Illustration Of Polyfunctional Properties of Insecticidal Strains Of Bacillus Thuringiensis

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Abstract: In this study, several economically efficient nutrient media based on local raw materials were selected. Phytohormone synthesis, biomass production, insecticidal activity against the Colorado beetle and effects on plant growth and development in vegetative experiments of Bacillus thuringiensis strains grown in these nutrients were studied. Bacterial strains of Bacillus thuringiensis grown on molasses medium showed the highest insecticidal activity, entomocidal activity was 31.4% on 7 days and 67.4% on 14 days. The average entomocidal activity of B. thuringiensis bacterial strains grown on nonfat dry milk-based medium was 34.5% against the Colorado beetle after 7 days of incubation, and 67.7% by day 14. According to the results of the experiment, it is possible to recommend nutrient media based on molasses and dry milk for the production of biopreparations based on Bacillus thuringiensis bacterial strains.

Keywords: Colorado Beetle, Bacillus Thuringiensis, Entomocidal Activity, D-Endotoxin, Food Environment, L. Decemlineata.

1. INTRODUCTION

Increasing soil fertility and improving the natural condition of the land is one of the main tasks facing the agriculture in our Republic. In the implementation of these works, the use of monocultures and complexes of rhizosphere microorganisms, which effectively affect the accumulation of plant nutrients in the soil and their growth, has a special place.

The most popular biological insect pest control agent is *Bacillus thuringiensis* (Bt), a gram-positive spore-forming bacteria. It is used to manage insects that are important to agriculture, forestry, medicine, and veterinary care. The creation of massive, proteinaceous crystalline inclusions during sporulation is a crucial characteristic of the bacterium. Many strains of *Bt* have insecticidal properties, and these crystals contain one or more proteins (Cry and Cyt proteins, having molecular weights of 60–130 and 20 kD, respectively). Crystalline poisons are digested by insect larva and broken down by midgut alkaline proteases before being transformed to the active form by gut proteases [1],[2]. In order to form transmembrane leakage pores that result in cell swelling and lysis as well as the insect's eventual death, the active toxin first attaches to insect-specific receptors that are exposed on the surface of the plasma membrane of midgut epithelial cells. Due to the high commercial importance of *Bt* endotoxins and their high efficacy against pest larvae, strain selection and a variety of cultural conditions have been extensively researched to increase the *production* of *Bt* endotoxins [3],[4]. *Bt* is produced commercially on media made of complicated carbon and nitrogen sources.

The medium used to produce *Bt* biomass include energy-producing carbohydrates and nitrogenous sources that are simple for bacteria to consume. In addition to a wide range of inorganic sources that serve physiologically as protein, nucleic acid, and co-enzyme constituents as well as cofactors of several enzyme processes and bacterial cytochromes, *Bt* needs these sources for optimum growth [5],[6]. However, the cost of the media ingredients prohibits their use in the mass production of *Bt*. Therefore, it is necessary to substitute less expensive agricultural

products or byproducts from the agroindustrial sector. Since they will affect the end product's cost-benefit analysis, the price of the components used in fermentation is very crucial.

Endophytic and free-living soil bacteria that can either directly or indirectly promote plant development are known as plant growth-promoting bacteria. Numerous strategies by which bacteria stop phytopathogenic microorganisms from impairing plant growth and development include indirect encouragement of plant growth. Direct stimulation may include providing plants with fixed nitrogen, iron that has been sequestered by bacterial siderophores, soluble phosphate and other nutrients, and the ability to produce suitable amounts of the plant hormones such as indole-3- acetic acid (IAA), gibberellic acid and cytokinins and to lower the levels of the plant ethylene hormone mediating 1-aminocyclo- propane-1-carboxylate (ACC) deaminase activity [7],[8].

R. Felipe et al. used *Azotobacter chroococcum* AC1 and AC10 strains to study the properties that improve seed germination and cotton growth. They found that microbial inoculation had a greater effect on plant biomass ($p < 0.05$). The use of *Azotobacter chroococcum* strains together with natural fertilizers allows to reduce nitrogen mineral fertilizer doses up to 50% in cotton growth [9]. However, the multifunctional activity of *Bt* has not been fully studied.

2. STUDY PROBLEM AND QUESTIONS

The problem of the study is Phytohormone synthesis, biomass production, insecticidal activity against the Colorado beetle and effects on plant growth and development in vegetative experiments of *Bacillus thuringiensis* strains grown in these nutrients.

