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# STUDY BIOLOGICAL ACTIVITY OF EXTRACT ACTIVE SUBSTANCES OF ORANGE LEAVES IN CONTROL OF BLACK STEM DISEASE OF GRAPE PLANTS

**Wasen Fawzi Alpresem**
Medicinal and Aromatic Plants Unit, College of Agriculture, University of Basrah
*[1] **Correspondence:** wasen.fadel@uobasrah.edu.iq

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**Abstract:**

This study was conducted in the medicinal and aromatic plants unit laboratories at the College of Agriculture, University of Basrah, to investigate the effect of using orange leaf extract on controlling the blackening of the stem disease of grapes. A number of grape trees infected with stem blackening disease were selected by alcoholic extract of orange leaves at concentrations (0, 100, 200, 300 ml l-1). The alcoholic extract content of orange leaves of the active substances was estimated by GC-MS using a gas chromatograph coupled with mass spectrometry. The components were identified using the National Institute of Measurement and Technology (NIST) database by comparing the resulting spectrum of the unknown component with the known stored components in the NIST library. The detection showed the presence of 8 compounds in orange leaves, and the highest peak area of the extract was at 2.25 minutes for n-Hexane, and the lowest peak area was at 17.07 minutes for Copaene. The compound Ethyl Acetate appeared at 2.61 minutes with an area of 19.77 and Tetradecane at 19.47 with an area of 0.54. The different compounds varied in the time of their appearance, and the appearance of these eight compounds did not recur in different periods. The results of the study also showed that the treatment of orange leaf extract with a concentration of 300 ml l-1 recorded the highest percentage of response to extract, amounting to 70.94%, the highest percentage of the carbohydrate content of leaves amounted to 28.24%, the lowest percentage of phenols amounted to 0.65%, and the highest percentage of leaves content of chlorophyll amounted to 17.83.

**Keywords:** Biological activity; extract of active substances; orange; black stem; grape.

## INTERACTION

Grape *Vitis vinifera* L. is a temperate zone fruit tree in the blueberry family. It is believed that the original home of the grape plant is the region between the southern Black Sea and the Caspian Sea, and all known grape varieties originated from it, and then its cultivation spread to the rest of the world (Perl and Eshdat, 1998). In Iraq, its cultivation dates back to the fifth millennium BC, when it was intercropped with date palm trees and other fruit trees (Al-Rawi and Al-Douri, 2000). Grapefruits are characterized by nutritional and economic importance, as they constitute an important part of human food, and eating them is necessary because they are rich in sugars, vitamins, organic acids, fats, proteins, fibers, minerals, and calories (Al-Saeedi, 2000).

Citrus fruits belong to the Rutaceae family, one of the evergreen fruit trees. It is believed that the original home of citrus is the tropics and subtropics in Southeast Asia (Agha *et al*., 1991). Trees and the quantity of annual production, and even in terms of the quality of the fruits and the content of its leaves of active gradients (Al-Khafaji *et al*., 1990). Some studies have indicated that the active substances extracted from plants give better results than the same substance manufactured by chemical methods, which may be accompanied by toxic side effects, which indicates the possibility of the active substances in the secondary compounds contributing to enhancing the effective role of a plant (Ngegba *et al*., 2018).

Black leg disease is one of the most common bacterial diseases, and its danger increases in wet areas. The cause of this disease is the bacterium Erwinia Carotovora Atroseptica. Infection with the black leg occurs in all stages of plant growth, especially when excessive irrigation, as the causative bacteria live in the soil and plant residues for a period not exceeding three months at a temperature of up to 2 c (Wach, 2009).

## MATERIALS AND METHODS

This study was conducted in the medicinal and aromatic plants unit laboratories and the department of horticulture and landscaping in the College of Agriculture, University of Basrah, to investigate the effect of using orange leaf extract on combating the blackening of stem disease in grapes. The field study was conducted in one of the orchards of Abo-Al-Khasib, where a number of infected trees were selected, and homogeneity was considered as much as possible regarding tree ages and service operations.

