American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

The Effect of the Bio-Extract of Bacteria Bacillus Subtilis & Pseudomonas Fluorescence on the Tomato

Ban Star Saleh, Wesal Ali Attia
University of Karbala College of Science Department of Biology

Ali Ahmed abd alwahed
University of Kufa College of Science Department of Biology

Mustafa Nabil Karim
Al-Mustaqbal University College of Engineering, Department of Medical Device Engineering

Received: 2024, 15, Mar
Accepted: 2025, 21, Apr
Published: 2025, 28, May

Annotation: This study includes the possibility of producing a biological product from the two strains of Bacillus and Pseudomonas bacteria, with the aim of using and circulating it economically and in commercial scope by the beneficiaries in the country after determining the basic elements for the production of the preparation represented by the fermentation medium which is selfishly composed of the extract of rennet. Our test results. Is that the wheat extract acts as a fermentation medium and also increases the density of bacteria. When testing the substance Caco3 as a carrier of the biological product, calcium carbonate showed a higher efficiency than the corrosion, as the density of bacteria increased by loading them with calcium carbonate, while it decreased and by a small percentage when using talc as a substance. Haha, the case of Ki. The results of the experiment showed the ability of the biological product to give high rates of growth as it increased The dry and fresh

weight of tomato foliage, and the average dry weight of tomato plant when treated with the preparation was 0.24 g germination when using 8 g / 100 g seeds. The lean weight of tomato plants, using a concentration of 8 g / 100 gm of tomato seeds, increased to 1.8 g.

Introduction

Microbiological diversity in the soil not only had a negative impact on plant growth, but there were also some organisms, whether bacteria, parasites, or fungi, with a positive effect on plant growth. Therefore, the presence of these organisms in the soil provides very important nutrients for plants and their growth and intensity.

These organisms can stimulate substances responsible for the growth of stems and leaves and accelerate the flowering and fruit, which justifies how all existing organisms evolved and emerged to offer several benefits. Accordingly, scientists and researchers have furthered many ways to isolate these organisms, develop them, and impel them to produce plant growth stimuli to promote plant growth

As human needs for plants are increasing, scientists and researchers have made efforts to produce natural plant-growing substances from surrounding organisms without adversely affecting consumers, be human or animal. Many plants produce different substances, organisms, and bacteria. Recently, the efficiency of some varieties of Bacillus pseudomonas was tested to produce a preparation that proved highly efficient in the promotion of plant growth. Some studies, such as Weller, 1984, Campell et al., 1987, Cook, 1993, Al-Otaibi, 1999, and Jassim, 1999, confirmed that the use of these bacteria increases plant growth.

To this end, the following procedures have been followed

1. Identifying an appropriate fermentation culture
2. Choosing a suitable carrier of the bio-bacterial preparation .
3. Testing preparation efficiency by increasing the tomato starters
4. Checking the influence of that preparation on plant growth

Review of references:

1. Pseudomonas

The cells in pseudomonas, individually straight or curved with pseudomonas fluorescens are 0.7 to 0.8 μm long, are bacillary gram- negative bacteria commonly found in organic soils having adapted to stable balance with plant roots (Broadbeet *et al*., 1971). Having single or double rods, mostly with non-polar rods, these non- fermentative, catalysis-positive, and non-spore bacilli possess an oxidative metabolism and can produce a variety of spores in the environments in which they grow, particularly in iron-free nutritive

environments such as nutrient gars. (Govan, 1997)

Even though the PH of pseudomonas is from 7 to 9, these bacteria, however, being obligatory aerobic in growth, can grow in PH- saturated cultures.

2. Genus Bacillus

Bacilli are slightly rod-shaped organisms with the ability to move with lateral rods which are 1.5 to 3.5 μm long. These bacteria are native to soils, wastes, and degradable and non-degradable organic wastes. Bacillus bacteria can produce internal spores that are resistive to extreme surroundings, external weathering, and growth-neutral conditions including temperature, bases, and extreme acidities. (Collee *et al*., 1996)

Additionally, bacillus bacteria can develop gram-positive and spore-forming species including B. anthracis, B. subtilis, and B. megaterium. (Al-Juboory, 1990, and Tobar, 2003).

