USE OF BACTERIAL EXOMETABOLITES FOR SUPPRESSION OF FUSARIUM WILT AND PHYTO STIMULATION OF EGGPLANT (*Solanum melongena* L.)

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Abstract. Exometabolites of strains *B. velezensis* CNMN-BB-16, *M. yunnanensis* CNMN-BM-19 and *Actinomadura* sp. 36, were tested in experiments of antagonism against *F. oxysporum* CNMN-FF-06 and *F. solani* CNMN-FF-07; and determination of plant growth promoting activity on *S. melongena* L. (Classic F1). According to the results, almost all experimental variants totally suppressed developing and proliferation of phytopathogenic strains *in vitro*. Eggplant seedlings had shown almost no symptoms of Fusarium wilt. In case of phytostimulation, the length of stems was by 0.83-29.58% more than in control. Significant increases in length were recorded by using solutions of the exometabolites of *B. velezensis* CNMN-BB-16 in concentration of 3.0% and *M. yunnanensis* CNMN-BM-19 in concentration of 2.0%. The dry weight of aerial part, increased further and ranged by 31.25-119.42% more. For root dry weight, the data had increased significantly, by 9.09-154.95%. The number of leaves on plants is significantly greater than in the control, and the plants are better developed. Obtained data had demonstrated efficacy of exometabolites both as *Fusarium* antagonists and as phytostimulators of *Solanum melongena* L.

Keywords: actinobacteria; bacteria; eggplant; Fusarium wilt; plant growth promoting.

INTRODUCTION

Nowadays food security is challenging with numerous factors like: food prices, population growth, pressure on food production, water scarcity, biofuel production, soil degradation, and various diseases of agricultural crops [18, 25]. The latter factor is given much attention, as it represents a more serious danger in terms of the speed of spread and direct damage to the plant organism.

Among plant diseases, it is worth noting fungal diseases, since fungi have a wide array of enzymes for lysis, intoxication and death of plants [8]. It is worth noting representatives of the genus *Fusarium*, which is dangerous due to the synthesis of mycotoxins that penetrate the tissues of plants and fruits, which subsequently pose a risk to the end consumer [8, 47]. In the field conditions, *Fusarium* species produce mycotoxins before harvest. *Fusarium* species are highly adaptable to climate conditions, thereby infecting crops all over the world [7]. Thus, this genus can be considered a serious problem for agriculture.

A healthy diet, approved by the Food and Agriculture Organization (FAO) listing the most important qualities as: adequate, safety, diverse, and balanced in necessary nutrients. From which it follows that such an agricultural crop as eggplant (*Solanum melongena* L.) should be included in the human diet [40]. In recent years, the cultivation of this crop has declined due to various problems, including diseases caused by various pathogens such as fungi [21, 31].

Fusarium spp. cause next damages for this crop: leaf spot, fruit spot, root spot, root rot which lead to plant wilting. Fungus uses a variety of strategies to get into plant organism by spores. The degree of disease development depends on such factors like soil quality, pH, availability of chemical elements, chemical action, availability of physical wounds or through stomata [43]. The self-defense of crop is influenced by stage development of plant and defense signaling mechanisms which were activated as immune response to intruder [24]. To ensure plant viability and avoid crop losses, methods to control the disease cycle such as biological control, chemical control, use of nanoparticles, and application of organic fertilizers are being studied [9, 44].

For the plant protection by using biological methods is the most preferable, as it is a natural process with minimal consequences for plants and the final consumer. Therefore, scientists are paying their attention to saprophytic bacteria and their exometabolites for use as biocontrol of *Fusarium* species, as well as for promoting plant phytostimulation and strengthening the immune system [39].

According to numerous literature sources, various strains of bacteria have been successfully used, which have had a positive effect on the growth and development of crops. For example, *Bacillus* sp. SV101 and *Bacillus tequilensis* SV104 isolated from the *Solanum elaeagnifolium* had shown significant results against Fusarium wilt [2]. According to other data, exometabolites of *Micrococcus luteus* were used in the combating of *Fusarium oxysporum* f. sp. *ciceris*, which causes Fusarium wilt of chickpea. The following characteristics were found in this strain: synthesis of indole-3-acetic acid (IAA), ammonia production, potassium and phosphate solubilization, glucanase and chitinase production, along with antagonistic potential against the mentioned pathogen. *M. luteus* showed significantly positive effect on vegetative parameters of plants and inhibitory effect against pathogens [36].

Mycelial bacteria (actinobacteria) are also used for these purposes. They have similar properties for successful biocontrol and phytostimulation of agricultural crops [13].

According to the research, the strain *Actinomadura* ACD1 had shown high activity against mycotoxigenic and phytopathogenic fungi, including strains of the genera *Aspergillus* and *Fusarium*, as well as other pathogenic microorganisms. The kinetics of

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antimicrobial activity was studied on ISP-2, Bennett and TSB media, which is very important for synthesize of certain substances. The highest antimicrobial activity was obtained using a butanol extract from the ISP-2 medium after seven days of culture fermentation. The minimum inhibitory concentrations of this antibiotic against pathogenic microorganisms were also determined [22].

Thus, use of saprophytic microorganisms that stimulate plant growth and protect them from various phytopathogenic fungi has been proven. In the present study, strains of the genera *Bacillus*, *Micrococcus* and *Actinomadura* were investigated on their growth-promoting potential on *Solanum melongena* L. and testing for their ability to control soil phytopathogens *Fusarium solani* and *Fusarium oxysporum*, which causes wilt and rot of eggplant crop was assessed.

