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دراسة تأثير مستخلص وزيوت نبات الطبي المحلي (الزعتر) في حفظ بعض الاسماك

Study of the effect of extracts and oils of the local medicinal plant (thyme) in preserving some fish

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Abstract

There is a growing interest in natural ingredients that can be used as preservatives in the food industry and preservation. Among the natural materials that can be used for preservation are plant extracts that are extracted from some natural plants, such as thyme (*Thymus vulgaris*), The current work aimed to study the efficiency of extracts and oils of local medicinal plant (thyme) in preserving fish. Two species of fresh, economical fish writhe*(sparus aurata)* sardines,*(sardine pilchardus)* that are widely consumed were collected during May, 2023, each species divided into two group type, one of the two were treated with the extracts of these plants and the other group were treated with the oils of studied plants demonstrated the effectiveness of these herbs in preserving these fish. A comparison was made between the effectiveness of extracts and oils from these plants in preserving the fish studied. The results showed that there were differences between the samples before and after treatment. The results showed that there were differences between the types of solutions, indicating that the alcoholic extract is better than the aqueous and oil extracts in the process of preserving genetic fish and sardines. There are no differences between the types of plant used in the experiment, which are (thyme,) Whereas a concentration of 20% is better in the preservation process than a concentration of 10%.

Introduction

There is a growing interest in natural ingredients that can be used as preservatives in the food industry and preservation. As well as prompting consumer demand for natural preservatives in processed meat and fish products to search for alternative options that can maintain health and safety standards. A preservative is a substance added to products such as food products, including fish, pharmaceutical products, biological samples,…etc., in order to prevent and protect against biodegradation resulting from bacterial growth, or from any unwanted chemical changes. [1]

A lot of research has been conducted to find ways to increase the preservation period of fish, including the addition of chemically manufactured aids to preservation, which are herbal extracts, organic acids, or aromatic oils, as well as resorting to preserving fish in a cooled or vacuum-aired atmosphere[1] Recently, interest in the fish industry writhe*(sparus aurata)* sardines*,(sardine pilchardus)* has increased, and this coincided with an increase in interest in natural compounds, either for direct addition to them, or use as a natural antioxidant and anti-microorganisms. Herbal extracts have been used as effective oils against bacteria [2].

Among the natural materials that can be used for preservation are plant extracts, such as thyme (*Thymus vulgaris)* [3]. Thyme is considered one of the aromatic plants with multiple uses. Solo, and with other aromatic plants; the active substances in thyme are many, and among the strongest are thymol and carvacrol, and from them come most of the medical benefits of thyme, as it is anti-fungal, anti-bacterial, and antioxidant, there is also many researches that proves that using garlic extract to reduce microbial contamination of food during storage. It has been shown that garlic delays the rate of microbial spoilage and extends the shelf life of fish fillets for a period of six days during refrigerated storage [4].

importance of study:

The importance of the study can be found in two influential factors, namely the health factor and the economic factor. on the health factor side, natural materials with plant extracts are used to preserve wraith fish`(*sparus aurata*) sardines, (*sardine pilchardus*) be beneficial to consumer health, then it is compared to the medicinal oils of plants. as for the economic aspect, it is the use of simple and inexpensive materials, such as the use of medicinal plants such as Thyme (*Thymus capitaatus*)

Aims of the study

1. Determine the possibility of using medicinal plant extracts as natural preservatives for fish instead of chemical preservatives.
2. Make a comparison to find out the best natural plant used in the study in terms of their efficiency in the process of preserving wirtha fish and sardines.
3. Using essential oils from the same natural plants used in the study to see their effect on preserving writhe fish and sardines.
4. Comparison in terms of efficiency between plant extracts and medicinal oils of the same study plants in the process of keeping wirtha fish and sardines.

Methods and materials

3.1 Study area:

This study included different samples of natural plants, namely thyme, garlic, and rosemary were collected from two areas: first area was Sabratha which located approximately 70 kilometers

(43miles) west of the modern city of Tripoli on the Mediterranean Coast. The 2nd area was Asabaa which located in the central part of the former Western Mountain District, about 120 km southwest of the city of Tripoli,