Most bacillus bacteria are chemoheterotrophic where they can break down organic substances such as carbohydrates, fat, and amino acids to exploit them as a source of carbon and energy.

Furthermore, these bacteria can also ferment carbohydrates heterogeneously to force them to produce glycerol and butanol. (Tobar, 2003)

Bacillus species are mostly mesophiles in nature, which means they adapt to medium temperatures and prefer to grow between 30 and 45 °C. Bacillus bacteria can be easily isolated using the available laboratory techniques. A specimen of soil, from which bacillus bacteria are to be isolated, can be pasteurized at 80 °C and for 15 minutes to dispose of other bacterial organisms and vegetative bacillus-related cells, conduct laboratory dilution of the sample, and transplant the last dilution in a nutrient agar culture.

(Tobar, 2003)

Scientific investigations have shown that most subtypes of bacillus bacteria can grow in soil, within root tissue, or in root surroundings. (Coombs *et al*., 2003, Nicholson, 2003). Additionally, these bacteria can be found in abundance away from roots at rhizospheres (Qassim and Ali, 1989) at a rate of 10-103 soft cells/gram. The most important organisms productive of bacillus species are B. subtilis, B. cereus, B. megaterium, and B. cirulans. (Nicholson et al., 2000, Nicholson et al., 2002).

2.3. Promotion of Plant Growth

the use of tomato-growing organisms increases tomato resistance against pathogenic fungi, particularly F. oxysporcum and

Lycopersicum. In the same vein (Minxiao, *et al*, 2023) posited that treating tomatoes with pseudomonas fluorescens may enhance tomato productivity, reduce infection with disease-causing fungus, and increase tomato weight, freshness, and tenderness.

Moreover, among the studies conducted on Bacillus subtilis, were those that have found that these bacteria increase tomato production by yielding tender, fresh, and weighty tomatoes

Pérez-Rodriguez, *et al*. (2020) found that treating tomatoes with a genus of Pseudomonas fluorescens increased the production of tomatoes by 500% in greenhouses

In Iraq, Al-Hitti *et al*. (1996) found that the addition of a pseudomonas fluorescens-based preparation helped grow rice class 33 in fields in the 1990-1991 harvest seasons using two methods. In the first method, the preparation has been added to grains before planting in the soil. This led to a significant effect on rice yielding by increasing the plant height, multiple branching, and multiple seeds. In the second method, the preparation has been added to the soil. This method increased the dry weight of roots, which resulted in a significant increase in the final crop. The final increases hit 50 to 75% in the 1990-1991 harvest season and 52.7 to 78% in the 1991-1992 harvest season following the addition of the preparation to the grain and to the soil respectively.

3.1. Study supplies

3.2. The devices and chemicals used in the test

S	Name of scientific system	The manufacture company	Of Origin
1	Sensitive Balance	Sartorius	Germany
2	Autoclave	Melage	Germany
3	Compound microscope	Olympus	Japan
4	Incubator	Memmert	Germany
5	Electric Oven	Memmert	Germany

Chemicals used in conducting experiments contained in this study

S	Subject	The manufacture company	Of Origin
1	Talic	Baby care	USA
2	Agar	Difco	UK
3	Formalin	Fluka	Switzer land
4	Caco3	Laboratory gypsym Noura karbala	Iraq
5	Cotton		Turkia
6	Antiseptic		China
7	Gauze		Iraq
8	Alcohol ethyl	BDH	U.k