2.1. Objectives of the Study

In our experiment, selected cotton seeds were inoculated with *Bt* bacterial strains in special containers in the open field. The aim of this study was to investigate the insecticidal activity of local strains of the entomopathogenic bacteria *Bt* against the Colorado potato beetle, synthesis of phytohormones and plant growth and development by growing local strains of entomopathogenic bacteria *Bt* in different nutrient media based on inexpensive local raw materials. In this study, 8 strains representing several subspecies (ssp.) and wild isolates were used to investigate *Bt* potential as a biostimulator and biofertilizer bacterium that could boost plant growth.

3. METHOD AND PROCEDURES

3.1 Research Object

Local bacteria *Bacillus thuringiensis* Bt1, Bt18fo, Bt26, Bt31, Bt81, Bt82, Bt91 and Bt94 and 2-3-year-old Colorado beetle larvae (L. decemlineata).

Composition of the feed medium (%):

1. Standard nutrient medium (PB) (%): Peptone-1.0; glucose- 0.6; NaCl-0.5; K2NR04-0.05; MgSO4-0.02, (pH-7.0).

2. Nutrient medium based on molasses (%): molasses -0.5, K2NR04-0.05; MgSO4-0.02; NaCl-0.5 g; pN- 7.0-7.2.

3. Nutrient medium based on potato extract (%): extract - 20, glucose - 0.5; NaCl-0.25; rN- 6.8-7.0.

4. Nutrient medium based on skimmed dry milk: milk-1.0, sucrose-0.5; NaCl-0.5; K2NR04-0.05; MgSO4-0.02; (pH-7.0).

Cultures were grown at 29-31°C on a shaker at 150 rpm.

3.2 Entomicidal Activity

Insecticidal activity of local strains of *Bt* V. Guli et al. determined by the method [14]. Alfalfa leaves treated with 70% ethanol were used as food for larvae. 4 g of alfalfa leaves were placed in a 0.5 l container and thoroughly mixed with 0.8 ml of bacterial suspension with a titer of 2x108 spores and chrys./ml, and 10 test insects starved for 16-20 hours were subjected to 3 repeated experiments [15].

As pre-cultures (50 l each) were put into sterile culture media, a little amount of Bt was inoculated individually in 2 ml of each nutrient medium and allowed to develop for 12 h at 37°C. The cultures were allowed to develop in an orbital shaker at 37°C with continuous agitation (120 rev/min). At 6-hour intervals from 0 to 72 hours, culture samples (2 ml) were taken from each culture medium. A digital pH meter and UV-VIS spectrophotometer were used to measure the pH and turbidity of the culture. Additionally, spore-crystal combinations were looked for microscopically [12],[13],[18].

3.3 Planting and Setting Up a Growing Environment

A 1% sodium hypochlorite solution is used to surface sterilize healthy seeds of the same size for ten minutes. It is then washed in distilled water that has been sterilized. The seeds are allowed to develop seeds in simple water. In plastic containers with a diameter of 17 cm and a depth of 10 cm, germinated seeds are planted in the soil together with a suspension of active cultures that has been cleaned with 5 grams of alcohol. Below are some examples:

T1. Control

T2. Bt1+ seed

T3. Bt18fo + seed

T4. Bt26+ seed

T5. Bt31+ seed

T6. Bt81+ seed

T7. Bt82+ seed

T8. Bt91+ seed

T9. Bt94+ seed

Three duplicates of each sample were created. Plant growth requires that the soil relative humidity be between 53% and 57%, the storage temperature be between 27° and 21°C day and night, the light intensity be between 100 and 200 mol m-2 s-1, and the atmospheric CO_2 level be between 300 and 410 mol mol-1. Every other day, deionized distilled water was used to water all potted plants.

3.4 Evaluation of Plant Growth Markers

Each pot's wheat plants were harvested after 30 days. With distilled water, dirt particles were removed from plant roots. Utilizing an electronic analytical balance (EP214C 224S-CW, Switzerland), their stem and root length as well as wet biomass were then measured. After six days of drying at 70°C in a drying oven, root and stem dry weight samples were weighed to measure their mass [10],[11].