MATERIALS AND METHODS

The *Bacillus velezensis* CNMN-BB-16 (Bv16), *Micrococcus yunnanensis* CNMN-BM-19 (My19) and *Actinomadura* sp. 36 (A36) are maintained in National Collection of Non-pathogenic Microorganisms (NCNM) at the Institute of Microbiology and Biotechnology of the Technical University of Moldova. The strains were tested for several biochemical properties involved in suppression of growth and development of *Fusarium* spp., as well as phytostimulation of eggplant crop.

The eggplant seeds used in this investigation are *Solanum melongena* L. – cultivar Classic F1. Seeds were purchased from AgroArt (S.R.L. Leonis Prod). The variety is included in the Catalog of Plant Varieties for 2024 by the State Commission for Testing Plant Varieties of the Ministry of Agriculture and Food Industry of Republic of Moldova.

Cultivation of strains

Bacteria Bv16 and My19 were incubated on nutrient agar at 35±1°C for 24 h (Chemsolute). For obtaining bacterial suspension, *inoculum* was transferred in nutrient broth: meat peptone – 5,0 g/L; NaCl – 5,0 g/L; beef extract – 1,5 g/L; yeast extract – 1,5 g/L. Cultivation at 30±1°C for 48 h, on orbital shaker at 180 rpm.

Cultivation of A36 for performing bacterial suspension involved next stages: **1.** incubation of the actinobacteria strain on Gause agar medium (soluble starch – 20.0 g/L, KNO_3 – 1.0 g/L, K_2HPO_4 – 0.5 g/L, $MgSO_4$ – 0.5 g/L, NaCl – 0.5 g/L, $FeSO_4$ – 0.01 g/L, agar – 20.0 g/L, pH=7.2–7.4), for 14 days at 28°C; **2.** obtaining *inoculum* on Dulaney liquid medium (glucose – 20.0 g/L, $(NH_4)_2HPO_4$ – 7.5 g/L, NaCl – 5.0 g/L, K_2HPO_4 – 2.0 g/L, $MgSO_4 \cdot 7H_2O$ – 1.0 g/L, $CaCl_2$ – 0.4 g/L, $ZnSO_4 \cdot 7H_2O$ – 0.01 g/L, $FeSO_4 \cdot 7H_2O$ – 0.01 g/L, pH=7.0) for 3 days at 28°C, on orbital shaker at 180 rpm; **3.** cultivation of *inoculum* to log phase on starch nitrate-casein broth medium (SCNB) (starch medium – 10.0 g/L, casein – 0.3 g/L, KNO_3 – 2.0 g/L, K_2HPO_4 – 2.0 g/L, $MgSO_4 \cdot 7H_2O$ – 0.5 g/L, $FeSO_4 \cdot 7H_2O$ – 0.01 g/L, $CaCO_3$ – 0.02 g/L, NaCl – 2.0 g/L, soy flour – 30.0 g/L, pH=7.0) for 5 days at 28°C, on orbital shaker at 180 rpm.

The bacterial suspension was centrifuged (8000 rpm, 10 min.) for the separation of supernatant from biomass. The obtained samples of supernatant with content of exometabolites were used in antifungal and phytostimulation trials.

Biochemical analysis of strains

The next measurements of the experimental samples were performed after centrifugation and separation of the supernatant from the biomass.

Catalase activity (CAT) was measured by the spectrophotometric method based on the ability of hydrogen peroxide to interact with ammonium molybdate tetrahydrate salt to form a stable-colored complex [15].

Quantitative determination of IAA was performed by the spectrophotometric method proposed by Gordon, 1951. For this method was used Salkowski reagent (50 mL of 35% $HClO_4$ + 1 mL of 0.5 M $FeCl_3$). For obtaining of standard curve were used next concentrations of IAA: 0, 5, 10, 20, 40, 60, 80, 100 µg/mL. Each concentration and supernatant samples were mixed in ratio of 1 mL solution : 2 mL reagent Salkowski. The mixture was incubated in the dark at room temperature for 20 min. During this time, the color change to pink-reddish occurs. The absorbance measurement was performed at 530 nm [14].

Siderophore synthesized by bacteria were determined by Chrome Azurol S (CAS) assay method, being a common qualitative and quantitative approach. The CAS assay detects siderophores by their ability to chelate iron from a CAS-iron complex, resulting in a color change.

The CAS assay can be used to quantify siderophore production by measuring the decrease in absorbance of the CAS-iron complex at 630 nm, according to the following equation:

$$Siderophore\ units\ (\%\ SU) = [(Ar - As)/Ar] * 100$$

where: Ar – absorbance of uninoculated CAS solution; As – absorbance of the sample (bacterial supernatant) after mixing with CAS solution [45].

The protein content was determined spectrophotometrically according to the Lowry method, with Folin and Ciocalteu′s phenol reagent (Sigma Aldrich, USA) and crystalline albumin from bovine serum as the standard [28].

The intracellular lipids were extracted from biomass by Folch method modified in the laboratory. Fractional composition of the total lipids was determined by thin layer chromatography with “Sorbfil” plates in the solvent mixture hexane - diethyl ether - glacial acetic acid system (73:25:5), the quantity of each lipid fraction being recorded by densitometry method [6].

Antifungal activity

To assess biocidal activity of selected microorganisms, the antifungal activity was tested

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against *Fusarium oxysporum* CNMN-FF-06 (Fo06) and *Fusarium solani* CNMN-FF-07 (Fs07) stored at the same institution; considering that these pathogens have cause severe diseases and yield losses to numerous crops in Republic of Moldova.

Fusarium wilt symptoms on eggplant crop were studied by using test tube water agar method [49].

The supernatant of the strains containing secondary metabolites was diluted in sterile distilled water at a concentration of 2.0% and 3.0%; for the treatment of the seeds according to the blotter method, they were immersed for 2 hours in the solution, then distributed on moistened filter paper and cotton wool, in Petri dishes, until the first signs of germination appeared [12].