## PREPARATION OF PLANT EXTRACT

The healthy leaves were collected from orange trees, washed with plain water, distilled water, air-dried, and ground by an electric mill to obtain a vegetable powder. 50 g of dry vegetable powder was weighed and mixed with 500 ml of ethyl alcohol 70% in a glass flask with a capacity of 1000 ml and closed with cotton and aluminium foil, placed in a shaking incubator, and left for 24 hours at room temperature. After that, the mixture was sprayed using several layers of medical gauze to get rid of plankton, centrifuged at 3000 rpm for 10 minutes, and then filtered the extract using 0.1 Whatman NO. To obtain a clear solution for use in the GC-MS technique. GasChromatography-Mass Spectrometry (Hernández-Pérez et al., 1994). The active substances' leaves' content was estimated using a gas chromatograph coupled with mass spectrometry. The components were identified using the National Institute of Measurement and Technology (NIST) database by comparing the resulting spectrum of the unknown component with the known stored components in the NIST library.

## TREATMENT OF GRAPE TREES

Grape trees infected with stem blackening disease were sprayed with the plant extract at concentrations (0, 100, 200, 300 ml l-1) using a pump. The shoots were sprayed in the early morning until complete wetness with the use of spreading material, at a rate of twice between the first and second spraying, for two weeks, then studied the following traits:

1- Rate of response to treatment

The response percentage was estimated by knowing the manifestations of healing caused by this extract on the infected parts. The number of total trees and trees that gained healing was calculated, and the response percentage was estimated from the following equation:

% of response = number of trees that gained healing / Total number of trees x 100

2 - Estimate the percentage of carbohydrates in the leaves

The method of Dobius et al. (1965) was followed by taking 0.5 g of dried leaves in an electric oven (Galenhamp) at a temperature of 40 o C for 72 hours, then ground with an electric grinder, then placed in glass tubes with a capacity of 90 ml, and 70 ml of distilled water was added to it. The samples were placed in a bath aqueous at a temperature of 70 o C for one hour, after which it was left to cool to room temperature and filtered through filter paper; then, the method was completed after preparing the standard solutions.

3 - Estimate the percentage of phenols in the leaves

The method of Melo et al. (2005) was used to estimate the phenolic content of the leaves, as one gram of dried plant sample was taken using an electric oven type (Galenhamp) at a temperature of 40 o C for 72 hours. It was ground by an electric mill, after which 80 ml of distilled water was added and placed in a bath Aqueous type (Memmert). Take 1 ml of the prepared extract and add 1.5 ml of phenol reagent to it (diluted 10 times; after 5 minutes, add 1.5 sodium carbonate at a concentration of 6%). Spectrophotometer setting using the standard solution prepared from one ml of distilled water added 1.5 ml of phenol reagent and 1.5 ml of sodium carbonate 6% concentration. The absorbance reading of the samples was taken through a spectrophotometer.

4- Estimation of chlorophyll content of the leaves

The total chlorophyll content of leaf tissue was estimated according to the method described by Al-Najjar et al. (2021).

## STATISTICAL ANALYSIS

The study was designed using a complete randomized design (C.R.D) as a simple experiment. The means were compared using the Least Significant Differences Test (L.S.D) method under the probability level of 0.05 (Alrawi and Kalaf-alla, 2000).

## RESULTS AND DISCUSSION

### Qualitative analysis of chemical compounds in plant samples using GC-MS

Table (1) and Figure (1) show the chemical compounds present in the alcoholic extract of orange leaves, which were detected by gas chromatography using mass spectrometry, as the detection showed the presence of 8 compounds of orange leaves and the highest peak area of the extract was at 2.25 minutes for the compound n-Hexane. The lowest peak area was at 17.07 minutes for Copaene. The compound Ethyl Acetate also appeared at 2.61 minutes with an area of 19.77 and Tetradecane at 19.47 minutes with an area of 0.54. The different compounds varied in the time of their appearance, and the appearance of these eight compounds did not recur in different periods.

