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Effect of Temperature Difference on the Effectiveness of Lactobacillus bacteria in the Decomposition of Organic Waste and the Possibility of Using the Product as Agricultural Fertilizers

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Abstract

This experimental study was conducted to compare the effect of temperature on the effectiveness of Lactobacillus bacteria in decomposing organic waste and its potential use as a useful fertilizer for soil. Lactobacillus bacteria were isolated from the mouth of a donor and identified. Then, a number of organic wastes (cucumber peels and banana peels) were collected after washing, cleaning, drying and cutting. Lactobacillus bacteria were placed in a normal saline solution with a concentration of 0.90 ml and 15 ml of it was taken with a concentration of 5 McF and placed on organic waste weighing 100 grams and placed in containers, then covered and left. This waste was divided into two parts and prepared at a temperature of 37°C and another part at a temperature of 20°C to prove the effect of temperature differences on the effectiveness of Lactobacillus in decomposing waste. After the decomposition that lasted (1-1.5) months, this waste was taken, dried, ground, then mixed with soil and radish seeds were planted in the soil. After five days, the soil containing decomposed banana peels at 37°C showed the beginning of growth. On the seventh day, the normal soil showed growth. No growth was shown in the fields containing decomposed cucumber peels at 37-20°C and decomposed banana peels at 20°C during the first week. The plants withered without fertilizer after nine days of growth, while the plants in the fields containing decomposed banana peels at 37°C remained upright and healthy, highlighting their potential as a useful fertilizer.

Keywords: Lactobacillus, Organic Waste Decomposition, Temperature Effect, Soil Fertilizer, Plant Growth

تأثير اختلاف درجات الحرارة على فعالية بكتيريا اللاكتوباسيلس في تحلل النفايات العضوية وإمكانية استخدام الناتج كسماد زراعي

أسامة غازي عباس الزهيري [1] ، فرقد وكاع فجار الاعاجيبي [2] ، كوثر وكاع فجار الاعاجيبي [3]

المستخلص

أُجريت هذه الدراسة التجريبية لمقارنة تأثير درجة الحرارة على فعالية بكتيريا اللاكتوباسيلس في تحليل النفايات العضوية، وإمكانية استخدامها كسماد مفيد للتربة. عُزلت بكتيريا اللاكتوباسيلس من فم أحد المتبرعين، وتم تحديد نوعها. ثم جُمعت عدد من النفايات العضوية (قشور الخيار والموز) بعد غسلها وتنظيفها وتجفيفها وتقطيعها. وُضعت بكتيريا اللاكتوباسيلس في محلول ملحي طبيعي بتركيز 0.90 مل، وأُخذ منها 15 مل بتركيز 5 ماكفرلاند، ووُضعت على نفايات عضوية وزنها 100 غرام، ووُضعت في حاويات، ثم غُطيت وتركت. قُسِّمت هذه النفايات إلى قسمين، وجُهِّزت عند درجة حرارة 37 درجة مئوية، وقسم آخر عند درجة حرارة 20 درجة مئوية، لإثبات تأثير اختلافات درجات الحرارة على فعالية بكتيريا اللاكتوباسيلس في تحليل النفايات. بعد التحلل الذي استمر (شهرًا إلى شهر ونصف)، أُخذت هذه النفايات وجُففت وطُحنت، ثم خُلطت بالتربة وزُرعت بذور الفجل فيها. بعد خمسة أيام، بدأت التربة التي تحتوي على قشور الموز المتحللة عند درجة حرارة 37 درجة مئوية بالنمو. في اليوم السابع، بدأت التربة العادية بالنمو. لم يُلاحظ أي نمو في الحقول التي تحتوي على قشور الخيار المتحللة عند درجة حرارة 37-20 درجة مئوية، وفي قشور الموز المتحللة عند درجة حرارة 20 درجة مئوية خلال الأسبوع الأول. ذبلت النباتات بدون سماد بعد تسعة أيام من النمو، بينما ظلت النباتات في الحقول التي تحتوي على قشور الموز المتحللة عند درجة حرارة 37 درجة مئوية منتصبة وصحية، مما يُبرز إمكاناتها كسماد مفيد.