In the cultivation tubes (150 x 16 mm), for the infected variants, suspensions of *F. oxysporum* CNMN-FF-06 and *F. solani* CNMN-FF-07 (volume 0.1 mL, 10^6 CFU/mL) were inoculated by pour method with 10 mL of Knop medium (agar concentration of 0.6 %, pH=7.0). The concentration of *Fusarium* spp. CFU/mL was determined by serial dilution, subcultured in Petri dish on wort agar. Three seeds were placed individually in each tube and incubated for 10 days. The cotton plug was removed after the seedling reached the edge of the tube and the condition and presence of Fusarium wilt symptoms were examined [19].

Each variant was performed in triplicate, at 26.5±2°C, with 12 hours of light followed by 12 hours of darkness.

Fusarium wilt symptoms were scored using a scale from 0 to 4, where: 0 (healthy seedling); 1 (<25% of the seedling surface infected); 2 (26-50% of the seedling surface infected); 3 (51-75% of the seedling surface infected); and 4 (>75% of the seedling surface infected) [42].

The disease severity index (DSI) was calculated according to McKinney's formula:

$$DSI\ (\%) = (\sum vn)/(NV) * 100$$

where: v – represents the numerical value of the disease index scale, n – is the number of seedlings assigned to the disease index scale, N – is the total number of seedlings and V – is the numerical value of the highest disease index scale. The level of resistance of each treatment to Fusarium wilt was determined according to its DSI; EE: extremely effective (DSI = 0%), HE: highly effective (DSI = 0.1 to 5%), E: effective (DSI = 5.1 to 25%), I: ineffective (DSI = 25.1 to 50%) and HI: highly ineffective (DSI = 50.1 to 100%) [17].

Phytostimulation of eggplant crop

Solanum melongena L. seeds were treated with bacterial strains by soaking in bacterial solution (concentrations of 2.0% and 3.0%) for 2 hours. The soaked seeds were placed equidistantly on filter paper in Petri dishes (9 seeds each) and incubated in the dark at 26.5±2°C. The seeds were transferred of 6 pieces per pot (volume 1 L – soil 500 g), 5 pots (total 30 plants). The plants were hydrated once every 2 days (14 hours of light / 10 hours of darkness), at 28.0°C. After 40 days, the plants were washed, the length of the stems and roots was measured. Plant organs (roots, stems with leaves) were weighed separately and dried at 65.0°C for 2 days (to determine dry weight/100 plants) [25].

Statistical analysis of data

Data are displayed as mean ±SEM. The statistical significance was evaluated using a one-way ANOVA with post hoc Tukey test. A $p \leq 0.05$ value was considered statistically significant. Data were analyzed using PAST 3.26 statistical software [16]. The entire experiment was performed in 3 replicates, 3 times.

RESULTS

Soil microorganisms play a key role in plant health and soil fertility. Some rhizosphere microbes are essential for nutrient cycling, soil enrichment, plant growth promoting, and biological control of plant pathogens [23].

Before the experiments on eggplant crop, the strains were analyzed biochemically. In the supernatant obtained from the cultivation of the strains (*B. velezensis* CNMN-BB-16, *M. yunnanensis* CNMN-BM-19, *Actinomadura* sp. 36) on liquid media, CAT activity and the synthesis of phytostimulatory compounds such as auxins IAA and siderophores were determined. In the biomass of the above-mentioned strains, the quantity of proteins and the presence of important lipid fractions were determined, which are precursors and serve as evidence of the presence of other exometabolites synthesized in the bacterial suspension (Table 1).

The unit mmol H_2O_2/min/mg protein measures the rate of hydrogen peroxide detoxification by catalase enzymes, which is crucial in host defense against fungi. A higher rate of this detoxification indicates that the host is effectively breaking down hydrogen peroxide, an oxidative stress molecule, thereby preventing excessive build-up that could lead to host tissue damage. In this context, a greater mmol H_2O_2/min/mg protein reflects stronger antioxidant enzyme activity and a more robust defense response against fungal invasion [26, 38]. As could be seen in table 1, the catalase activity of studied strains varied within values of 59.71-139.38 mmol H_2O_2/min/mg protein. The soaking of seeds in a catalase-containing exometabolite solution creating a protective environment during imbibition.

Table 1. Biochemical composition of bacterial metabolites

Strain	Supernatant				Biomass				
	CAT mmol H_2O_2/min/mg protein	IAA µg/mL	Siderophores % SU	Proteins mg/mL	Proteins % ADB	PL %/total lipids	MG and DG %/total lipids	TG %/total lipids	ST %/total lipids
Bv16	**139.3±1.1**	Negative	**45.6±0.8**	2.3±0.3	**28.2±1.8**	15.1±0.3	3.4±0.4	3.6±0.4*	4.6±0.4
My19	75.3±1.4	**36.5±1.7**	10.9±1.3	3.9±0.2	12.9±0.9	9.2±0.4*	5.4±0.4*	10.6±0.1	4.4±0.3
A36	59.7±3.0	Negative	6.7±0.7	**5.8±0.7**	24.7±2.2	**15.2±0.2**	**6.7±0.2***	**14.3±0.3**	**12.0±0.1**

Note: *p ≤ 0.05.

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Plant hormones regulate the plant physiology, shape and associated microbial environment. However, some microorganisms show symbiosis, and release some essential plant hormones contributing to a better growth and development. IAA are synthesized by diverse bacterial genera. In various plants, microorganisms present in the rhizosphere can synthesize IAA as the secondary metabolite. As an example of commercial IAA production, *Streptomyces griseoviridis* K61 and *Streptomyces lydicus* WYEC108 were used under trade name Mycostop [34]. Based on the results of IAA determination, only the *M. yunnanensis* CNMN-BM-19 strain showed the presence of the active substance at a concentration of 36.53 µg/mL.