Table (1) GC-MS analysis of an alcoholic extract of orange leaves

| Chemical formula | compound name | space | detention time | the top |
|---|---|---|---|---|
| $C_6H_{14}$ | n-Hexane | 55.06 | 2.25 | 1 |
| $C_4H_8O_2$ | Ethyl Acetate | 19.77 | 2.61 | 2 |
| | unknown | 3.11 | 2.92 | 3 |
| $C_{15}H_{24}$ | Copaene | 0.19 | 17.07 | 4 |
| $C_{14}H_{30}$ | Tetradecane | 0.54 | 19.47 | 5 |
| $C_{15}H_{32}$ | Pentadecane | 0.70 | 19.99 | 6 |
| $C_{15}H_{26}O$ | Hedycaryol | 0.99 | 22.13 | 7 |
| $C_{15}H_{24}O$ | Spathulenol | 1.06 | 22.90 | 8 |

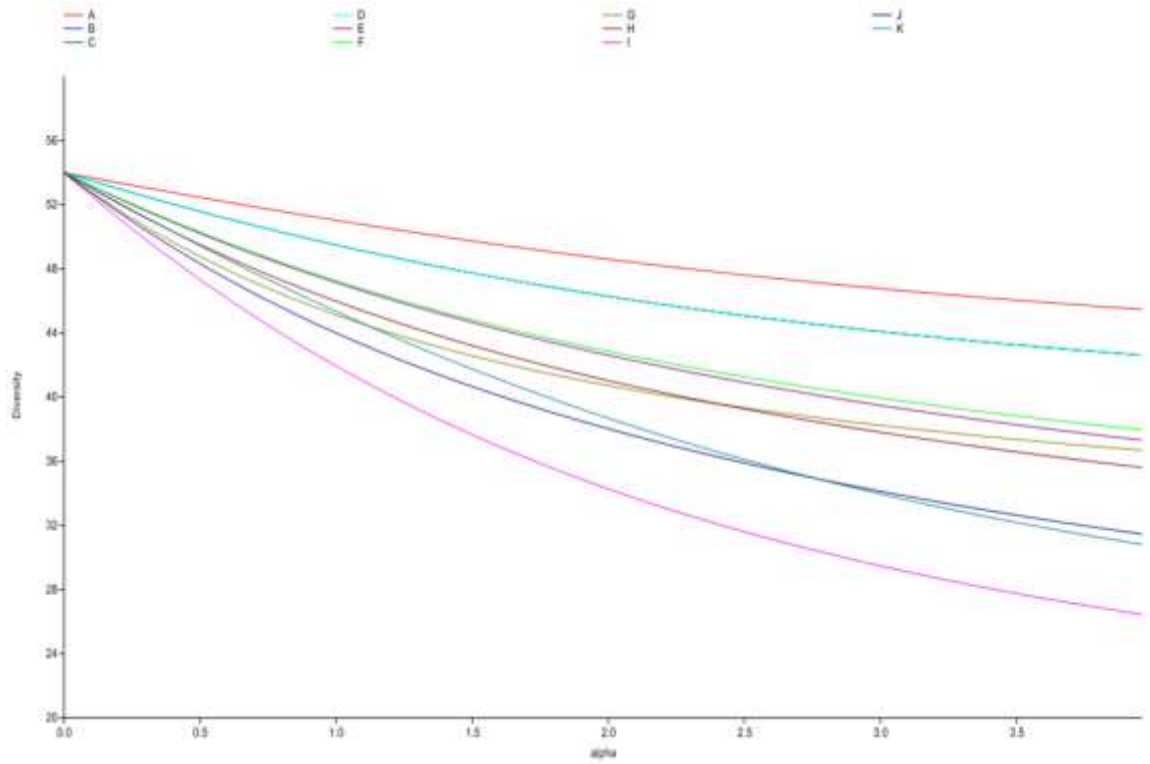

Figure (1) GC-MS analysis of an alcoholic extract of orange leaves

## Rate of response to treatment

It is clear from the results of Table (2) that the plant extracts have a significant effect in decreasing or increasing the response percentage in the treatment of black leg disease in grape trees, as the comparison treatment recorded the lowest response rate of 1.45%. In comparison, the treatment of orange leaf extract with a concentration of 300 ml l-1 recorded the highest response rate to the extract, amounting to 70.94%, with a significant difference from the rest of the concentrations. The treatment of the extract with a concentration of 200 ml l-1 recorded a response rate of 55.37%, while the concentration of 100 ml l-1 caused a response rate of 22.25%.