2.3: Used implant pads

1. ***Nutrient Agar.***
2. **Nutrient Broth.**

3.3. The microorganisms used and how to isolate them:

1. Bacillus Subtilis, 2. Psudomonace Fluorecense Two specific areas were selected from Najaf governorate, which are Al-Buhdari and Al-Hawat, to isolate the species belonging to the genus Bacillus pseudomonas by taking three size samples (**100** gm) of soil with a depth of **10** cm and **20** cm and for each of the specified sites and each of the sites were identified. The samples are in a fully sterilized, single-use, disposable plastic package and mix the samples belonging to one site with each other, and then bring (**1** g) of the mixture and a series of dilution was performed on it by sterile test tubes containing (4 ml) of water and these tubes were numbered from (1-3) A series of dilution was performed on it (0.001-10), 1 ml of the last dilution was withdrawn and cultivated on three petri dishes, container with 20 ml of medium NA, sterile at a temperature of 181 ° C, pressed 1 atmosphere for a period of 20 minutes, and spread by the planning method by a glass diffuser. It was sterilized and then the dishes were incubated in the incubator at a temperature of C for a period of 24 hours, and this process was repeated three times for each soil sample and for each site according to a study (1996. Collee et al.). It was found during cultivation that the dishes belonging to the Albuhdari area had turned the color of the medium to yellow Fatih as for the epitopes after isolation and cultivation, bacterial colonies of pale-yellow color were circular or oval in shape, and phenotypic and biochemical tests were performed. We obtained the genus Fluorecense Bacillus subtilis pseudomonas and confirmed the identification of both sexes. 4. : a study **3, 4: Bacillus** microorganisms: Samples of bacterial colonies that were confirmed to be belonging to **Bacillus** bacteria were tested by taking a web swab, spreading it on a sterile glass slide, fixing it, and then staining it with a cream stain and examining the shape of the bacterial cell and the location of the blackboards in it. Blue pigmentation is an indication that it is a bacterium belonging to the Cram-positive group, in which the bacteria dyed red in the color of Cram is financial for the cram stain. Jubouri 130. (Collee et. 1996)

5.3: The fermentation medium was used wheat extract :.

As 20 grams of wheat seeds were crushed by an electric mill and soaked in 1 liter of sterile distilled water for 4 hours (Hamid 2000, Ashur 2005). The extract was filtered with a sterile gauze pad and then sterilized by an autoclave at a temperature of 151 ° C and a pressure of 1 atmosphere for a generation of 20 minutes, and then the medium was cooled and inoculated with 4 colonies of bacterial growth Subtilis A. After that, a series of pseudomonas Fluorecense was grown on NA medium with a 4-hour age for each of these fermentation media. The fermentation media were incubated for 24 hours at a temperature d dilution. 1 ml of the last dilution of the fermentation medium was selected and grown on NA medium with three replications. Incubation at a temperature of ٣٧C for a period of 24 hours, after which the average number of bacteria was calculated according to the Clark 1965 equation.

(Bacillus subtilis, pseudomonas Fluorecense)

6.3: Determination of the suitable loading material for the production of the biological product:

The efficiency of two substances, calcium carbonate and alkalization, as the bearers of the biological preparation, were tested and the best ones were selected for loading, and the test included taking 100 grams of each of the two materials calcium carbonate and talc in sterile containers and these materials were sterilized by an electric oven at a temperature of 160 m For an hour and left to cool, as for the wheat leftover powder, it was sterilized with the cauldron at a temperature of 121 m and a pressure of 1 atmosphere for a period of 30 minutes, then added to each of them 100 ml of fermentation medium stimulated in advance at the age of 8 hours, after which the pots were transferred to an electric dryer at a temperature of 37 ° C for a period of 5 days until they were well dried. Then the powders loaded with bacteria were crushed in a sterile room (Hood). Then a series of dilutions was prepared from each carrier (0.001 - 10) and then transferred from the last dilution of the bacterial suspension in the dilution to sterile glass dishes containing the nutrient medium NA. Three replications and the dishes were incubated at a temperature of 8 ° C for a period of 24 hours (Al-Zaidi and his group, 1987. Hamid 2001). Then the numbers growing per gram of each carrier were estimated in order to calculate the average number of colonies growing in each plate multiplied by the reciprocal of the thickness dilution 1983, (Clark 1965)