Siderophores are important because they chelate iron and help alleviate iron deficiency in plants [11]. Those synthesized by microorganisms also protect plants from diseases by inducing systemic resistance and reduce availability of iron for plant pathogens [32]. The amount of siderophores synthesized by strains was very wide – 6.73-45.62%.

Proteins that are crucial for the production of beneficial extracellular metabolites with phytosimulation and antimicrobial activity include enzymes like protein kinases and protein phosphatases, which regulate signaling pathways involved in metabolite production. While specific genes and proteins directly responsible for all exometabolite production are not fully detailed in general literature, key proteins are involved in the synthesis and release of these compounds [35, 46, 48]. Their quantity in supernatant varied within 2.3-5.82 mg/mL and in absolute dry biomass (ADB) 12.97-28.21% in dependence of strain.

Lipids perform a variety of functions, regulating cellular processes in the plasma membrane and mediating signaling pathways. They also participate in complex interactions between plants and surrounding microorganisms, with which plants engage in various forms of symbiosis. As chemical signals, lipids facilitate the stages of rhizosphere interactions — from plant root to microorganism, from microorganism to microorganism, and from microorganism to plant root —and modulate plant defense responses upon exposure to or contact with beneficial or phytopathogenic microorganisms [29]. The main lipid fractions identified were mono- (MG), di- (DG), and tri- glycerides (TG), as well as phospholipids (PL) and sterols (ST). They are important for various metabolic processes during plant growth and defense.

Biological control of Fusarium wilt in various agricultural crops through the use of antagonistic bacteria has become widespread. In early studies, *B. velezensis* CNMN-BB-16, *M. yunnanensis* CNMN-BM-19 and *Actinomadura* sp. 36 strains were tested for their antagonistic ability to inhibit the growth and development of *Fusarium* strains using the disc-diffusion method [4, 5]. In the current study was demonstrated a significant suppression of Fusarium wilt *in vitro* using three bacterial isolates (supernatant in concentrations of 2.0% and 3.0%). Uninfected seeds (negative) and seeds infected with *Fusarium* spores (positive) served as controls (Table 2, Figure 1).

According to the results, one of the seeds in the negative control was contaminated with spontaneous microflora, resulting in a DSI of 2.78%, in comparison with the positive control 0.02-0.03% / %. The degree of contamination in the positive controls Fo06 and Fs07 was 100% and 83.3%, respectively. The images show that germination had begun in the contaminated samples, but with the development of phytopathogenic strains and the release of metabolites into the medium, this leads to growth stagnation and gradual tissue degradation (Figure 1 – a, b, c). In the experimental samples, it is evident that seeds treated with supernatant solutions showed a significant suppression of phytopathogen development in almost all experiments. With the exception of only one seed in the Bv16 3.0% variant, slight contamination could be occurred for individual reasons (Table 2, Figure 1 – d, e, f).

The next step involved assessment of phytostimulation effect of bacterial supernatant solutions on eggplant crop. After 40 days of growth, plants from the experimental groups were compared with the control sample. Stem length, root system length, and fresh and dry weights of these were measured separately (Table 3).

According to the results of stem length, the solution of exometabolites of strains in different concentrations showed diverse results in the experimental variants. Positive effects ranged by 0.83-29.58% more than the control. Significant increases in length were recorded by using solutions of the exometabolites Bv16 at a concentration of 3.0% and My19 at a concentration of 2.0%.

In contrast, in terms of root system length, almost all variants showed a decrease in root system length by 2.82-30.99%. Only variant A36 (2.0%) showed a slight increase in root system length of only by 1.4% in comparison with control. The availability of exo-

Table 2. The effect of bacterial exometabolites on *Fusarium* strains inhibition by test tube water agar

Variant of experiment	DSI Fo06, %	DSI, % / positive control 100 % ^{Fo06}	DSI Fs07, %	DSI, % / positive control 83.3% ^{Fs07}
Negative control	2.7	0.02	2.7	0.03
Bv16 2.0%	0.0	0.0	0.0	0.0
Bv16 3.0%	2.7*	0.02	0.0	0.0
My19 2.0%	0.0	0.0	0.0	0.0
My19 3.0%	0.0	0.0	0.0	0.0
A36 2.0%	0.0	0.0	0.0	0.0
A36 3.0%	0.0	0.0	0.0	0.0

Note: *$p \leq 0.05$.

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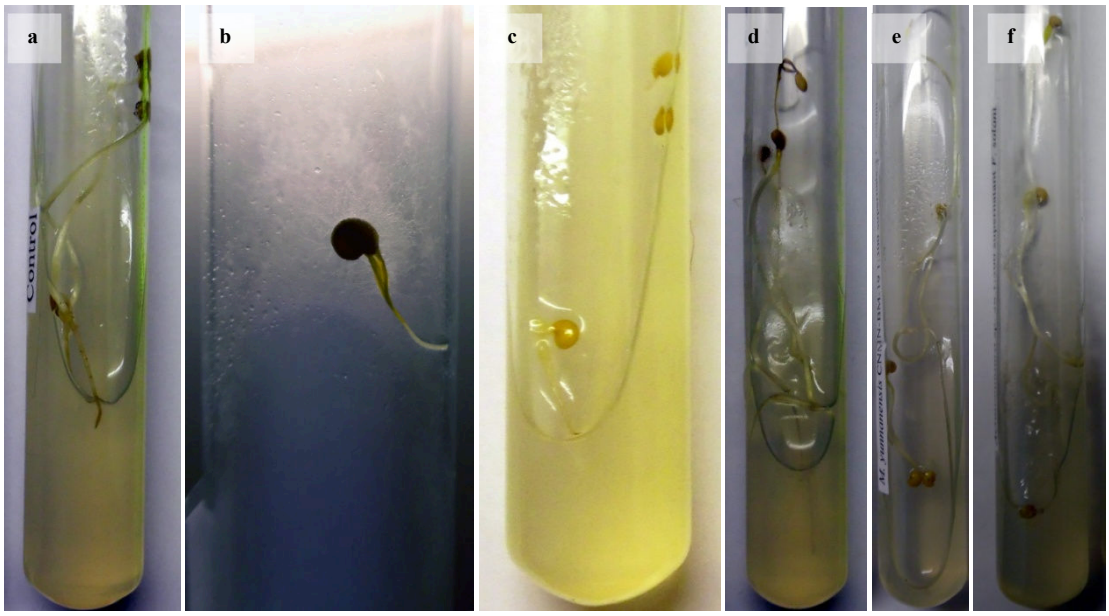