4. STUDY RESULTS AND DISCUSSION

One of the determinants of bacterial growth in nutrient media is the availability of carbon and nitrogen (C/N). In this study, the effect of the composition of different nutrient media on the entomocidal activity of local strains of *B. thuringiensis* bacteria was investigated. A standard medium based on peptone is undoubtedly universal for the cultivation of most microorganisms. It is known that peptone contains many amino acids that have a positive effect on the growth and development of bacteria, as well as amino acids that allow the production of cry toxins.

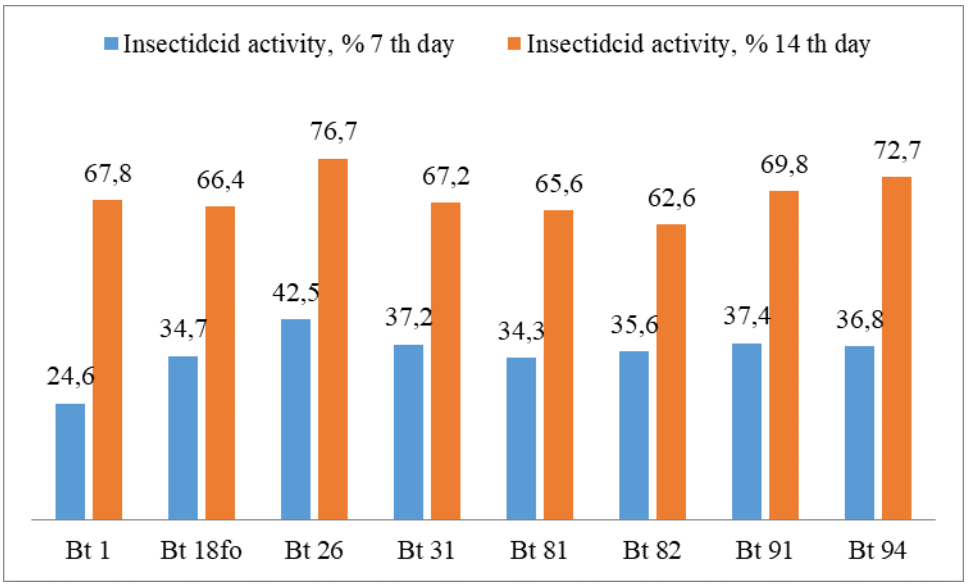

Figure 1. Insecticidal activity of *Bt* bacterial strains against Colorado potato beetle grown on pepton nutrient medium

According to Figure 1, bacterial strains Bt26, Bt91, and Bt94 grown in peptone medium had 76.7%, 69.8%, and 72.7% entomocidal activity against L. decemlineata larvae, respectively, over 14 days of exposure compared to other strains. The strains Bt1, Bt18fo, Bt31, Bt81 and Bt82 showed the lowest insecticidal activity, 67.8%, 66.4%, 67.2%, 65.6% and 62.6%, respectively. The average insecticidal activity of the bacterial strain *B. thuringiensis* grown for 7 days in peptone medium was 37.05%, and after 14 days it was 79.2%.

a

b

c

d

e

f

Figure 2. a – treatment of the 2nd and 3rd generations of the Colorado beetle with B. thuringiensis 18 fo strain; b – dead insects; c – treatment of the 2nd and 3rd generations of the Colorado beetle with B. thuringiensis strain 26; d – dead insects. e – the pupa after treatment of the 2nd and 3rd generations of the Colorado beetle with B. thuringiensis strain 26; f - the view of the colorado beetle emerging from the pupa, the external morphology of having undergone mutation and alteration into a damaged state.

Thus, under the influence of *Bt* strains of bacteria, it was observed that some larvae emergence to the pupa without dying, and the beetles that emerged from the pupa in the next generation changed their external morphology and became deformed (Figure 2). This means that although the toxins of the bacteria do not affect some Colorado beetles in the first generation, they can affect the genome structure of the insect, mutate it, and cause disability in the insects that come out of the pupa. It is important to note that the mutated insects were not observed to lay eggs and produce offspring.

Synthesis of β-exotoxin in *Bt* strains may be due to increased insecticidal activity or production of crystalline d-endotoxin, which has a specific effect on hard-winged (Coleoptera) pest insects. Strains with high activity are considered an important source for preparing bioinsecticidal preparations against the Colorado beetle, a pest in the agricultural field.

