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A physiological study of the interaction effect of acetate and phosphorous on the growth of some chemical characteristics of (Ocimum basilicum vara viride) and its effectiveness against pathogenic bacteria

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Abstract

In this study, two experiments were conducted, one in a field and one in a laboratory. The field experiment took place on 10/1/2023 in a private farm in the agricultural village of Sayed Hussein in the Al-Rifai district, Dhi Qar Governorate, using a plastic canopy. The laboratory experiment involved studying chemical compounds in Al-Fadil Foundation for Training and Development in Babylon Governorate and bacterial compounds in Al-Rifai General Hospital. The study examined the effects of different levels of potassium acetate (CH3COOK) and phosphorous (NPK High phosphorous) on the content of flavonoids, tannins, and phenols in leaves, as well as the inhibitory activity of basil extract against two types of pathogenic bacteria: Streptococcus pneumonia (Gram-positive) and Salmonella paratyphi (Gram-negative). Results showed that acetate spraying at level A3 significantly increased the content of flavonoids, tannins, and phenols in leaves, and inhibited both positive and negative bacteria. Similarly, higher levels of phosphorous (P3) led to increased content of these compounds in leaves and significant inhibition of the pathogenic bacteria. Furthermore, the interaction between acetate and phosphorous levels had a significant effect on the characteristics of the leaves, with the highest effect observed at level A3P3. This combination also showed significant inhibition of pathogenic bacteria. Overall, the study demonstrated the positive effects of acetate and phosphorous levels on the content of beneficial compounds in leaves and their inhibitory activity against pathogenic bacteria.

Keywords:

Ocimum basilicum vara viride, pathogenic bacteria, physiological study, acetate, phosphorous.

Introduction

The genus Ocimum basilicum vara viride belongs to the Lamiaceae family, which includes about 200 species. With regard to Its spread, the plant's original homeland in different regions, of Assia, West Africa and America [1, 2], and then moved to Europe through the commercial navigation movement. It is grown in gardens as ornamental plants of to, the genus Ocimum, scents such as the scent of rose, lemon , licorice, eucalyptus, sweat weevils and cloves [3]. Basil contains medically active containing medically such as glycosides. Alkaloids and terpenes. Basil is also used as a medicinal plant to treat headache, gastritis, nausea, earache, diarrhea, and cough, and as a folk remedy for indigestion, and to give flavor food [4]. It showed a positive effect in relieving pain similar to the effect of aspirin [5] .The watery and ethanolic extract of all parts of the basil plant showed an increased in the healing of stomach ulcers when applied to experimental mice [6]. That the use of the extract of the watery and alcoholic vegetative parts of the basil plant has a role in retrieval of the memory of mice, and that the effect of these extracts is due to the effectiveness of the antioxidant compounds

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of Flavonids, Tannis and Terponedes found in the basil extract [7].And the study showed that the volatile oil in the basil plant a role in inhibiting the growth of clinical isolates of bacteria Enterococcus Staphylococcus, pseudomonas [8].

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 [6]. And that crude methanolic extract of all parts of the plant has an effective effect in inhibiting Mycobacterium tuberculosis H3RV and others [9].

Materials and Methodology

1- Method of preparation of flavonoids

Leaves were dried for 48 hours, then grinded and weighed 5 grams, then extracted by Soxhlet and 300 ml ethanol solvent at (50-55) degrees Celsius for 3-4 hours and then filtered by filter paper.

A rotary evaporator was used to concentrate the extract under low pressure at a temperature of 40 degrees, then the extract was weighed after the concentration process (2.6 g), and then stored in tightly closed bottles at 4 degrees Celsius until use. Flavonoids were determined according to the method [10] in the crude extract by aluminum chloride in the presence of the rutin. As 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 distilled water was added to it[11] .

A solution of 20% sodium nitrate solution and (0.3 ml) of 20% aluminum chloride, after which the mixture was placed in the incubator for (10) minutes, after then 2 ml of sodium hydroxide (1molar) solution was added to the mixture and then the volume was added to 10 ml of distilled water [12], then the absorbance was recorded model aiong at a length of 510 nm, and then calculate the concentration of total flavonoids in relation to the titration curve and for rutin[13] in units (mg.g^{-1} dry weight).

2- Determination of the tannins content of leaves

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, the wavelength 500 nm was read. Then determine the level of the two by a curve extrapolation that is done using a catechin solution [14].