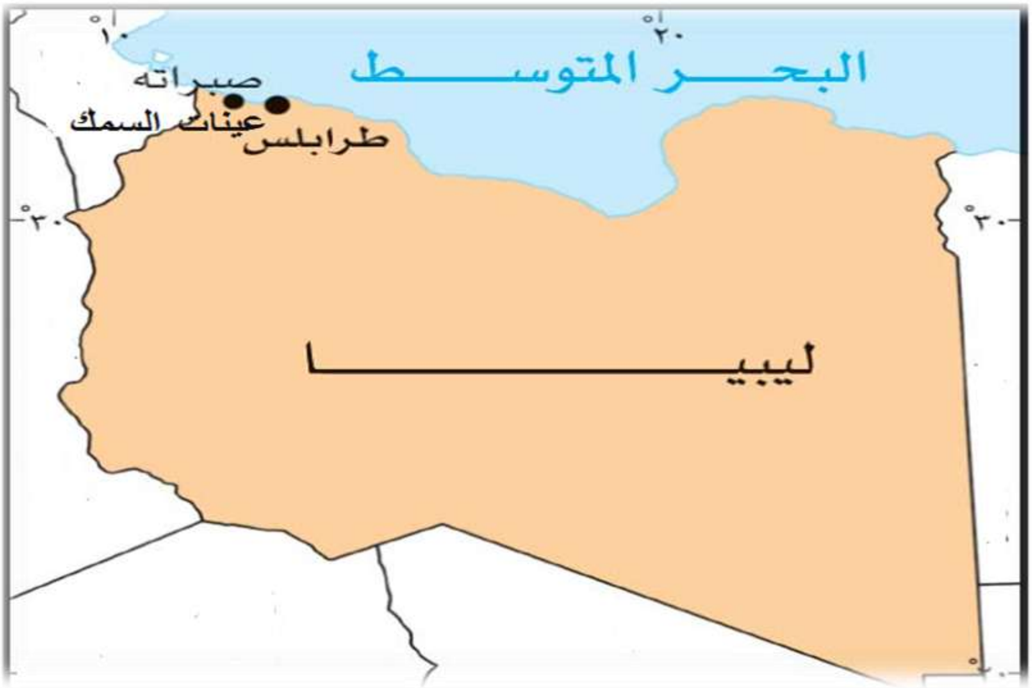

Figure Study area

3.2 Samples collection:

The experiment was conducted in the laboratories of the Zawia Medical Research Center. Also the study included 220 samples of Wraith (*sparus aurata*) and Sardines, (*sardine pilchardus*) were divided into 110 Sardines and 110 Wraith, and it were fresh, free-range type were taken from the fish market in the city of Sabratha on 3/5/2024. Oil of the same studied plants, was taken from the herbal pharmacy in the Ajilat city. Plant samples were collected from the western mountains of Libya, specifically the Asabaa city on 27/3/2023. They included thyme, The study included a comparison of the extract of the studied plant with the oil of the same plant in the process of preserving fish.

3- Classification of Thyme plant :

Kingdom: Plantea

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Lamiales

Family: Lamiaceae

Genus: *Thymbra*

Species**:** *Thymus capitatus*

1- Classification Fish Sardine:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Clupeidae

Genus: *Sardina*

Species *Sardina pilchardus*

2- Classification Wraith fish :

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Genus: *Sparus*

Species *sparus aurata*

Figure (3.4)

plant thyme

Figure fish Wraith

Figure fish sardines

Sample preparation:

Preparation of plants:

Cold extraction is carried out in the following steps:

1- The active ingredients are extracted by washing the used plant organs with plain water (tap water) to remove dust and impurities. They are washed with distilled water and then dried in the shade at a room temperature until the moisture is removed, Or placed at 28% for two days.

2- The dry samples are ground using a milling device, then the plant powder sample (100 grams) is weighed and then placed in 500ml of sterile distilled water and 70% ethanol for 24 hours.

3- The extract was placed in a 1000 ml opaque glass flask, closed with aluminum foil, and the mixture was left in a shaker water bath at 37°C for 24 hours. Then, it was placed in test tubes and centrifuge at 2500 rpm for 30 minutes. After that, the total filtrate was concentrated using a rotary vacuum evaporator, converting it into a thick liquid to get rid of the solvent, whether it was alcohol or water. Then, it was placed in an incubator at 37°C in glass Petri dishes. After drying, it was collected in glass bottles and placed in the refrigerator until use; The name of the plant, the weight of the extract, and the date and time of extraction were written on it. I did process was repeated several times to obtain a sufficient amount of the extract. [5]

Preparation of Fish:

After the fish arrived at the experimental site, they were placed in the fish storage refrigerator with a sterile atmosphere containing ice cubes inside the container. After an hour of placing them in the container, the experiment was cleaned and cut. The head, tail, and body were removed. Muscles used

in the experiment were cut into small pieces, approximately 5 grams from each sample of Sardine,*(sardine pilchardus)* and Wraith *(sparus aurata)*. Then, subsequently, these pieces were immersed two times, with distilled water once and alcohol extract another once.