Figure 1. The effect of bacterial exometabolites on *Fusarium* strains inhibition: a) control; b) infected with Fo06; c) infected with Fs07; d) treated with Bv16 2.0%; e) treated with My19 3.0%; f) treated with A36 3.0%.

Table 3. The effect of bacterial exometabolites on growth promotion of eggplant (after 40 days growth)

Variant of experiment	Control	Bv16 2.0%	Bv16 3.0%	My19 2.0%	My19 3.0%	A36 2.0%	A36 3.0%
Stem length, cm	24.0±2.1	22.9±3.5	31.1±2.0**	30.8±2.4	24.2±2.6*	23.8±2.4	25.9±2.5
GPE, %	100	95.4	**129.5**	**128.3**	100.8	99.1	107.9
Root length, cm	7.1±1.0	5.1±1.2	4.9±0.8**	5.2±0.5**	6.1±1.0	7.2±0.9*	6.9±1.0
GPE, %	100	71.8	69.0	73.2	85.9	**101.4**	97.1
Stem fresh weight, g	219.4±7.3	209.4±8.6	387.1±12.4*	429.0±17.9*	238.0±4.2	213.3±11.4*	282.3±9.5
GPE, %	100	95.4	**176.4**	**195.5**	108.4	97.2	**128.6**
Stem dry weight, g	18.9±1.8	17.7±2.3*	37.0±3.1*	41.5±3.6**	30.0±3.0*	24.8±4.4	35.1±4.8*
GPE, %	100	93.8	**195.6**	**219.4**	**158.8**	**131.2**	**185.3**
Root fresh weight, g	25.5±3.7	27.5±4.1	19.5±1.4	26.6±2.5	39.7±4.3*	35.5±2.6	21.9±0.9
GPE, %	100	107.6	76.5	104.1	**155.4**	**139.0**	85.8
Root dry weight, g	2.2±0.3	2.4±0.4	2.9±0.1	4.0±0.1*	5.6±0.5**	5.0±0.7**	3.2±0.2
GPE, %	100	109.0	132.4	**182.8**	**254.9**	**225.2**	145.0

Note: *$p \leq 0.05$, **$p \leq 0.01$, GPE – growth promoting effect.

metabolites creates conditions in which the root system does not need to develop deep into the soil to find them, thereby contributing to a reduction in length.

According to data of green fresh weight of developed stems, an increase in weight by 8.49-95.52% was observed in comparison with the control sample. Significant values of over 25.0% were observed after bacterization with A-6 (3.0%), Bv16 (3.0%), and My19 (2.0%) exometabolite solutions. The dry weight of aerial part, with the exception of the Bv16 (2.0%) variant, increased further and ranged from 31.25% to 119.42% more. Even in the A36 (2.0%) variant, where fresh weight was 2.79% less than in the control, dry weight showed a 31.25% increase. Positive results for fresh root weight in comparison with the control ranged from 4.18% to 55.42% more than in the control. Significant results were observed with the use of My19 (3.0%) and A36 (2.0%) exometabolite solutions. For root dry weight, the data increased even more significantly. Positive results were observed in all experimental variants, exceeding the control sample by 9.09-154.95%. As with fresh weight, significant results were observed in experimental variants My19 (3.0%) and A36 (2.0%). Also noteworthy is the Bv16 variant (3.0%), where the fresh weight content was by 23.49% lower than in the control; however, in terms of dry weight, the experimental sample exceeded the control by 32.43%.

As could be seen in figure 2, by comparing plants from control variant a) with experimental samples b), c), and d); the last variants are more developed. While the root system is more developed in the control variant, the green aerial part is more developed in the experimental samples. Plants from the experimental samples have longer stems, and the stems themselves are wider and more stable in diameter.

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Figure 2. The effect of bacterial exometabolites on growth promotion of eggplant: a) Control; b) treated with Bv16 3.0%; c) treated with My19 2.0%; d) treated with A36 3.0%

The number of leaves is significantly greater than in the control, and they are more developed. It is also worth noting that the dry weight of both the green aerial part and the root system is significantly higher, confirming a higher metabolic rate of plants due to the use of phytostimulating exometabolites of bacterial origin.

DISCUSSION

Protection from phytopathogens and phytostimulation of plants using preparations of bacterial origin are gaining increasing popularity.