3- Determination of the polyphenol content of the leaves

The total phenolic content of dry extracts was estimated using Folin ciocaltem, 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 was 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 was read a wavelength of 760 nm according to the method [14].

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 grams of the crushed powder for each concentration and dissolve it in 100 ml of concentrated ethanol. The solution returned and then placed in a water bath, at 40°C for 3 hours ,it is filtered with a piece of gauze to remove large parts , then filtered by filter paper,the filtrate was collected in opaque glass dishes until use [15].

4 -2 Testing the biological activity of the extract was tested on bacteria

The activity of basil plant extract was tested on Gram-positive and Gram-negative bacteria using the drilling method. Wall in the medium of solid agar [16].Dig out the nutrient medium by dissolving 28 g of it in a liter of water, then dissolved 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.

Bacteria were cultured on nutrient media using Cotton Swab. It is completely planned to ensure even distribution of bacteria on the medium, and we make small holes with a diameter of 9 ml at 5 in each dish using a corkborer and add 0.5 ml of each extract that has been made, according to the required concentrations The dishes were placed in the oven at 37 degrees for 24 hours, and then measured The diameters of the inhibition zoneswere measured in millimeters and using the graduated ruler.

Results and Discussion

The result from table No (1) indicate 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 A_3 level, which amounted to (29.53 mg. g^{-1}) in the lowest rate was at level A_0 and reached (22.79 mg. g^{-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 P_3 and it reached (30. 37mg.g^{-1}) compared to the lower rate at the P_0 level of (19.33 mg. gm $^{-1}$). Also, the interaction between the two experimental factors had a significant effect, as it reached the highest rate for treatment A_1 P_3, which amounted to (30.9 mg. g^{-1}) compared to the control treatment (the comparison) which amounted to (15.80 mg, g^{-1})

Table (1): Effect of acetate and phosphorous spraying on the leaves content of flavonoids (mg/g^{-1}) 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	
19.33 d	28.8 e	16.85 l	15.90 m	15.80m	P_0
23.58 c	29.24 d	22.87 jk	22.36 j	20.60 k	P_1
28.1 b	29.6 da	27.72 g	29.90 i	225.18 h	P_2
30.37a	30.5 b	30.5a	30.91 a	29.58 d	P_3
	28.48 a	24.48 b	24.76 c	22.79 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.

Leaves content of tannins.

The results of Table No (2), showed that the spraying of acetate had a significant effect on the content of leaves tannins and the highest value was at the A_3 leve and it reached (32.93 mg. g^{-1}) and the lowest rate for acetate effect at level A_0 was (27.34 mg.gm).

As we can see from the table that Phosphorous has an effect significantly, and the highest value at the P_3 level was (35.93 mg .g^{-1}) compared to the lowest rate at the P_0 level.

Which amounted to (27.09 mg.g^{-1}), and the interaction between the two experimental factors had a significant effect, the highest rate was (36.7 mg.gm^{-1}) . The lowest rate when treating the coupling was (20.40 mg.g^{-1}).

Table (2): Effect of acetate and phosphorous spraying on the leaves content of tannin (mg/g-^{1}) 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	
25.17 d	29.20 j	26.72 i	24.37 k	20.40 d	P_0
28.42 c	32.25 bc	27.52ab	28.81da	25.13 h	P_1
31.24b	33.60 ab	32.11dc	31.18 d	28.08 a	P_2
35.34a	36.70 ab	34.90da	35.0cd	36.77ba	P_3
	32.93a	30.31 b	29.89 c	27.09 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.

The content of leaves from phenols.

The results of Table No. (3) also indicated that the spraying of acetate had a significant effect on the phenols content of the leaves content, and the highest average value at level A_3 amounting to (43.08 mg.g^{-1}), while the lowest value was at level A_0, it reached to (37.04 mg.g^{-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 P_3 level (45.14 mg.gm-^{1}), while the lowest rate was at the P_0 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 A_3P_3 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 (3): Effect of acetate and phosphorous spraying on the leaves content of polyphynol (mg/g-^{1}) 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	
37.46 d	41.80 e	39.30 l	35.58 m	33.16 m	P_0
38.55 c	43.84 d	39.39 jk	36.69 j	34.30 k	P_1
40.12b	43.25d	43.40 g	37.50 i	36.4 h	P_2
44.1 a	44.6 b	44.30a	43.30 a	44.30 d	P_3
	43.08 a	41.59 b	38.26 c	38.07 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

Effect of alcoholic extract of basil plant in inhibiting pathogenic bacteria Streptococcus pneumonia class gram-positive

We note through Table No. (4) the role of acetate extract in inhibiting Streptococcus pneumonia, and the highest rate of this inhibition was at level A_3 (22 mm) compared to the lowest rate at level A_0, which amounted to 12.75 mm.