Table: plant species, fished species used in the study:

Scientific name	Used part
Thymus capitatus	Leave
sparus aurata	Muscles
sardine pilchardus	Muscles

The effect of Thyme extract in preserving fish (Sardines, Wirath):

A aqueous and ethanol plant extracts were prepared at a concentration of 10% and 20% from the Rosemary plant, then immerse the pieces of fish 5 grams in the alcohol extract, and distilled water for 30 minutes and placed in a refrigerator at $4c^0$ Celsius. After that, the samples were dried, the Rosemary extract. The fish pieces were placed in a sterile glass and petri dishes then returned to the refrigerator. After 24 hours of observation of the pieces immersed in the water extract or alcohol, I noticed they maintained their consistency shape and excellent smell. It also maintains its consistency after 48 hours . It considered excellent. After 3 days, I noticed that it also maintains its consistency, shape and smell for 6 days immersed in the Rosemary extract. After that I noticed a change in the shape, there was a pallor in the fish, its color began to turn to yellow color , the beginning of rot, was evident, and the smell changed. [5]

6 The effect of Thyme oil in preserving fish (Sardines, Wirath):

I prepared a package of the same plant oil, it was 30 ml., then immerse 5 grams of the fish pieces in Thyme oil for 30 minutes and placed them in a refrigerator at $4c^0$ Celsius. After that, I dried the samples of Thyme oil. I placed the fish pieces in a sterile glass petri dishes and returned them in the refrigerator again. After 24 hours of observing the fish immersed in Thyme oil, I noticed that it maintains their texture in terms of shape and nod excellent smell . This texture was also maintained after 48 hours considered excellent. I noticed that after 3 days, the texture, shape and smell for 5 days immersed in the Thyme oil. After I noticed a change in the shape, there was a pallor in the fish, its color turned to yellow, the beginning of rot was evident and the smell changed. [5]

Figure, fish extract thyme

figure fish in oil thyme

Testing the effectiveness of plant extracts and comparing their oils in preserving some fish:

Testing the effectiveness of plant extracts in preserving fish by number of bacterial colony , The culture and bacterial count were performed according to the Ranjan method.[6]. as follows:

The samples were subjected to examination directly in the laboratory, and when this was not possible, they were kept in the refrigerator to complete the work on the second day. The fish samples were minced inside a glass tube with a piece of glass under sterile conditions. The samples were mixed until they were homogeneous. 1 gram of each sample was added before and after treatment with the extract and with the oil of the same plant, ground in 9 ml in saline solution (nutrition). Dilutions of

10^{-1} to10^{-4}were made by taking 1 ml of each sample for 9 ml of saline solution, and 1 ml was withdrawn by pipetting from the first dilution and added to the tube containing 9 ml of the dilution of physiological saline solution to obtain the required dilution. From each dilution, 1 ml was transferred to Petri dishes. Three repetitions for each dilution, each repetition was repeated in paragraph 2.1 above. 10 ml of the medium was added under sterile conditions to each Petri dish and the sample was thrown well using concave glass sticks forward and backward until the sample was distributed. Complete on the middle, the plates were incubated upside down in an incubator at $37c^0$ for 24 hours. After the incubation period was over, the number of Colony Forming Units (CFU) was calculated. The two replicate plates were selected in which the number of colonies in each plate ranged between 300-300 colonies. Then, the number of colonies in each plate was calculated, and the arithmetic mean of the number of colonies in the two plates was extracted and multiplied by the inverse of the dilution factor.

The same method was also used to prepare the plant extract experiment in the process of the number of bacterial colony on plant oils, but without the 10% and 20% dilutions oil of the same plant.

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Statistical methods used in the study

To achieve the objectives and hypotheses of the study and to analyze the data collected from the samples, many appropriate statistical methods were used based on the use of the Statistical Package for the Social Sciences software "Statistical Package for Social Sciences (SPSS) version 26, and the following is the set of statistical methods that the researcher used:

Arithmetic mean

The arithmetic mean is "the sum of values divided by their number." It is one of the measures of central tendency and is used to estimate population parameters or test statistical hypotheses .