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الكلمات المفتاحية: اللاكتوباسيلوس، تحليل النفايات العضوية، تأثير درجة الحرارة، سماد التربة، نمو النباتات

Introduction

Waste is a byproduct of population increase, urbanization, and economic growth ,Approximately 2.59 billion tons of waste will be generated globally in 2030, which is predicted to reach 3.4 billion tons by 2050, doubling from 2016 and tripling by 2100 [1]

Soil carries out an important ecological services for the sustenance and survival of life. Soil health management is vital for the maintenance of biodiversity and safeguarding sustainable agricultural production. So, retaining and preserving soil health has prime importance for ecosystem sustainability. The health of soil is regulated by soil properties, that is, physicochemical and biological properties. Modern agriculture is largely dependent upon fertilizers [2].

The excessive use of chemical fertilizers and long-term cultivation has, to some extent, damaged the soil environment. This condition not only changes the soil's physicochemical characteristics and microbial community structure but also limits the sustainable development of agriculture[3].

Lactic-acid bacteria, including Lactobacillus spp., Enterococcus spp., Lactococcus spp., Pediococcus spp. and Leuconostoc spp., can work rapidly to decompose plant material and any type of organic waste in factitively anaerobic condition. Bacterial species in this group prefer a more anaerobic condition to decompose organic waste that is rich in carbohydrate and sometimes work together with yeast species such as Saccharomyces spp. and Schizosaccharomyces spp [4].

Fruits and vegetables are abundant in essential nutrients and contain high levels of water, soluble carbohydrates, fiber, minerals, vitamins, polyphenols, and other bioactive compounds Despite this, they are often considered waste once they experience changes in color, undergo biochemical reactions, become infested with microbes, experience breakage or frostbite, are subjected to heat treatment, or reach levels of ripeness that make them unacceptable to consumers [5]

As banana peels contain 42 percent potassium, they can be used as nutrient source for plant. Along with nitrogen and phosphorus, potassium is one of the most important components of fertilizer, and banana peels are the best organic source of potassium. A banana peels approximate N-P-K ratio is 0-3-42, hence the Banana peels make up 18-33 percent of the total weight of the fruit and are considered a waste product [6].

Materials and methods

Sample collection

The waste samples used in this study were collected from the Iraqi houses, which are both banana peels and cucumber peels

Isolation and diagnosis

At first the swab was taken from the donor's mouth; it was activated with Nutrient broth Broth and which were incubated for 24 hours and she while implanted on the acre of lactobacillus then added caco3 and incubate her anaerobically for 24 hours with a temperature of 37 degrees Celsius.

Microscopic examination

Microscopic examination was performed to determine the dryness of the bacterial isolate using

Gram stain. A sample was taken from a portion of a colony grown on a gastric medium (nutrient agar) using a carrier medium. A bacterial smear was made from this sample on a clean glass slide and treated with Gram stain in all its steps. Then, it was placed under a microscope to determine whether the bacteria were Gram negative or Gram positive [7] .

Biochemical tests

Some biochemical tests had been performed to diagnose the bacteria, by the catalase test. The catalase test was performed by placing a few drops of 24 h grown culture on a microscopic slide, and then placing 1 drop of 3% H2O2 on the culture using a dropper. After the addition of H2O2, [8]

Preparation of waste samples for

After collecting the samples of banana peels and cucumber peels, they were washed to remove impurities, cut them in order to increase the area and dry them at room temperature for 24 hours.

Preparation of bacterial solution

Isolates of lactobacillus bacteria were taken and mixed in normal saline so that their cobs were made equal to the concentration of 5 MacFarland by comparing them with McFarland tubes.

Application of bacteria to waste for decomposition

After the waste was washed and cut and the bacterial solution was prepared, the waste was placed in glass cans and in equal sizes, where the 100 grams represented the size chosen in this study for each type of waste, then 15 ml of bacterial solution was placed on the waste .