Present research demonstrated the effectiveness of bacteria and actinobacteria (*Bacillus velezensis* CNMN-BB-16, *Micrococcus yunnanensis* CNMN-BM-19, and *Actinomadura* sp. 36) against *F. oxysporum* CNMN-FF-06 and *F. solani* CNMN-FF-07. Pre-treatment of seeds showed normal germination and seedling development, and virtually no signs of phytopathogenic development were observed. According to the thematic articles, the inhibitory effect of diffusible and volatile secondary metabolites from *B. subtilis* against some plant fungi including *F. oxysporum* was demonstrated [1]. Inoculation of *F. oxysporum* onto the agar medium mixed with a dilution series of a suspension of soil microorganisms significantly indicated in some variants the efficiency against spinach Fusarium wilt [33]. These data provide useful information on the suppressive activity of bacteria against Fusarium wilt.

As for phytostimulating substances, indole-3-acetic acid and siderophores play an important role in plant growth and development. IAA is a phytohormone responsible for next functions, like: root initiation, cell division, and cell enlargement. Siderophores are tiny iron-chelating compounds synthesized by soil microorganisms. They could influence plant growth by increasing availability of iron near the root or by inhibiting the colonization of roots by phytopathogen and making iron unavailable to them. Availability of siderophore is an important factor for phytopathogen antagonism and plant growth promoting [20].

Recent studies confirm that *Micrococcus yunnanensis* GKSM13 produces indole-3-acetic acid (47 µg/mL) [30]. *Micrococcus yunnanensis* CNMN-BM-19, on the other hand, produces less indole-3-acetic acid (36.5 µg/mL), but a positive effect on both plant length and dry weight was noted.

Another trial conducted by Liu *et al.* (2024), confirmed the ability of *B. velezensis* YL2021 strain to synthesize several types of siderophores, which exhibit broad-spectrum of antimicrobial activities against Gram-positive and Gram-negative bacteria, the oomycete *Phytophthora capsici*, and phytopathogenic fungi [27]. Thus, confirming the antifungal activity of the *Bacillus velezensis* CNMN-BB-16 against *Fusarium* spp.

Proteins and lipids are important precursors of over exometabolites with phytostimulatory and antimicrobial effect. Among growth promoting substances are highlighted phytohormones, hormone-like substances, proteins, peptides and amino acids, fatty acids, which demonstrated potential plant biostimulatory effects [3]. Also, plant growth promoting bacteria synthesize antibacterial proteins and enzymes that damaging the cell wall of phytopathogens [10].

The presence of such lipid fractions as phospholipids, mono-, di- and tri- glycerides seems to be particularly important, since they are involved in many metabolic processes.

Phospholipids are critical components of the plasma membrane bilayer; they act as binding sites for intra- and inter- cellular proteins and are involved in cell metabolism and signaling, after sensing a pathogen, phospholipid-hydrolyzing enzymes are mobilized to trigger signaling cascades vital for cellular responses [37].

Mono-, di-, and tri- glycerides are comprise the bulk of oil storage in microorganism and plant tissues [29].

Sterol lipids are participating in signal transduction. The most well-known examples are phytosterol in plants and cholesterol, such as steroids with specific roles as hormones and signaling molecules [29].

All of the above substances were detected in the studied strains, with the exception of IAA, which was synthesized in significant quantities only by the *M. yunnanensis* CNMN-BM-19 strain.

According to the results of Santhosh *et al.*, treatment of eggplant seeds with *Bacillus amyloliquefaciens*

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(SS_BL08) metabolites resulted in a two-fold increase in root and seedling length [41].

Another study revealed that, the *P. oxalicum* HZ06 significantly promoted eggplant seedling growth under greenhouse conditions. An obvious increase in leaf size and root length in comparison with control was noted. The plant blossomed abundantly after 27 days of colonization. According to the results on plants, an increase of 22.35%, 39.26%, and 47.69% in the length, dry and fresh weights of stems, respectively, and of 0.83%, 41.30%, and 28.72% in the length, dry and fresh weights of roots in comparison with control was registered [25].

In comparison of literature results with the obtained data, it should be noted that the experimental samples showed similar results in stem length (by 29.58% more than the control) using solution of the Bv16 at a concentration of 3.0%. There was also a weak stimulation of root system length by 1.4% (variant A36 (2.0%) in comparison with control). However, the fresh and dry weights in the various treatments differed significantly from the control sample. Fresh and dry weight exceeded the control in some experimental variants by 1.5-2.5 times.

Exometabolites of *Bacillus velezensis* CNMN-BB-16, *Micrococcus yunnanensis* CNMN-BM-19, and *Actinomadura* sp. 36 strains have demonstrated efficacy both as *Fusarium* antagonists and as phytostimulators of eggplant crop. Trials will be continued to identify the best phytostimulating effect for each strain.

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REFERENCES

[1] Aydi-Ben-Abdallah, R., Jabnoun-Khiareddine, H., Daami-Remadi, M., (2020): Fusarium wilt biocontrol and tomato growth stimulation, using endophytic bacteria naturally associated with *Solanum sodomaeum* and *S. bonariense* plants. Egyptian Journal of Biological Pest Control, 30(113): 1-13.

[2] Aydi-Ben-Abdallah, R., Jabnoun-Khiareddine, H., Nefzi, A., Mokni-Tlili, S., Daami-Remadi, M. (2016): Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Solanum elaeagnifolium* stems. Journal of Phytopathology, 164(10): 811-824.

[3] Brito-Lopez, C., van der Wielen, N., Barbosa, M., Karlova, R., (2025): Plant growth-promoting microbes and microalgae-based biostimulants: sustainable strategy for agriculture and abiotic stress resilience. Philosophical Transactions of the Royal Society B, 380(1927): 20240251.

[4] Byrsa, M., Balan (Batir), L., Bogdan-Golubi, N., Moldovan, C., (2025): Actinobacteria with antagonistic properties against representatives of the genus *Fusarium*. pp. 370-374. In: International scientific-practical conference "Education through research for a prosperous society", Ed. 12th, Vol. 1. Chisinau: CEP UPSC.