The use of peptone-based media for industrial production of *Bt* bacteria is expensive. Preparation of nutrient medium for the production of bacterial preparations on the basis of cheap, available wastes of industrial enterprises causes the cost of biopreparations to be several times cheaper. Potato extract-based nutrient medium is one of the inexpensive and nutritious nutrients for growing bacteria of the *Bt* group [16]. It is known that potatoes contain the following (%): protein - 8.35%, carbohydrates - 81.21%, fats - 0.06%, ascorbic acid - 0.0836%, thiamin - 0.001%, riboflavin - 0.00011%, niacin - 0.0061, water - 6.51% [17]. Group *Bt* bacteria grow very well and produce high biomass in potato medium for 2 days. The growth of bacteria differs from that in other nutrient media. After about 32

hours of cultivation on potato medium, formation of spores and crystals begins, followed by 95% spore formation after 55 hours. The formation of spores in a short time is probably related to the lack of nitrogenous substances compared to carbohydrates in the potato nutrient medium. According to the experimental data, it was found that *Bt* bacterial strains grown in the potato nutrient medium have different levels of insecticidal activity against the Colorado beetle (Leptinotarsa decemlineata).

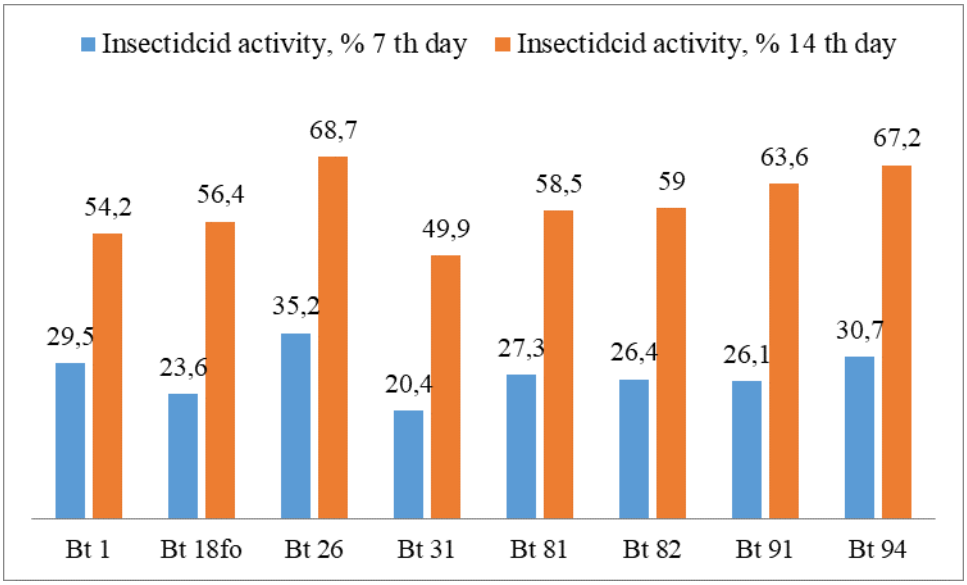

Figure 3. Insecticidal activity of *Bt* bacterial strains against Colorado potato beetle grown on potato nutrient medium

According to Figure 3, the insecticidal activity of the studied cultures ranged from 49.9% (Bt31) to 67.2% (Bt94). The average insecticidal activity of bacterial strains grown in potato medium for 7 days was 27.4%, and for 14 days it was 59.7%. In our next study, a study was conducted on the nutritional value of the sugar production residue - molasses medium. Analysis of the chemical composition of molasses showed that it contained 80% dry matter and 20% water. It is known from the literature that the content of these dry substances is 60.0% sucrose, 16.7% nitrogen-free organic matter, 14.8% nitrogenous matter, and 8.5% mineral matter [18]. In addition, molasses has been found to contain about 17 sets of amino acids.