The arithmetic mean is found by the following equation

$$\bar{x} = \frac{\sum_i^n xi}{n}$$

Standard deviation

The standard deviation for a set of observations is "the positive square root of the sum of the squares of the deviations of the values from their arithmetic mean divided by (n – 1)." It is one of the measures of dispersion, and is used to find out the closeness or divergence of the responses of a sample item on a particular option. The standard deviation is found from the equation Next

$$S = \sqrt{\frac{\sum_{i=1}^{n} f(Xi - \bar{x})^2}{n-1}}$$

Test(t):

The value of (t) for two independent samples is found using the following equation

$$t = \frac{(\bar{x}-\bar{y})-(\mu_1-\mu_2)}{s_p\sqrt{\frac{1}{m}+\frac{1}{n}}}$$

Analysis of variance and F test

To test the significance of the difference between several means (three or more means), the F test can be used, which is mainly based on the analysis of variance method. Analysis of variance is defined as dividing the sum of the squares of the total deviations from the general mean of the variable under study into two parts relative to the source causing the difference, based on the following equation:

$$\sum_i\sum_j(y_{ij}-\bar{y}_{..})^2 = n\sum_i(\bar{y}_{i.}-\bar{y}_{..})^2+\sum_i\sum_j(y_{ij}-\bar{y}_{i.})^2$$

Where the left side of the equation represents the sum of the squares of the total deviation and is denoted by the symbol SST

While the first part of the right side represents the sum of the squares of the deviation for the processors and is denoted by the symbol SSt, and the second part of the right side represents the sum of the squares of the deviation of the random error and is denoted by the symbol SSe. The F test is known as the following statistic:

$$F = \frac{SSt/(k-1)}{SSe/k(n-1)}$$

After obtaining the calculated F, it is compared with the tabular F value in order to make a decision regarding accepting or rejecting the null hypothesis, which states that the means of the group of variables are equal.

If the calculated F value is less than the tabulated F value with two degrees of freedom (k-1) and (k(n-1)) and an assumed significance level used in most studies of 0.05, the null hypothesis is accepted, meaning that the means of the group of variables are equal. However, if the calculated F value is greater than the tabulated F value, the alternative hypothesis is accepted, meaning that the means of the group of variables are not equal.

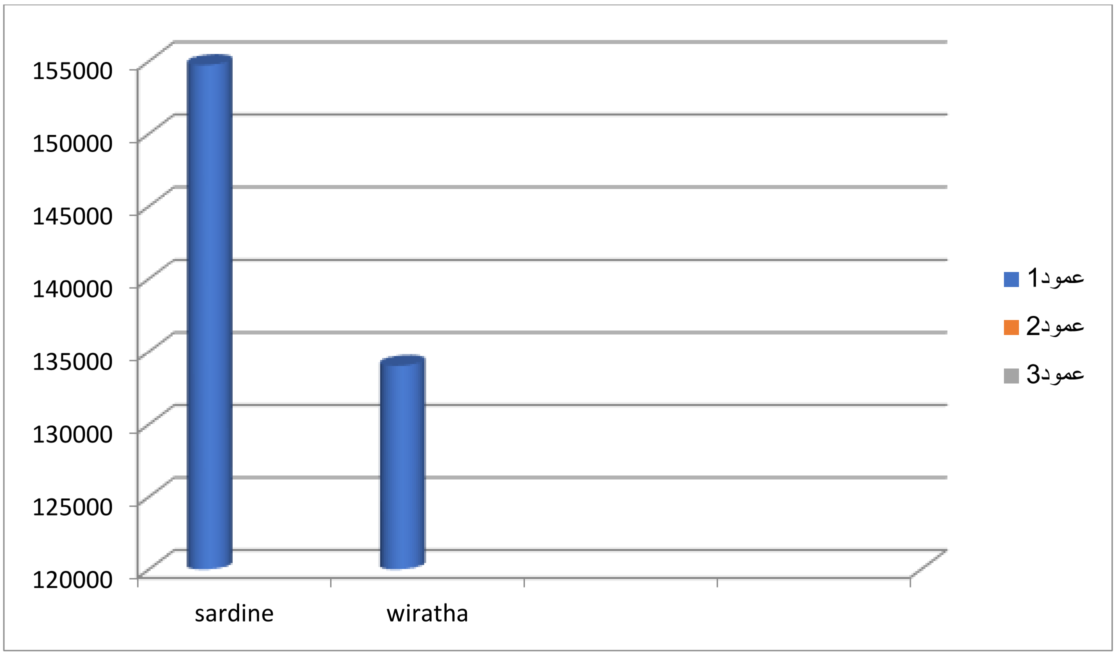
Results and Discussion:

Statistically significant differences between the sardine sample and the genetic sample in the bacterial size of the thyme plant:

To find out if there were differences between the sardine sample and the genetic sample in the size of the bacteria in the thyme plant, the (t) test was used to compare the means of two independent samples, and Table No. () shows that.