7.3: The final product:

Production of the final product: The wheat extract medium was selected as a fermentation medium and calcium carbonate as the carrier of the biological product and for the purpose of conducting the laboratory and field evaluation ... A kg of the biological product was prepared as follows: We prepare 500 grams of carbonate Calcium and put it in large aluminum utensils at a rate of250 grams for each vessel that was sterilized in the electric oven at a degree of 160 m for a period of an hour, and at the same time we prepare 1 liter of fermentation medium (wheat extract) after sterilizing it with an autoclaved at a degree of 121 m and a pressure of 1 atmosphere for a period of 20 minutes. Then the fermentation medium was inoculated with 100 ml of Bacillus subtilis vaccine grown on N.A food medium at an age of 28 hours.

And 100 pseudomonas Fluorecense bacteria grown on the Nutrient broth at the age of 48 hours The fermentation medium was incubated at a temperature of 37 ° C for 48 hours, after which the fermentation medium in which the bacterial vaccine was grown was added to the carrier material Caco3, and then the final product was dried with an electric dryer. At a degree of 40 m and for a period of 6 days, the product was milled by an electric mill sterilized with alcohol inside the sterile culture room and put the product in it for use, 1987. Leben et (2005)

The plates containing colonies ranging between (20-500) bacterial colonies were selected and then the Clark equation layer to calculate the number of bacterial cells.

9.3: Applying it to the plant:

The tomato plant was cultivated, and the biological catalyst was added to it with the cultivation of the tomato plant without adding the biological preparation to it for the purpose of calibration

The following was calculated: the percentage of germination = the number of plant seedlings / the total number of seedlings planted x 100, as well as the calculation of the average growth after 3 weeks of the germination process, including the calculation of:

1_the height of the plant with a drawing. The crown is to the developing summit.

2_ Dry weight of the vegetative mass: the seedlings were dried the broth to measure the dry weight at a temperature of 20 m for a period of 24 hours, using the sensitive balance, and the dry weight was recorded.

3_ Fresh weight of shoots: Three seedlings were taken randomly. The shoot was cut and measured by a sensitive balance.

10.3: Bio-chemical tests to determine the sex of Bacillus.

The bacterial isolate Bacillus subtilis was diagnosed with a confirmatory diagnosis by testing its ability to ferment the sugars in the test strip manufactured by India Himedia / Rapid for the consumption of a large group of carbohydrates by micro- organisms, especially Bacillus bacteria. All the tests on the test strip with Bacillus subtilis vaccine are blown on the liquid medium Nutrient broth at a age of 24 hours and then incubated for a period of 4-hours at a temperature of 37C and the color changes that occurred in the pits in the test strip are observed which are positive or negative evidence of the occurrence of fermentation. In polysaccharides that include turning red to yellow is evidence of collee. et. 2000)

Result and discussion

1,4: Determination of the appropriate loading material:

From Figure (1), it includes that calcium carbonate showed high efficiency as a carrier of the biological product, compared to talc, as the density of bacteria loaded on CaCo3 significantly exceeded the density of bacteria loaded on talc. In addition to calcium carbonate being a good carrier, it inhibits the growth of pathogenic fungi by indirect effect such as changing the nature of the culture medium, the growth of the base conditions when they are hydrolyzed by giving them negative hydroxyl ions (Awad 1986) and since the fungi prefer to grow in a neutral or slightly acidic medium (Ankald 1980) The change in the pH value changes the nutrients needed by the fungi, or the calcium carbonate substance may have a direct effect on making the water voltage in the medium more negative, according to Keller et al. (1982), as well as it is cheap and available locally.

