

## BIOMASS PRODUCTIVITY OF STREPTOMYCETES ISOLATED FROM SOILS OF REPUBLIC OF MOLDOVA AND FEATURES OF THEIR EXOMETABOLITES ON PHYTOSTIMULATING, ANTIFUNGAL, ANTIBACTERIAL AND ANTINEMATICIDAL ACTIVITIES

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**Abstract.** Streptomycetes isolated from soil of Republic of Moldova were tested on their ability to synthesize soluble pigment on Czapek's medium, oatmeal agar, starch-ammonia agar (SAA), potato glucose agar (PGA). Only *Streptomyces* sp. 11 synthesized soluble pigment with different colors on media oatmeal agar. However, *Streptomyces* sp. 22 and *Streptomyces* sp. 47 were able to synthesize soluble pigment only on SAA medium and oatmeal agar respectively. All studied strains have shown the abilities to accumulate biomass on complex medium M-I. Results show a range in amount from 5.38 g/l to 12.5 g/l air-dry biomass. Secondary metabolites of streptomycetes are antagonists of pathogenic bacteria, fungi and increase mortality of plant parasitic nematodes. Furthermore, all studied strains showed ability to suppress the growth of *Botrytis cinerea* (diameter of detained zones of growth – 11.0-16.0 mm) and *Fusarium oxysporum*. Determination of antifungal activity of the studied strains showed the slower growth caused by *S.* sp. 22 (zone 16.0 mm). Moreover, these strains obtain the antibacterial activities against *Clavibacter michiganensis*, *Agrobacterium tumefaciens*, *Erwinia carotovora* and *Xanthomonas campestris*. The most intensively antibacterial activities of these strains were against *Xanthomonas campestris* and *Clavibacter michiganensis* (delayed zone of growth 13.0-16.0 mm and 13.5-15.0 mm). Also the influence of exometabolites of *Streptomyces* sp.11 and *Streptomyces* sp.154 showed an increase in length of rootlets of tomato seeds, among all strains. The influences of secondary metabolites of all studied strains were effectively in different degrees on mortality rate of invasive stage juveniles of root-knot nematodes in greenhouse. The results showed that secondary metabolites of *S.* sp. 11, *S.* sp. 22, *S.* sp. 47, *S.* sp. 76 possessed the nematocidal effects on second stage juveniles of *Meloidogyne incognita*.

**Keywords:** *Streptomyces*, exometabolites, phytostimulation, antimicrobial and nematocidal activities, invasive juveniles of *Meloidogyne incognita*.

### Rezumat. Productivitatea biomasei streptomicetelor izolate din solurile Republicii Moldova și caracteristica exometaboliților lor asupra capacităților fitostimuloare, antimicotică, antibacteriană și nematocidă.

Streptomicetele izolate din solurile Republicii Moldova au fost testate pentru abilități de a sintetiza pigmenți solubili pe medii - Chapik, agar cu fulgi de ovăz, agar cu amidon și amoniac și glucoza de cartof pe agar. Dintre speciile investigate *Streptomyces* sp. 11 a sintetizat pigmenți solubili de diverse culori pe mediul agar cu fulgi de ovăz. Totodată *Streptomyces* sp. 22 și *Streptomyces* sp. 47 au avut capacitatea de a sintetiza pigmenți solubili pe mediul nutritiv de agar amidon amoniac și respective din mediul de agar cu fulgi de ovăz. Toate tulpinile investigate au posedat abilități de acumulare a biomasei pe complexul medium M-I. Rezultatele obținute reflectă o gamă de valori cuprinsă între 5,38 g/l la 12,5 g/l biomasa uscată. Metaboliții secundari ai streptomicetelor sunt antagoniștii bacteriilor, cupercilor fitopatogene și omit nematodele fitoparazite la plante. În plus toate tulpinile cercetate au demonstrat capacitatea de a suprima creșterea micozelor *Botrytis cinerea* (diametrul zonelor reținute în creștere – 11,0-16,0 mm) și *Fusarium oxysporum*. Determinarea activității antimicotice a tulpinilor studiate au posedat o creștere mai lentă tulpina *S.* sp. 11 (zona 19,0 mm), mai mult decât atât, aceste tulpini obținute, posedă acțiuni antibacteriene contra agenților patogeni *Clavibacter michiganensis*, *Agrobacterium tumefaciens*, *Erwinia carotovora* și *Xanthomonas campestris*. Eficiențe mai avansate antibacteriene ale acestor tulpini au fost în impact cu bacteriozele *Xanthomonas campestris* și *Clavibacter michiganensis* (zona de reținere în creștere de la 13,0 -16,0 mm și de la 13,5 - 17,0 mm). De asemenea, influența exometaboliților de *Streptomyces* sp. 11 și *Streptomyces* sp. 154 au reflectat o creștere în lungime a rădăcinilor din semințe de tomate, printre toate tulpinile. Influența metaboliți secundari ai tulpinilor studiate au fost efective în grade diferite pe rata de mortalitate a larvelor invazive ale nematodelor galicole. Rezultatele au aratat că exometaboliți *S.* sp. 11, *S.* sp. 22, *S.* sp. 47, *S.* sp. 76 au posedat eficiență nematocidă la stadiile invazive ale speciei nematodului *Meloidogyne incognita*.