### The percentage of carbohydrates in leaves

The results of the statistical analysis in Table (2) showed that the plant extracts had a significant effect on the carbohydrate content of grape leaves infected with black leg disease, as the comparison treatment recorded the lowest percentage of 9.28%, while the orange leaf extract treatment with a concentration of 300 ml l-1 recorded the highest percentage. It reached 28.24%, significantly different from the rest of the concentrations. The treatment of the extract with a concentration of 200 ml l-1 recorded a percentage of 21.38%, while the concentration of 100 ml l-1 caused a response rate of 15.51%.

The difference in the percentage of carbohydrates for grape leaves may be attributed to the difference in the conditions surrounding the infection for each treatment, which in turn affects the vital system and the construction of carbohydrates, as it is noted that the percentage of carbohydrates in the leaves increased in the treatments that recorded the highest percentage of response to the plant extract, and on the contrary for the treatments that the lowest response rate was recorded, which recorded the lowest percentage of carbohydrates (Table 2), and therefore differences appeared in the percentage of carbohydrates (Abdul Qader et al., 1982; Motar, 1991).

### *The percentage of phenols in the leaves*

The results of the study showed in Table (2) that the plant extracts had a significant effect on the phenols content of grape leaves infected with black leg disease, as the comparison treatment recorded the highest percentage of phenols amounted to 7.33%, while the orange leaf extract treatment with a concentration of 300 ml l-1 recorded the lowest percentage. It reached 0.65%, significantly different from the rest of the concentrations. The treatment of the extract with a concentration of 200 ml l-1 recorded a percentage of 2.34%, while the concentration of 100 ml l-1 caused a response rate of 5.15%.

From the results of the study, it is noted that the percentage of phenols in the leaves increased in the treatments that recorded the lowest percentage of response to the plant extract and, on the contrary, for the treatments that recorded the highest percentage of response and that recorded the lowest percentage of phenols (Table 2). The high percentage of phenols in the leaves of infected trees may be due To the effect of pathogenic bacteria in the plant, which leads to an increase in phenolic substances among the natural chemical defences found in the plant. (Nizamuddin et al., 1983).

### Leaves content of chlorophyll

The results of the statistical analysis in Table (2) showed that the plant extracts had a significant effect on the chlorophyll content of grape leaves infected with black leg disease, as the comparison treatment recorded the lowest percentage of 6.33%, while the orange leaf extract treatment with a concentration of 300 ml l-1 recorded the highest percentage. It reached 17.83%, significantly different from the rest of the concentrations. The treatment of the extract with a concentration of 200 ml l-1 recorded a percentage of 13.67%, while the concentration of 100 ml l-1 caused a response rate of 9.35%. As sufficient levels of carbohydrates (Table 1) increase the chlorophyll level in the leaves (Zhiming and Fengbin, 2014).

Table (2) Effect of treatment with orange leaf extract on some biochemical properties of grapes

| Orange leaf extract (ml l-1) | % response rate | Carbohydrates % | Phenols % | chlorophyll mg 100g-1 fresh weight |
|---|---|---|---|---|
| 0 | 1.45 | 9.28 | 7.33 | 6.33 |
| 100 | 22.25 | 15.51 | 5.15 | 9.35 |
| 200 | 55.37 | 21.38 | 2.34 | 13.67 |
| 300 | 70.94 | 28.24 | 0.65 | 17.83 |
| effect rate of extract | 37.50 | 18.60 | 3.87 | 11.80 |
| L.S.D. | 11.12 | 4.36 | 1.14 | 2.24 |

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