Transactions

The shape (1) Shows the effect of the carrier on the density of the forbia in the weight unit

: Evaluating the penance of the Chinese preparation

1.2.4: Estimating the density of bacteria per gram of the microbial preparation Immediately after production:

after taking 9 mg of the biological preparation and it was suspended in 1 ml of distilled water and then a series of dilutions (0.002_ 0.1) He took 1 ml of the last dilution and planted it in a petri dish, as well as 1 ml of dilution D (10) and planted in a petri dish containing 5 ml of NA and after incubation for 24 hours at a temperature of 37 ° C, the containers were taken On (30 - 300) bacterial colony and promised bacteria. It was found that in dilution (0.1) more bacteria than in the last dilution (1960) bacterial cells in 1 ml of the biological preparation. As for the number of bacteria in dilution 0.001, it reached (1024) bacterial cells in 1 ml of the biological preparation.

4.2.2: Test the efficacy of the biological product in the growth of tomato plant:

The results showed that the treatment of the preparation was significantly superior to the treatment of **8 g / 100** g in terms of the increase in the density of bacteria. The results showed an increase in the growth of the plant.

***Rate of plant height**:*

During seedling germination, it became clear that there was a significant difference in the rate of height of the plant working with the bacterial biosimilar at concentrations of 8 g / 100 g seeds of the biostimulator that the highest rise in the concentration of (8) reached 20 cm, while the untreated plant With the biological preparation, its height does not exceed (12 cm). As for the concentration of (5), it was noticed that there are significant differences, as the height of the leg reached (16) cm. The etymological relationship illustrates this:

High plant

Product concentration

The shape (2) Show difference in height is for concentration The fresh weight of the shoot mass:

The statistical analyzes showed that the fresh weight of the shoot mass was superior to the plant working with the biological preparation at a concentration of 8 g / 100 g liters of seeds, where the weight was 1.8 g germination. As for the concentration of 5g / 100 g seeds, it is 1.07 g, the germination of Al-Otaibi 1999.Leben et al1987.

Soft weight

Focus of vital improvise 100 /gm seeds

The Shape (3) The difference shows for the light weight of the focus in the preparation

Dry weight of the vegetative set

The experiments related to the dry weight tests indicated that there was a significant superiority in the plants treated with the bacterial biological preparation at a concentration of 2g / 100 g with light on the plants treated with other concentrations, as the fresh weight reached 0.24 g / plant, while the weights decreased at a concentration of5 g / 100 g. Seeds to 0.12 g / plant. This superiority is due to the concentration of (8) the density of bacteria per unit weight of the product compared to the concentration of (5) g / 100 g seeds, and this is due to the susceptibility caused by the bacteria by increasing the plant's ability to compete for nutrients and its effect on growth-stimulating hormones, and this result is similar to what he mentioned (1984 Weller) as well as (Jasim 1999)

Conclusions and recommendations: conclusion and Recommendation Conclusions:

It was concluded that wheat extract is of great importance in the preparation of fermentation media, especially used in the cultivation of Bacillus bacteria used in the experiment. Pseudomonas It was concluded that calcium carbonate (Caco3) overtakes talc is the first to have the highest efficiency in being a carrier of the biological preparation. The clear superiority in growth for plants that were treated with the bacterial biological preparation over plants grown naturally without using the biological preparation.

Recommendations:

We recommend using the product at a concentration of 3 g / 100 g seeds in fogging tomato plant to increase growth and combat the fungus F. Lycoperisici F. Oxysporum We recommend that studies be carried out on this biological product that has the ability to increase the fresh and dry weight of tomato plant and to combat pathogens, especially root pathogens. And vascular wilt.