**Cuvinte cheie:** *Streptomyces*, metaboliții secundari, capacități fitostimuloare, antimicrobiene și activități nematocide, larve invazive *Meloidogyne incognita*.

## INTRODUCTION

Actinomycetes are a unique group of microorganisms that combine the molecular, chemical and physiological features of bacteria and fungi (VALAGUROVA et al., 2003). Nearly all the currently known genera of actinomycetes are isolated from soil or found in soils. Actinomycetes are an integral part of the complex microbial soil, making ¼ of bacteria that grow on nutrient media used for sowing soil suspensions. Actinomycetes, except thermophiles, are included as members of the genus *Streptomyces*, which are characterized by a relatively low rate of growth. Active development and significant quantitative increase in the number of actinomycetes and their development were observed by the presence of mycelium in the soil or by a nutrient culture medium, such as starch, chitin or organic residues. It is known that number of actinomycetes and their activities enhance decomposition of humus, nitric balance of soil, synthesis and production of antibiotic substances. Moreover many soil microorganisms including actinomycetes take

part in accumulation of biological active substances (VETLUGINA et al., 1989; ZENOVA, 1992; BURTEVA, 2002; PETRUK, 2005).

Actinomycetes are gram-positive filamentous bacteria comprising a group of streptomycetes that have been involved in antagonistic relations with plant pathogens. Especially in agricultural soils populations of *Streptomyces* form  $10^5 - 10^7$  colonies/gram of soil. In fact the species of streptomycetes account more than 70% of the total antibiotic production. It was proven that some species of release compounds that inhibit the growth of plant-pathogenic fungi (JONES et al., 1996; CRAWFORD et al., 1993), bacteria (LIU et al., 1997; LORANG et al., 1995) and preserve plants from bacterial (LIU et al., 1997), fungal diseases (NEMEC et al., 1996) and plant parasitic nematodes (DICKLOW et al., 1993).

Their colonies can be differentiated by light colors, caused by releasing of colored substances in the culture or pigmentation of cells. This ability to form pigments is determined genetically and can be used as criteria in diagnostic analysis. Colored forms can be easily recognized and identified. These pigments play protective role, preserving cells from the effect of light and UV rays. There is very close connection between pigmentation and formation of the secondary exometabolites. Moreover, formation of antibiotics and other biologically active substances can be expected with the high probability in the presence of pigmentation (VALAGUROVA et al., 2003; GAUSE et al., 1983; KRASILNIKOV, 1970; MUROMTSEV & DANILINA, 1994). The color of the pigment excreted in the culture medium is one of the diagnostic features of actinomycetes. The number of stained cultures is about 70-80%. Completely colorless cultures are relatively low. The color of pigment correlates with the color of the air mycelium and the substrate mycelium, and color can be an indicator of any specific properties (ZENOVA, 1992). For example, some cultures produce prodigiosin that produce biomass with red-orange color; cultures with violet, red-violet color form atracyclines and mitomycins; green color cultures consist of ferroverdin and viridomycin, and possess antimicrobial properties (GAUSE et al., 1983). The study on the formation of pigment of actinomycetes form series Fradie showed stimulates germination of cotton seeds (HOJAMURATOVA, 1997).

Thus, the pigment is not only considered as a diagnostic character, but also had great practical value. The pigments preserve the culture from effect of UV rays, and also involved in gas exchange (absorb  $O_2$  in redox processes, are carriers of  $O_2$  (carotenoids). Moreover, pigments belong to different classes of compounds, those that are soluble and those that are insoluble in water. Their chemical nature has received very limited attention and it is only in recent years that metabolites, particularly the ones with antibiotic properties, became interesting for studies (HARTJEN, 1998).

Streptomycetes are the major group of actinomycetes producing secondary metabolites that decrease the invasive juveniles of root-knot nematodes from genus *Meloidogyne*. Root-knot nematodes cause severe damage to a wide range of crops and increase yield losses worldwide (BARKER, 1998; SUN et al., 2006). Their populations can build up in the short period of time under the perfect conditions of humidity and temperature and majority of these populations and generations (6-8) are detected in annual temperature range of 24-30°C with humidity near 70% (NICKLE, 1991; EISENBANK et al., 1981). Especially in the intensive cropping system is very important to control pests and plant diseases. Nowadays root-knot nematodes are the main problem that causes yield losses in the greenhouse among all organisms; reduce plant resistance to other stresses and decline in quality and quantity of the crop yield. Studies done by JAYAKUMAR (2009) showed that isolate Manp from *Streptomyces* caused 68.58% mortality *Meloidogyne incognita* J2 and up to 15.73% their hatching. Therefore, streptomycetes' isolates can be considered as prospective candidates for biological control agents (SASSER & KIRKPATRICK, 1982) instead of chemical control.