[5] Byrsa, M., Balan (Batir), L., Bogdan-Golubi, N., Moldovan, C., (2024): Antagonistic activity of bacterial isolates against *Fusarium* pathogens. pp. 486-490. In "Genetics, Physiology and Plant Breeding", Ed. 8th. Chisinau: Editorial-Printing Center of the State University Moldova.

[6] Eggers, L.F., Schwudke, D., (2016): Liquid extraction: Folch. pp. 1-6. In Wenk, R.M., (eds.): Encyclopedia of Lipidomics. Springer, Netherlands.

[7] Ekwomadu, T.I., Gopane, R.E., Mwanza, M., (2018): Occurrence of filamentous fungi in maize destined for human consumption in South Africa. Food Science and Nutrition, 6(4): 884-890.

[8] Ekwomadu, T.I., Mwanza, M., (2023): *Fusarium* fungi pathogens, identification, adverse effects, disease management, and global food security: A review of the latest research. Agriculture, 13(9): 1810.

[9] Elmer, W., Ma, C., White, J., (2018): Nanoparticles for plant disease management. Current Opinion in Environmental Science and Health, 6: 66-70.

[10] Fanai, A., Bohia, B., Lalremruati, F., Lalhriatpuii, N., Lalrokimi, Lalmuanpuii, R., Singh, P.K., Zothanpuia, (2024): Plant growth promoting bacteria (PGPB)-induced plant adaptations to stresses: An updated review. PeerJ Life and Environment, 12: e17882.

[11] Garg, G., Kumar, S., Bhati, S., (2021): Siderophore in plant nutritional management: Role of endophytic bacteria. pp. 315-329. In Maheshwari, D.K., Dheeman, S., (eds.): Endophytes: Mineral Nutrient Management, Volume 3. Sustainable Development and Biodiversity. Springer, Cham.

[12] Gonzales, L.M.R., (2015): Germination response of eggplant (*Solanum melongena* L.) seeds to different vinegar concentration as seed priming agents. International Journal of Scientific and Research Publications, 5(3): 1-4.

[13] Guilherme, S.C., Kitano, I.T., Ribeiro, I.A.F., Lacava, P.T., (2022): The potential use of actinomycetes as microbial inoculants and biopesticides in agriculture. Frontiers in Soil Science, 2: 833181.

[14] Gusmiaty, G., Restu, M., Payangan, R.Y., (2019): Production of IAA (Indole Acetic Acid) of the rhizosphere fungus in the Suren community forest stand. pp. 1-6. In pp. Conference Series: Earth and Environmental Science. Purpose-Led Publishing.

[15] Hadwan, M.H., Hussein, M.J., Mohammed, R.M., Hadwan, A.M., Saad Al-Kawaz, H., Al-Obaidy, S.S.M., Al-Talebi, Z.A., (2024): An improved method for measuring catalase activity in biological samples. Biology Methods and Protocols, 9(1): 1-12.

[16] Hammer, Ø., Harper, D.A.T., Ryan, P.D., (2001): PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1): 1-9.

[17] Hajji-Hedfi, L., Rhouma, A., Hajlaoui, H., Hajlaoui, F., Rebouh, N.Y., (2023): Understanding the influence of applying two culture filtrates to control gray mold disease (*Botrytis cinerea*) in tomato. Agronomy, 13(7): 1774.

[18] Hussain, M.A., Li, L., Kalu, A., Wu, X., Naumovski, N., (2025): Sustainable food security and nutritional challenges. Sustainability, 17(3): 874.

[19] Khare, M.N., (1996): Methods to test seed for associated fungi. Journal Indian Phytopathology, 49(4): 319-328.

[20] Kshetri, P., Roy, S.S., Sharma, S.K., Singh, T.S., Ansari, M.A., Sailo, B., Singh, S., Prakash, N., (2018): Feather degrading, phytostimulating, and biocontrol potential of native actinobacteria from North Eastern Indian Himalayan Region. Journal of Basic Microbiology, 58(9): 730-738.

[21] Kumar, R., Khan, A., Singh, P., Singh, A., Srivastava, A., (2024): Fusarium infection of eggplant: disease cycle and management strategies. pp. 281-306. In: Shahid, M., Gaur, R., (eds.): Molecular Dynamics of Plant Stress and its Management. Springer, Singapore.

[22] Lahoum, A., Aouiche, A., Bouras, N., Verheecke, C., Klenk, H.P., Sabaou, N., Mathieu, F., (2016): Antifungal activity of a Saharan strain of *Actinomadura* sp. ACD1 against toxigenic