Waste incubation

Each type of mixture of waste and bacterial solution were divided into two parts, and all of it weighed 100 grams, one of them was a brood at a temperature of 37℃ and the other section at 20℃ to achieve the goal of the study and show the extent to which temperature affects the effectiveness of bacteria in the decomposition of organic waste .

pH measurement

The acidity of the waste was measured as a kind of decomposition indicator and device (pH meter).

Decomposing waste drying

Once the decomposition is complete, the decomposing waste was taken and dried for several days at room temperature .

Fertilization and cultivation

After the waste dried, it was ground with an electric grinder and added to the soil, where 2.5 grams of waste were added to every 150 grams of soil, moistened with water and left to homogenize for a day.

Agriculture Removed

Several fields were planted with each decomposition product and also a sample of commercial fertilizers was taken and planted with them also for comparison with preference and also all these fertilizers were compared with fertilizer-free soil.

Result and discussion

Bacterial isolation results

After 24 hours of the test, a clear bacterial turbidity appeared in one of the tubes as in the figure (1)

Figure (1): Activation of isolate on nutrient broth

The shape of the LAB has been identified through its appearance on the MRS dish after adding Caco3, where small colonies seemed to be somewhat large with white circular edges where these results agreed with what it came [9] where he explained that were found as whitish, small to large size, the circular margin on MRS media, as shown in Figure (2).

Figure (2): Lactic Acid Bacteria Colonies on MRS Agar with Caco3

Microscopic examination

And by examining the isolate under a microscope, it was found to be rod-shaped and positive for the Gram stain. This description agreed with what was stated by [10] where he described it as whereas Lactobacillus presented a uniform morphology of gram-positive, large rod-shaped bacteria. As shown in Figure (3).

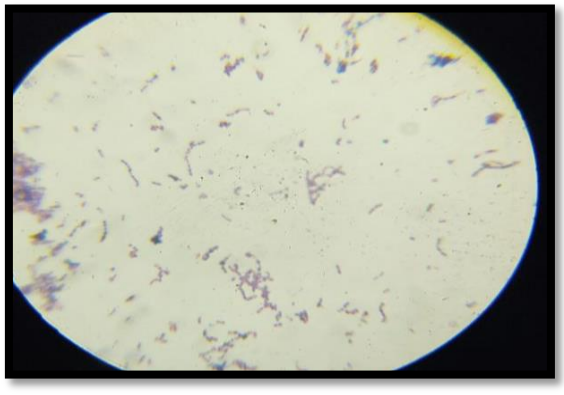

Figure (3): Microscopic Image of Rod-Shaped Lactobacillus Bacteria

Biochemical tests (catalase test)

The bacteria in this test showed a negative result, which is consistent with [11]which showed that In the catalase test, the results were negative, as shown in Figure (4)

Figure (4): Catalase Test Results for Lactobacillus Bacteria

In the early days of interaction with bacteria, plants showed changes in color, smell, pH, and texture as shown in Figure (5). The intensity of remove changes varied between the temperature range (20-37℃) with the highest intensity at 37 degrees.

In this figure Change in the texture of cucumber peels after five days of adding bacteria and incubating them 37°C. as shown in Figure (5)

Figure (5): Texture Change in Cucumber Peels After 5 Days at 37°C

Figure (6) Change in color and increase in moisture of banana peels one day after adding bacteria to them and incubating them at 37°C.The change in texture, odor, and acidity was much less at an incubation temperature of 20°C compared to that at 37°C .

Figure (6): Color and Moisture Change in Banana Peels After 1 Day at 37°C

Figure (7) Change in color and increase in moisture of banana peels occurred one day after bacteria were added and incubated at 20°C.

Figure (7): Color and Moisture Change in Banana Peels After 1 Day at 20°C

Figure (8) Change in the texture of cucumber peels after five days of adding bacteria and incubating them at 20 °C.