Reference

1. the narrator, Khashi Mahmoud and Abdulaziz Khalaf Allah 1980. Design and analysis of agricultural experiments.
2. Al-Zubaidi, Hamza Kazim, 1980. Bio-pest resistance, Dar Al-Kutub for printing and publishing.
3. Al-Taie, Abdul-Hussain Hamza Mutlaq, 1990. Diagnosis of strains of Fusarium oxysporum F.sp. Lycopricis (sacc) Snyder and.
4. Hansen and the rooting of tomato varieties resistant to wilt. Adel Abdul-Ghani Lutf 1999. Integrated resistance in tomato-tailed patients caused by Fusarium oxysporum F. sp. Lycopricis (sacc) Snyder and Hansen
5. Al Hiti, Iyad Abdul Wahid, Muhammad Amer Fayyad, and Ali Salem Al-Ghalibi 1996 Application of Psendomonas florescens pollination technique on rice plants and its effect on bacterial production capacity.
6. Ancold, S. T . 1980. Biology of fungi (translated by Sarhan, Abd Al-Radi Taha, Muhammad Sharif
7. Jassim Naji Salem 18. Biochemical and chemical resistance to Fusarium (graminearum) schwab that causes fusarium blight in wheat
8. Diwan, Majid Mutab, and Ali Hussain Al-Bahadli1985.
9. Diwan, Majid Mutaib, 1994. Numerical density of bacteria associated with tomato roots and their relationship to tails disease.
10. Robert, Daniel and Karl Butheroid 1987. Basics of plant diseases (translated by Ibrahim Jalal al-Din and Kamal Jalal Muhammad)
11. Awwad and Kazem Mashhout 1986. Principles of soil chemistry
12. Ma, M., Taylor, P. W., Chen, D., Vaghefi, N., & He, J.Z. (2023). Major soilborne pathogens of field processing tomatoes and management strategies. *Microorganisms*, *11*(2), 263.
13. Baker, R., y eland and B.sneh 1986. physical biological and host factors on iron sidrophorese and plant disease. plenum press. Newyork.p.77-84.
14. Cook, J.1993. Making greater use of interoduced Microorganism for biological control of plant pathogens. phytopathology. 31: 53-80.
15. Colle, j.g Fraser, A.G Marmion B.p., simmons, A1996. Mackic & MaCartney Practical Medical Microbiology, Pearson profacssional limited 14 ed
16. Dewan, M.M. and K. Sivasithamparam 1989. Occurrence of special of Aspergellus and Pencillium in root rot caused by Gammannomyces gramees var trici. Duij
17. Duijff. B.J 1994, Suppression of Fusarum Wilt by Psudomonas Spp.
18. Bowen, G.D. and A.D-rovira 1976 Microbial Colonization of plant root.
19. Broant bent, P.F, K. Brake and Y. Water Worth 1971. Bacteria and Actenomycetes Antagonestic to fungal root pathogens in Australian Soials.
20. Campell. J.W. , Coss D.D. and peteya, as Dj.1987. Clark, F.E. 1965. Agar - plats Method for Total microbial Black 1965. .
21. Keel, Cs. M Maurhofer, C. Voisard, J. Lavill, U. Burger, p. wirthur, D. Hass and Gefago, 1992
22. Koller, WCR Alan and E. Kalattukudy 1982. Protection of Pisum sateyum from fusarum solani.

23. Leben, S. D., JA Wadi and G, D eason. Keel, c.sm Mauirof, C. Voisad, J. laville, U.
24. Koller. W.C. R Allan and p. E. Kalattukudy 1982.
25. Leben S, D, J. A Wadi and G.D. Easton. 1987.
26. Govan, J.RW. 1997. Psudomonas, of non - fermentaction In: media Microbiology a guide to microbial infaction.
27. Robert, R and P. Vidyasekaran 1996. Development of fermentation of Pseudomonas fluorescens.
28. Pérez-Rodriguez, M. M., Pontin, M., Lipinski, V., Bottini, R., Piccoli, P., & Cohen, A. C. (2020). Pseudomonas fluorescens and Azospirillum brasilense increase yield and fruit quality of tomato under field conditions. *Journal of Soil Science and Plant Nutrition*, *20*, 1614-1624.