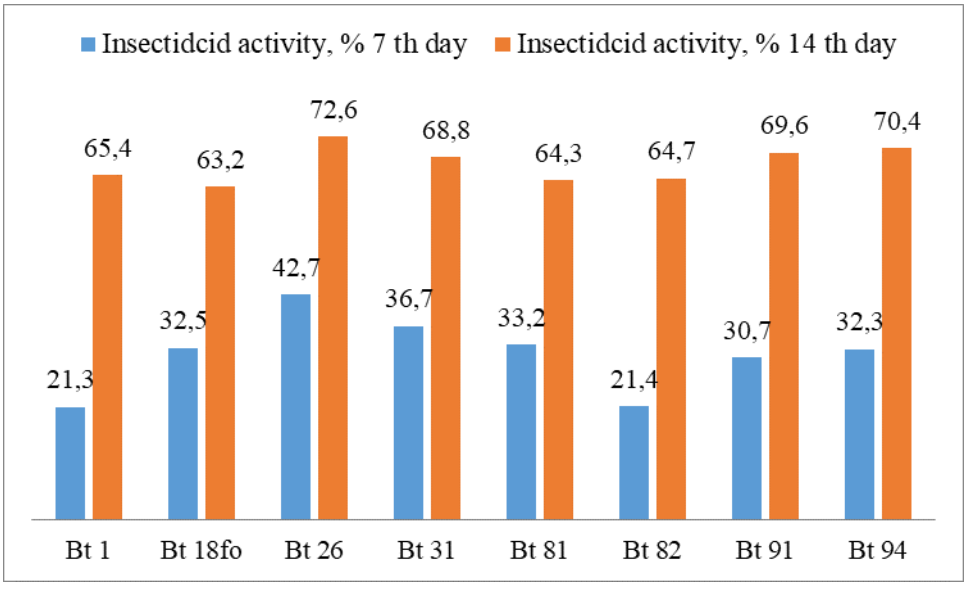

Figure 4. Insecticidal activity of *Bt* strains grown on molasses medium against the Colorado potato beetle

The entomocidal activity of *Bt* bacterial strains grown on molasses medium showed that bacterial strains *Bt91* and *Bt94* showed the highest insecticidal activity of 69.6% and 70.4%, respectively (Figure 4). The average entomocidal activity of all studied bacteria was 31.4% at 7 days of bacterial growth and 67.4% at 14 days. It should be noted that strain *Bt94* when grown on molasses medium had almost similar insecticidal activity against the colorado beetle to strains grown on peptone medium, but was found to be slightly higher than the insecticidal activity of strains grown on potato medium was studied. The composition of non-fat dry milk is as follows: 35% - proteins, milk sugar - 52%, fats - 1% and 6% - minerals [19],[20]. The growth of *Bt* strains on a medium based on skimmed milk began to form spores from 44 hours, and the formation of spores - crystals continued until 70 hours (95%). In particular, the bacterial strains *Bt26, Bt91* and *Bt94* grown in milk nutrient medium showed the highest entomopathogenic activity against the Colorado beetle on the 14th day of exposure and were analyzed to be 74.7%, 68.6% and 72.3%, respectively (Figure 5). Bacterial strains *Bt1, Bt18fo Bt31, Bt81* and *Bt82* showed slightly higher insecticidal activity compared to potato nutrient medium when cultured in milk nutrient medium, and almost equal entomocidal activity was noted in relation to peptone and molasses nutrient medium. For 7 days, the average entomopathogenic activity of the strains incubated against the Colorado beetle was 34.5%, and by the 14th day, this indicator increased to 67.7%.

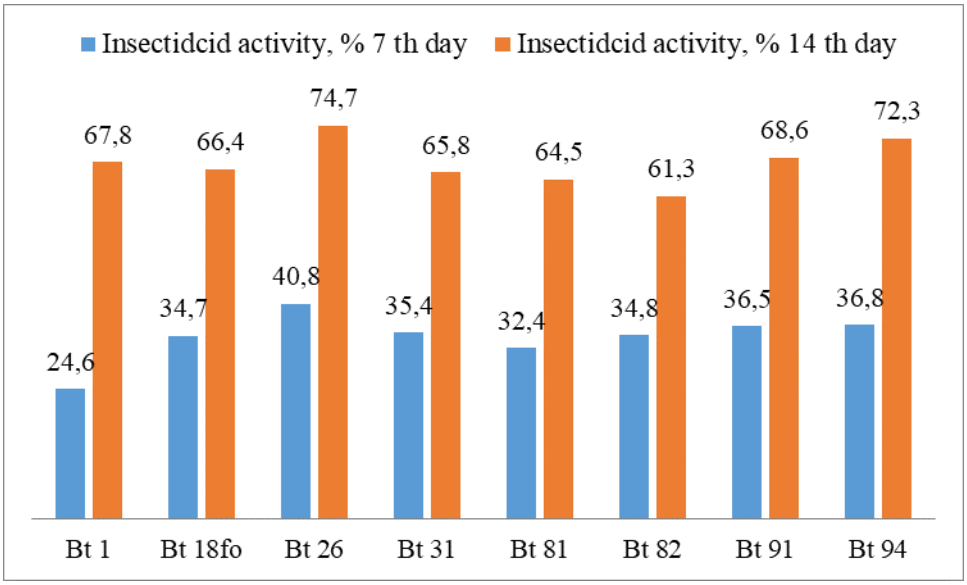

Figure 5. Insecticidal activity of *Bt* strains grown on skim milk-based medium against the Colorado potato beetle