Figure (8): Texture Change in Cucumber Peels After 5 Days at 20°C

Figures (7) and (8) present, removed that the change in temperature at 37°C was faster and greater than at 20°C, indicating that the activity of bacteria at 37°C in decomposing waste is much better than at 20°C. This study agreed with the study of [12] which showed that Microbial cells

grow at the lowest temperature (30 °C) and enjoy higher unsaturated fatty acids than saturated fatty acids. Since LAB growth is lower at 42 °C than at 30 °C and the production of organic acids follows a growth pattern, then growth at higher temperatures results in less acid production.

The pH decreased with the progress of decomposition from the first day, when it was 7.63 in cucumber peels and 7.5 in banana peels, to 6.4 in cucumber peels decomposed at 20°C, 7.7 in cucumber peels decomposed at

37°C, and 6.0 in banana peels decomposed at 37℃ . This study agreed with the study of [13] which showed that The result showed that the pH decreases as days of fermentation increases due to the production of acids.

In this study, the morphological stability of the decomposed waste and the pH stability within a certain range were used as indicators of complete decomposition. After a month of incubation, the pH level tended towards acidity at 20°C, being highly acidic in banana peels and slightly less acidic in cucumber peels. At 37°C, the pH level tended towards neutrality in banana peels and was slightly basic in cucumber peels. When decomposition was complete.as in table (1) and Figure (9).

Table (1): pH measurements during the waste decomposition period

Variable	Criteria			
	Banana Peels 20°C	Banana Peels 37°C	Cucumber Peels 37°C	Cucumber Peels 20°C
pH (Initial)	**7.5**	**7.5**	**7.63**	**7.63**
After 5 Day	**6.45**	**5.66**	**7.2**	**7.05**
After 10 days	**5.9**	**6.1**	**7.3**	**6.6**
After 15 days	**6.2**	**6.9**	**6.5**	**6.3**
After 20 days	**5.7**	**6.9**	**6.8**	**6.0**
After 25 days	**5.9**	**7.2**	**7.2**	**6.4**
After 30 days	**5.9**	**6.0**	**7.7**	**6.4**

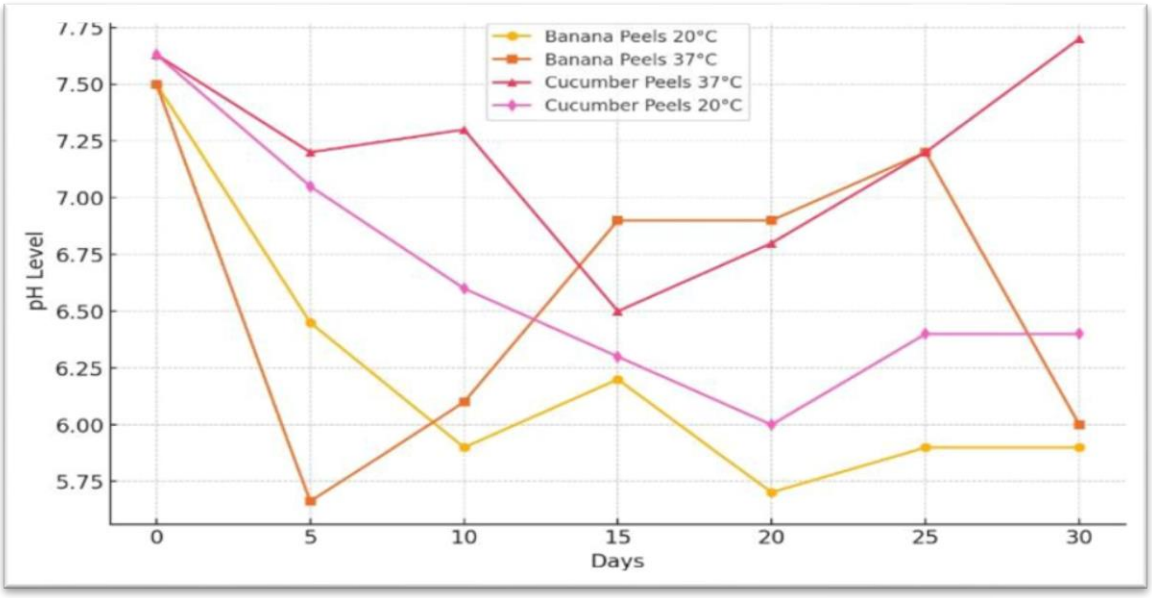

Figure (9): pH Measurements During Waste Decomposition Period

At this point, when the decomposition is complete, the decomposed products are taken and dried at room temperature as shown as shown in Figure (10), After it was dried, it was picked, ground, added to the soil, and then radish seeds were planted. As shown in Figure (11) and (12).

