1).

Table (2) effect of acetate and phosphorous spraying on the leaves content of tannin (mg/gm fresh weight.

Average effect of phosphorus (mg .L^{-1})	Acetate concentration (mg. L^{-1})				Acetate concentration (mg. L^{-1}) Phosphorus(mg.L^{-1})
	A_3	A_2	A_1	A_0	
25.88 k	31.26 j	27.84 i	25.27 k	19.18 d	P_0
29.07 a	33.36 bc	28.76 ab	30.02da	24.14 h	P_1
32.28 j	34.62 ab	32.22dc	33.12 j	29.19 a	P_2
35.93 eb	36.90 ab	34.81da	35.13eb	36.88ba	P_3
	34.03de	30.90 d	30.88 f	27.34 c	Average effect of acetate (mg.L^{-1})

Averages with similar letters that do not different from each other within the main factors or their interactions according to Duncan's polynomial test 0.05.

Table (3) effect of acetate and phosphorous spraying on the leaves content of flavonoids (mg/gm fresh weight.

Average effect of phosphorus (mg .L^{-1})	Acetate concentration (mg .L^{-1})				Acetate concentration (mg. L^{-1}) Phosphorus (mg. L^{-1})
	A_3	A_2	A_1	A_0	
18.34 d	27.8 e	15.75 l	14.92 m	14.88 m	P_0
22.47 c	28.24 d	21.97 jk	21.26 j	19.52 k	P_1
27.03 b	28.5 da	26.64 g	28.92 i	24.08 h	P_2
29.44a	29.4 b	29.8 a	29.89 a	28.68 d	P_3
	28.48 a	23.54 b	23.74 c	21.79 l	Average effect of acetate (mg. L^{-1})

Averages with similar letters that do not different from each other within the main factors or their interactions according to Duncan's polynomial test 0.05.

4- Effect of the alcoholic extract of basil plant in inhibiting pathogenic bacteria Staph. Aureus gram-positive cultivar

We note through Table No. (4) the role of acetate extract in inhibiting Staphylococcus aureus, and the highest rate of this inhibition was at level A3 (23 mm) compared to the lowest rate at level A0, which amounted to 12.25 mm. Phosphorous spray also had the effect of Significantly in inhibiting the above bacteria, the highest rate was at the P3 level (19.75 mm). While the lowest rate was at the level P0 (16.5 mm).It is evident from Table (4) that the interaction of the two experimental factors had a significant effect, the highest rate was at the A3P3 level, which amounted to (24) mm in the lowest effect when the comparison treatment was (9 mm)

Table (4) effect of alcoholic extratic of basil leaves treated with acetate and phosphorus on inhibiting bacterial growth..staphylococcus .aureus . .

Average effect of phosphorus (mg .L^{-1})	Acetate concentration (mg. L^{-1})				Acetate concentration (mg. L^{-1}) Phosphorus(mg.L^{-1})
	A_3	A_2	A_1	A_0	
16.5 f	22 ab	19 cd	16 ef	9 i	P_0
18.05 g	23.7 n	19 cd	17.5 c	12 h	P_1
18.75 c	23.5 aj	20.8k	17.7cb	13 gh	P_2
19.75 j	24 m	22 ab	18cd	15.fg	P_3
	23.3 a	20.2g	17.3 de	12.25 d	Average effect of acetate (mg.L^{-1})

Averages with similar letters that do not different from each other within the main factors or their interactions according to Duncan's polynomial test 0.05.

5 - the effect of the alcoholic extract of the basil plant in inhibiting pathogenic bacteria (E coli) Table No. (5) indicates that spraying acetate on basil plants has a significant effect in inhibiting the pathogenic bacteria E. coli, and the highest rate was at level A3 (24.25 mm), while the lowest rate was at level A0 (14.25 mm).) . We also note from the table that spraying phosphorous on basil plants had a significant effect on bacterial inhibition, the highest average value was at the P3 level (21 mm), while the lowest rate was at the P0 level of(17.25 mm), and through the results of Table (5) we note that there is

an interaction between the two factors The experiment had a significant effect on inhibiting the pathogenic bacteria E, coli, the highest rate was when the A3 P3 treatment was (26 mm), while the lowest rate was when the comparison treatment was (9 mm)

Table (5) effect of alcoholic extratic of basil leaves treated with acetate and phosphorus on inhibiting bacterial growth. Escherichia coli

Average effect of phosphorus (mg. L^{-1})	Acetate concentration (mg. L^{-1})				Acetate concentration (mg. L^{-1}) Phosphorus(mg.L^{-1})
	A_3	A_2	A_1	A_0	
17.25 b	22be	20 cd	18 ef	9 i	P_0
19.75a	24 ab	21 d	19de	15 h	P_1
20.57ad	25a	21.8 j	19.5 g	16 g	P_2
21.17 ac	26 e	21.4 n	20.3k	17 fg	P_3
	24.25 a	21.05b	19.2c	14.25d	Average effect of acetate (mg. L^{-1})

Averages with similar letters that do not different from each other within the main factors or their interactions according to Duncan's polynomial test 0.05.

5. Discussion

Flavonoids are secondary metabolic compounds produced in plants for their important role as an antioxidant and scavenge free radicals. Their production is in the daytime periods when It is secreted by the intensity of light, and it also plays a role in protecting the plant from ultraviolet rays in the early stages of the plant, as well as regulating some of the organs in the advanced stages.The root total and flavonoids were affected by the abundance of available elements for the plant from the soil during growth(Salas,perez,2018) . The effect of spraying organic fertilizer, which increases the content of the leaves of total flavonoids compared to plants that were not sprayed. The spraying process helped in the accumulation of carbohydrates in the plant, which enter the direct path in the production of phenols through Shilcimic acid (Barbero ,2014) .The presence of secondary metabolites in basil made this plant qualified to be used medicinally and in various fields. This study agrees with what was stated by (meghane & mobashera ,2015). The reason for the increase in secondary metabolites is flavonoids ,tannins and phenols(table 1,2and 3) in the content of the leaves of the basil plant to the role played by acetate and phosphorous when sprayed on the plant in regulating many cellular and physiological processes that lead to an increase in the primary and secondary metabolic compounds of the plant (Roje, 2006).

The acetate and phosphorous sprays indicated that the alcoholic extract of the basil plant was effective in inhibiting the pathogenic bacteria. The reason may be that the plant contains acids, including Ursolik, Capric, and Oleanic (Rizk et al ., 1995& Yahya et al., 2015), as the acidity dissolves and changes the nature of proteins in the cell membrane as well as affects the enzymes of the bacterial cell (Al-Sarraf, 1995 ; Musa et al ., 2002). The presence of phenols in the plant, which was attributed to it, kills and inhibits many microorganisms. (Kemp & Burden, 1986). The phenols work, by inhibiting the mechanism of the cell membrane of microorganisms, and thus to the inhibition of the growth of the microorganism (Sartortto et al.,2004) and the extract works by inhibiting the enzymes responsible for basic metabolic reactions by interfering with them that are not specific to proteins, so they work to denature the protein and then lead to the inability of microorganisms to continue to grow, and this The result is consistent with that of (Hammar, 1991) .The secondary metabolites, flavonoids, phenols, tannins, alkaloids and terpenes that it contains have the ability to inhibit some microorganisms (Cowan, 1999)

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