Table (1) Results of the t-test for comparison between the sardine sample and the genetic sample in terms of bacterial size for the thyme plant

Type of fish	Sample (N)	mean (x̄)	Std. Deviation (s.d)	t-test	p-value
sardine	34	154742.64	116407.83	0.727	0.470 (Not statistically)
wiratha	34	134009.80	118770.77		



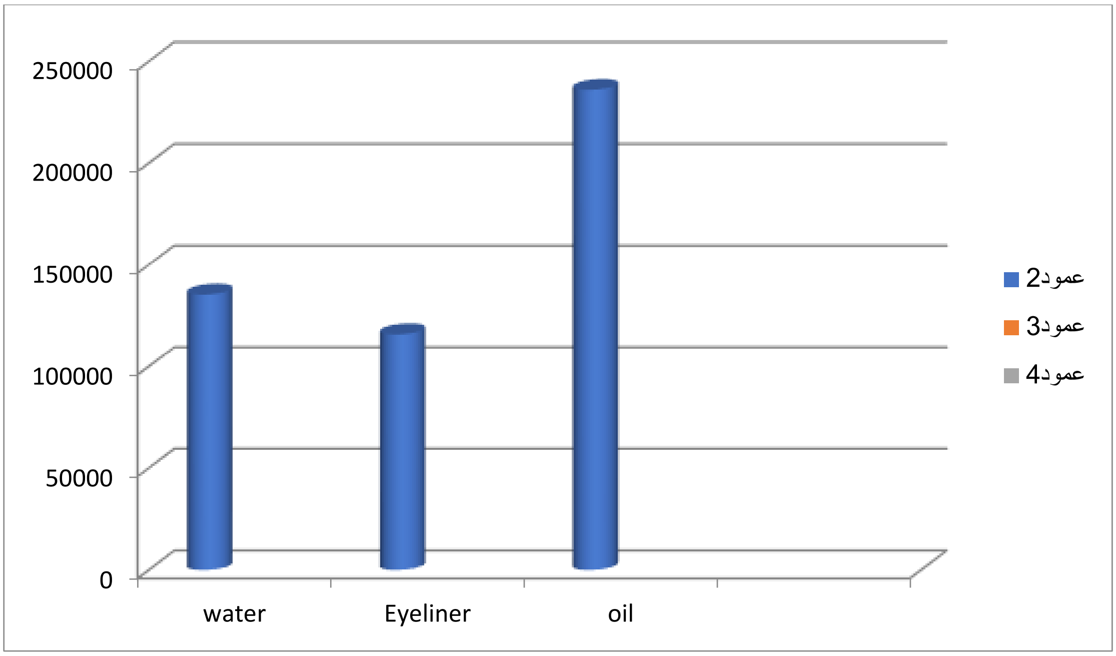
It is clear from Table. (1) that the arithmetic mean of the sardine sample is equal to (154742.64), with a standard deviation of (116407.83), while the arithmetic mean of the genetic sample is equal to (134009.80), with a standard deviation of (118770.77), and the calculated (t) value (0.727) is less than the tabular (t) value, which is equal to (1.671), Since the level of significance is equal to (0.470), it is not statistically significant because it is greater than (0.05), the level of significance adopted in the study, and based on the calculated value of (t) and the previously observed level of significance, it can be said that there are no statistically significant differences between the sardine sample and the genetic sample in the size of bacteria for the thyme plant.

Statistically significant differences between the types of solutions in the size of bacteria in thyme plants?