Phosphorous spray also had the effect of Significantly effect in inhibiting the bacteria above, it reached the highest rate at the P_3 level which is (19.0 mm). While the lowest rate was at the level P_0 (15.75 mm). It is shown thought Table (4) that the interaction of the two experimental factor had a significant effect, the highest rate was at the $A_3 P_3$ 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 in inhibiting bacterial growth. Streptococcus pneumonia

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	
15.75 d	21 ab	18 cd	15 ef	9 i	P_0
17.25 c	22.0 a	18 cd	16.0 c	13 h	P_1
17.75 b	22 a	20.0k	16.0cb	14 gh	P_2
19 a	23 b	21.0b	17 cd	15.fg	P_3
	22.0 a	19.0b	16.0 c	12.75 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

The effect of the alcoholic extract of the basil plant in inhibiting pathogenic bacteria (*Salmonella paratyphi*)

Table No. (5) indicates that spraying acetate on basil plant had a significant effect in inhibiting the pathogenic bacteria *Salmonella paratyphi*, and the highest rate was at level A_3 (23.25 mm), while the lowest rate was at level A_0 which amounted (13 mm).

We also notice through The table that spraying phosphorous on basil plants had a significant effect on bacterial inhibition, the highest average value was at the P_3 level (20 mm), while the lowest rate was at the P_0 level of (16.5 mm), and through the results of Table (5) it is noted that an interaction between the two factors.

The experiment had a significant effect on inhibiting the pathogenic bacteria *Salmonella paratyphi*, had the highest rate was when the A_3 P_3 treatment was (25 mm), while the lowest rate was when in the comparison treatment which was (9 mm).

Table (5): Effect of alcoholic extratic of basil leaves treated with acetate and phosphorus on inhibiting bacterial growth. *Salmonella parptyphi*

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 d	21b	19 cde	17 fg	9 i	P_0
18.5 c	23 a	20c d	18c	15 h	P_1
19b	24a	20 cd	18 c	16 g	P_2
20 a	25 e	20cd	19cde	17 fg	P_3
	23.25 a	19.75 b	18c	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.

Discussion

Flavonoids are secondary metabolic compounds are produced in plants for their important role as an antioxidant and free radical's inhibitors, and it also plays a role in protecting the plant from ultraviolet radiation in the early stages of the plant, as well as regulating some of the organs in the advanced stages [17]. Therefore, the increase in the content of flavonoids in the leaves is related to speed of growth of vegetative system and the root system. and the effect of flavonoids on the abundance of elements available to the plant from the soil during total and flavonoids were affected by the abundance of available elements for the plant from the soil during growth [18]. The effect of spraying organic fertilizer, which works increases the content of the leaves of total flavonoids, the application of organic fertilizer had a significant effect on increasing the tatail 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 [19]. The presence of secondary metabolites in basil makes this plant eligible qualified to be used medicinally and in various fields. This study agrees with what was stated by meghane & mobashera [20]. And The reason for the increase in secondary metabolites compounds flavonoids, ,tannins and phenols(table 1,2and 3) in the content of the basil leaves is attributed 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 plants [21, 22]. The reason for the increased of pathogenic bacteria when spraying acetate and phosphorous was attributed to the fact that the alcoholic extract of the basil plant was effective in inhibiting the warring bacteria. The reason may be that the plant contains acids, including Ursolik, Caprice, [23, 24], as the acidity dissolves and changes the nature of proteins in the cell membrane as well as affects the enzymes of the bacterial cell [25, 26]. The presence of phenols in the plant, which was attributed to the killing and inhibition many microorganisms [27]. val phenols work, by inhibiting of the cell membrane machinery microorganisms, and thus to the inhibition the growth of the microorganism, the extract also does works to inhibit the enzymes responsible for basic metabolic reactions by interfering with them that are not specific to proteins, so they work to mutate the protein and then lead to the inability of microorganisms to continue to growing , and this[28]. The result is consistent with that of [29]. The secondary metabolites have flavonoids, phenols, tannins, alkaloids and terpenes that it contain plants have the ability to inhibit some microorganisms [30].

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