Figure (10): Drying Process of Decomposed Waste

Five days after planting, growth was observed in soil enriched with decomposed banana peels at 37°C, as shown in Figure (11).

Figure (11): Plant Growth banana peels decomposed at 37°C as fertilizer

In contrast, normal soil without fertilizer showed growth after seven days, as shown in Figure (12).

Figure (12): Plant Growth in normal soil without fertilizer

There was no seed germination in the soil fertilized with decomposed cucumber peels at both temperatures 20-37°C After 5-7 Days. as shown in Figure (13).

Figure (13): soil fertilized with decomposed cucumber peels at both temperatures 20-37°C After 5-7 Days

Throughout the cultivation period, no growth was observed in soil fertilized with decomposed cucumber peels at 20 and 37°C, and with decomposed banana peels at 20°C. This demonstrates the benefits of using banana peels decomposed at 37°C as fertilizer as in figure (14), the relative abundance of nutrients and minerals in banana peels compared to cucumber peels, and the effectiveness of temperature in decomposing waste and producing fertilizers containing beneficial soil nutrients.

Figure (14): Plant Growth banana peels decomposed at 37°C as fertilizer After 10 Days

The soil containing artificial fertilizers showed clear wilting in its plants, as in Figure (15)

Figure (15): Plant Growth in artificial fertilizers soil After 10 Days

Plants wilt in the soil without fertilizers after ten days of growth, as shown in Figure (16).

Figure (16): Plant Growth soil without fertilizers After 10 Days

After 10 days of growth in each field, the plants in the soil without fertilizers began to wilt, as shown in figure (16). Similarly, the soil with commercial fertilizers also displayed noticeable wilting in its plants, as seen in Figure (15). In contrast, the plants in soil containing decomposed banana peel fertilizers remained perfectly straight and healthy compared to the others, as shown in Figure (14).

The speed of plant growth and continued healthy growth may depend largely on the fertilizers used and the elements they contain that are beneficial to the plant, such as: This study showed that banana peels decomposed at a temperature of 37 degrees are ideal for germination in terms of fertilization. These results are consistent with what was reported in the study of [6] which confirmed that , As banana peels contain 42 percent potassium, they can be used as nutrient source for plant. Along with nitrogen and phosphorus, potassium is one of the most important components of fertilizer, and banana peels are the best organic source of potassium, A banana peel's approximate N-P-K ratio is 0-3-42. Also, The pH also had an impact on the preference of fertilizers in terms of as previously indicated in this study that the organic fertilizers resulting from the decomposition of decomposed banana peels at a temperature of 37 ° C were the best for plant growth These fertilizers had an acidity point at the completion of 6.0 i.e. acidic, and this is what is consistent with the study [14] Who stipulated it Phosphate uptake by plant roots increases as the pH decreases..

Conclusions

1. A temperature of 37°C is optimal for the effectiveness of lactobacillus in decomposing organic waste
2. The agricultural wealth of banana peels and their use as fertilizers has surpassed commercial fertilizers.
3. Banana peels decomposed at 37°C had the advantage of being used as fertilizers compared to cucumber peels decomposed at both temperatures.
4. The pH of the waste decomposed at 20°C was more acidic than that of the waste decomposed at 37°C.

Recommendations

1. Showing greater interest in natural fertilization and reducing chemical and industrial fertilization.
2. Working on recycling waste in various fields to benefit and reduce environmental pollution.
3. Expanding research in the field of utilizing Lactobacillus bacteria in the field of fertilization.

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