To find out if there are differences between the types of solutions in the size of bacteria in the thyme plant, the (f) test (one-way analysis of variance) was used, and the following table (2) shows that:

table (2) results of the (f) test to compare the types of solutions in the size of bacteria for thyme plants

Solution type	Sample (N)	mean ($\bar{x}$)	Std. Deviation (s.d)	Test (f)	p-value
water	26	135285.25	108517.81	5.046	0.009 (statistically)
Eyeliner	28	115809.52	80026.65		
oil	12	235847.22	169280.54		
total	66	145306.81	118183.52		



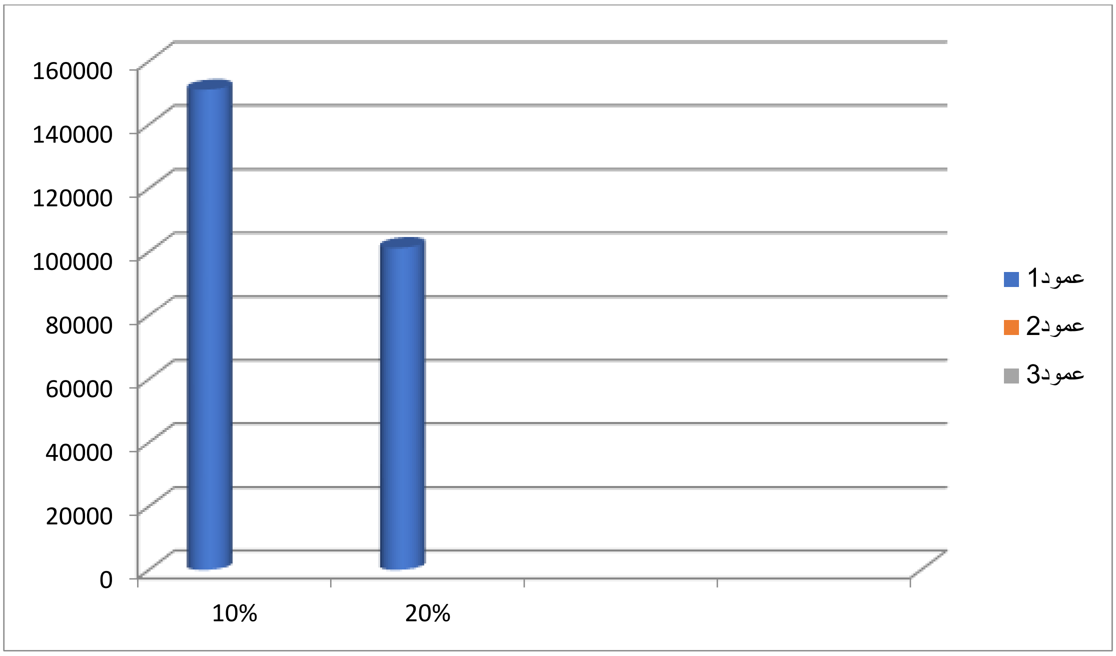
It is clear from Table (2) that the calculated value of (f) is equal to (5.046) which is greater than the tabular value of (f) with two degrees of freedom (0.02) and (65) and a significance level of (0.05) which is equal to (3.15), and since the significance level is equal to (0.009) it is statistically significant because it is less than (0.05) the significance level adopted in the study, which indicates that there are differences between the types of solutions in the size of bacteria for the thyme plant, as we find that the oil is the solution with the highest bacterial size followed by the aqueous solution and then the alcohol.

Statistically significant differences between the 10% and 20% concentrations in the bacterial volume of thyme plants?

To find out if there are differences between the 10% and 20% concentrations in the size of bacteria in thyme plants, the t-test was used to compare the means of two independent samples, and Table. (3) shows this.

table (3) results of the t-test for comparing the 10% and 20% concentrations in the bacterial volume of thyme plants

treatment	Sample (N)	mean ($\bar{x}$)	Std. Deviation (s.d)	t-test	p-value
%10	26	150961.53	114924.31	1.986	0.042 (statistically)
%20	28	101252.97	63517.20		



It is clear from Table. (3) that the arithmetic mean of the samples with a concentration of 10% is equal to (150961.53), with a standard deviation of (114924.31), while the arithmetic mean of the samples with a concentration of 20% is equal to (101252.97), with a standard deviation of (63517.20). Also, the calculated (t) value (1.986) is greater than the tabular (t) value, which is equal to (1.671), and since the level of significance is equal to (0.042), it is statistically significant because it is less than (0.05), The level of significance adopted in the study, and based on the calculated (t) value and the previously observed level of significance, it can be said that there are statistically significant differences between the 10% concentration and the 20% concentration in the size of bacteria in the thyme plant, as we find that the samples with a 10% concentration have a higher percentage of bacteria than the samples with a 20% concentration.

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