DOI: 10.61215/AUOFB.2026.1.10

fungi and other pathogenic microorganisms. Journal de Mycologie Medicale, 26(3): 193-200.
[23] Larekeng, S.H., Gusmiaty, G., Achmad, F., (2020): Production of IAA hormone in rhizosphere bacterial isolates of community forest stands. pp. 1-11. In IOP Conference Series: Earth and Environmental Science. Purpose-Led Publishing.
[24] Li, P., Lu, Y.J., Chen, H., Day, B., (2020): The lifecycle of the plant immune system. Critical Reviews in Plant Sciences, 39(1): 72-100.
[25] Li, X., Li, D., Yan, J., Zhang, Y., Wang, H., Zhang, J., Ahmed, T., Li, B., (2021): Effect of plant-growth-promoting fungi on eggplant (*Solanum melongena* L.) in new reclamation land. Agriculture, 11(11): 1036.
[26] Liang, S., Jiang, Y., Ling, C., Xian, M., Ye, H., Cao, Q., Zhang, C., Dong, Z., Wang, W., Zuo, J., (2025): Exogenous catalase supplementation alleviates *Fusarium graminearum* mycotoxins-induced oxidative stress in weaned piglets. Agriculture, 15(17): 1892.
[27] Liu, Y., Dai, C., Zuo, Y., Qiao, J., Shen, J., Yin, X., Liu, Y, (2024): Characterization of siderophores produced by *Bacillus velezensis* YL2021 and its application in controlling rice sheath blight and rice blast. Phytopathology, 114(12): 2491-2501.
[28] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951): Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry, 193(1): 265-275.
[29] Macabuhay, A., Arsova, B., Walker, R., Johnson, A., Watt, M., Roessner, U., (2022): Modulators or facilitators? Roles of lipids in plant root-microbe interactions. Trends in Plant Science, 27(2): 180-190.
[30] Majhi, K., Let, M., Bandopadhyay, R., (2024): Efficacious use of *Micrococcus yunnanensis* GKSM13 for the growth of rice seedlings under copper stress with elucidation into genomic traits. Current Plant Biology, 37: 100318.
[31] Mahadevakumar, S., Janardhana, G.R., (2016): The leaf blight and fruit rot disease of brinjal caused by *Phomopsis vexans* in six agro-ecological regions of South West India. Plant Pathology and Quarantine, 6(1): 5-12.
[32] Matilla, M.A., Krell, T., (2018): Plant growth promotion and biocontrol mediated by plant-associated bacteria. pp. 45-80. In Egamberdieva, D., Ahmad, P., (eds.): Plant microbiome: Stress response. Microorganisms for Sustainability, vol 5. Springer, Singapore.
[33] Mitsuboshi, M., Kioka, Y., Noguchi, K., Asakawa, S., (2016): An evaluation method for the suppression of pathogenic *Fusarium oxysporum* by soil microorganisms using the dilution plate technique. Microbes and Environment, 31(3): 307-313.
[34] Mukherjee, A., Gaurav, A.K., Singh, S., Yadav, S., Bhowmick, S., Abeysinghe, S., Verma, J.P., (2022): The bioactive potential of phytohormones: A review. Biotechnology Reports (Amsterdam, Netherlands), 35: 1-9.
[35] Nisha, P., Paul, E., Francis, B., Hyrunnisa, M.A., Shahma, R.M., Johnson, S., (2023): Bioactive exopolysaccharide from marine bacteria *Micrococcus* sp. MRN-01. Materials Today: Proceedings, 80(2): 1272-1281.
[36] Patel, P., Patel, K., Dhandhukia, P. Thakker, J.N., (2021): Plant growth promoting traits of marine *Micrococcus* sp. with bio-control ability against *Fusarium* in chickpea plant. Vegetos, 34: 94-101.
[37] Pokotylo, I., Kravets, V., Martinec, J., Ruelland, E., (2018): The phosphatidic acid paradox: Too many actions for one molecule class? Lessons from plants. Progress in Lipid Research, 71: 43-53.
[38] Riseh, R.S., Fathi, F., Vatankhah, M., Kennedy, J.F., (2024): Catalase-associated immune responses in plant-microbe interactions: A review. International Journal of Biological Macromolecules, 280(2): 135859.
[39] Saeed, Q., Xiukang, W., Haider, F.U., Kučerik, J., Mumtaz, M.Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., Mustafa, A., (2021): Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. International Journal of Molecular Sciences, 22(19): 10529.
[40] Saini, D.K., Kaushik, P., (2019): Visiting eggplant from a biotechnological perspective: A review. Scientia Horticulturae, 253: 327-340.
[41] Santhosh, C.R., Mahadevakuamar, S., Nuthan, B.R., Chandranayak, S., Sreedharamurthy, S., (2023): Eggplant (*Solanum melongena* L.) associated endophytic bacteria promote plant growth and counter soil-borne plant pathogenic fungi. Authorea, 10: 168329349.
[42] Segarra, G., Casanova, E., Borrero, C., Aviles, M., Trillas, I., (2007): The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants. European Journal of Plant Pathology, 117: 393-402.
[43] Srivastava, S., Kadooka, C., Uchida, J.Y., (2018): *Fusarium* species as pathogen on orchids. Microbiological Research, 207:188-195.
[44] Sun, A., Jiao, X., Ren, P., Yu, D., Li, F., Chen, Q.-L., Bi, L., He, J.-Z., Hu, H.-W., (2022): Organic fertilization regimes suppress fungal plant pathogens through modulating the resident bacterial and protistan communities. Journal of Sustainable Agriculture and Environment, 1(1): 43-53.
[45] Timofeeva, A.M., Galyamova, M.R., Sedykh, S.E., (2022): Bacterial siderophores: Classification, biosynthesis, perspectives of use in agriculture. Plants (Basel, Switzerland), 11(22): 3065.
[46] Tripathi, R., Aravind, T., Kumar, S., Keswani, C., Singh, S.P., Tewari, R., Tewari, A.K., Singh, K.P., Minkina, T., (2025): Microbial phytohormones: the potential orchestrators of plant growth and defense. Discover Plants, 2(180): 1-16.
[47] Wu, F., (2014): Perspective: time to face the fungal threat. Nature, 516(7529): S7.
[48] Yin, Y.R., Sang, P., Xian, W.D., Li, X., Jiao, J.Y., Liu, L., Hozzein, W.N., Xiao, M., Li, W.J., (2018): Expression and characteristics of two glucose-tolerant GH1 β-glucosidases from *Actinomadura amylolytica* YIM 77502T for promoting cellulose degradation. Frontiers in Microbiology, 9: 3149.
[49] Zanjare, S.S., Suryawanshi, A.V., Zanjare, S.R., Karjule, A.P., (2023): Evaluation of different seed health testing methods for detection of seed borne leaf spot causing organism in safflower. The Pharma Innovation International Journal, 12(3): 5757-5760.

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