According to the results of the research, the insecticidal activity of bacterial strains grown in molasses and dry milk based media is almost equal compared to the peptone media used as a sample, and these media can be recommended for the production of biopreparations. The lack of nitrogen compounds in the potato nutrient medium may be related to the decrease in the synthesis of cry-toxins. This, in turn, can lead to a decrease in insecticidal activity. In addition, production consistency can be adversely affected due to the inability to find potato product residues all the time.

The data above provide information on the range of effects of the strains used on potato growth, while below are comparisons of stem and root wet and dry biomass as a function of the same strains versus the control option (Table 1).

Table 1: Effect of *Bt* strains grown in potato extract on plant biomass

№	Name of strains used, average	Wet stem biomass (g), average	Wet root biomass (g), average
1	Control	0,83 ±0,32	0,092 ±0,18
2	Bt1	2,82 ±0,21	0,24 ±0,17
3	Bt18fo	2,59 ±0,25	0,308 ±0,13
4	Bt26	2,74 ±0,34	0,33 ±0,17
5	Bt31	2,38 ±0,16	0,23 ±0,43
6	Bt81	2,31 ±0,39	0,265 ±0,28
7	Bt82	2,09 ±0,13	0,234 ±0,33
8	Bt91	2,33 ±0,33	0,313 ±0,40
9	Bt94	2,03 ±0,15	0,213 ±0,14

In the control option, the average wet biomass of the cotton stem was 0.83 grams, and the root was 0.092 grams. As a result of the activity of the *Bt 1* strain, it was found that the wet biomass of the cotton stem was 3.4 times higher than the control, and the root was 2.67 times higher than the control.

According to the results, under the influence of strain *Bt 94*, which recorded the lowest result, the root and stem of cotton produced 2.3 and 2.4 times higher biomass compared to the root and stem of the plant in the control version, respectively, *Bt* strains not only have insecticidal activity, but also shows that stimulation activity is also high of these strains.

Table 2: Effect of *Bt* strains grown in skim milk-based medium on plant biomass

№	Name of strains used, average	Dried stem biomass (g), average	Dried root biomass (g), average
1	Control	0,21 ±0,26	0,072 ±0,43
2	Bt1	0,61 ±0,46	0,191 ±0,33
3	Bt18fo	0,56 ±0,32	0,087 ±0,12
4	Bt26	0,661 ±0,13	0,210 ±0,23
5	Bt31	0,607 ±0,45	0,186 ±0,17
6	Bt81	0,52 ±0,33	0,095 ±0,46
7	Bt82	0,41 ±0,32	0,053 ±0,16
8	Bt91	0,513 ±0,22	0,073 ±0,16
9	Bt94	0,41 ±0,32	0,066 ±0,46

In the control variant, the average dry biomass of cotton stems and roots was 0.21 and 0.072 grams. The difference between the average biomasses of shoots and roots of cottons of the control and treated variants with *Bt* strains took the following values, respectively: *Bt26* strain 3,14 and 2,91 times; *Bt 1* accumulated -2,904 and 2,65 times heavy biomass.

CONCLUSION

It should be noted that among the studied bacterial strains of *Bt*, local strains *Bt26, Bt 91* and *Bt 94* showed high insecticidal activity when grown in all used nutrient media and serve as a basis for the development of biopreparations against the Colorado beetle. Due to the high insecticidal activity of bacterial suspensions grown in skim milk-based medim, this medim can be used to grow bacteria on an industrial scale in large quantities.

Bt26 and *Bt1*strains were noted as bacteria that stimulate and increase the production of biomass in plants. Suspensions of these strains grown in potato extract and skim milk-based medim showed higher results on plant growth and development compared to other low-cost nutrient medim.

As a final conclusion, the above-mentioned medium- and high-acting biostimulant bacteria can be used to increase the resistance of agricultural crops to diseases and to obtain early and high yields.

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