Biological control can be considered as a prior option to control root-knot nematodes particularly when it is based on the substances that are able to accelerate plant development, stimulate germination of seed, enhance growth of rootlets, and simultaneously suppress development and growth of plant diseases to increase quality of production (DROPKIN, 1972; BABENCO et al., 1992; DELIU et al., 1997). Furthermore, streptomycetes with nematicidal effects (GALAGAN et al., 2009) are also antagonists of bacterial (NDONDE & SEMU, 2000), fungal diseases (SMITHER-KOPPERL et al., 2001; YU et al., 2008).

The proposal of this research is to study the abilities of streptomycetes isolated from soils of Republic of Moldova on accumulation of biomass and the evaluation of effects on phytostimulating, antibacterial, antifungal and nematicidal activities.

## MATERIALS AND METHODS

Thirty five strains of streptomycetes isolated from soils of Republic of Moldova (National Collection of Non-pathogenic Microorganisms, ASM) were used in the screening procedure. All studied strains were stored at +4°C (refrigerator) in laboratory conditions on different medium. Inoculum was obtained on medium Dulone during three days at 27°C in flasks 0,25l on agitator. Streptomycetes were cultivated on complex medium M-I containing corn flour 20 g/l (the source of carbon) in flasks Erlenmeyer on agitator within 5 days at 27°C. It was applied at 100-200 µL of spore suspension and transferred to solid medium. Plates were incubated at 28°C in thermostat for 4-6 days. After sufficient growth and sporulation, culture plates were wrapped. To maintain biomass productivity, 10 ml of inoculum was transferred into 250 ml Erlenmeyer baffled flasks. Erlenmeyer baffled flasks containing 100 ml of liquid media was sterilized by heating to 121°C for at 30 minutes. Further, culture flasks were incubated in a shaker at 28°C with 200 rpm for 120 hours. Biomass has been separated from cultural liquid (CL) on a centrifuge (7000 rev/min. during 20 min.) (EGOROV, 2004). The received CL containing the complex of the exometabolites (EM) of the studied strains was diluted

with distilled water 1:200. Tomato seeds of Leana were counted and soaked into CL solutions of the studied streptomycetes strains and distilled water as a control (3 replications) by standard method (VOZNIJAKOVSKY, 1989). Petri dish is covered with filter paper (or cotton wool) and 10 ml of distilled water. For each studied grade of tomato seeds three repetitions by 20 seeds were used. For studying their germination and growth of rootlets, covered Petri dishes with tomato seeds were stored in the thermostat during 4 days. The quantity of sprouted seeds was counted, and their weight and length measured. Recalculation of the received data was done for 100 seeds (VOZNIJAKOVSKY, 1989).

In the study of the shape, structure and size of the colonies, culturally sensitive streptomycetes, were examined on the nature of the growth of isolated 20-day-old colonies on organic and synthetic media. The shape and structure of the colonies and their size (diameter in mm), the degree of development of the aerial mycelium, its character and color, color of the substrate mycelium (colony) (ZHUKOVA et al., 1978, GAUSE et al., 1983; ZENOVA, 1992), the ability to paint medium were studied. In describing the colony, following features were such as form colonies (round, round with scalloped edge, round with a roller on the edge, amoeba-like, folded, wrong *etc.*), diameter in mm (if the size of colonies is not more than 1 mm, such colonies called point); color (allocated or not allocated pigment in the environment); surface (smooth, rough, wrinkled, lumpy), structure of the colony (homogeneous, fine-grained, coarse, striated, fibrous) and edge of the colony (EGOROV, 2004). For determination of antimicrobial properties of streptomycetes, the simplest and most affordable method of agar blocks are used. Test cultures were seeded in lawn in sterile Petri dishes. Agar blocks with streptomycetes are cut with mirror drill and placed on the surface of another agar plate in a Petri dish inoculated with the test organism. Then these Petri dishes are placed in thermostat at a temperature of 27 °C. The zones without formed growth were measured in the diameter (EGOROV, 2004).

Phytopathogenic bacteria were used as a test cultures such as *Clavibacter michiganensis* 10 (agent of bacterial canker of tomato), *Agrobacterium tumefaciens* 8628 (agent root of cancer and cancer of the fruit of beet roots); *Xanthomonas campestris* 8003 (activator of vascular bacteriosis of cabbage) (ZENOVA, 1992).

For nematocidal test the invasive juveniles of *Meloidogyne incognita* were extracted from the infected tomato roots with galls, using Baermann method (BEZOOIJEN, 2006). Suspension with second stage juveniles (about 50) were collected of *M. incognita* and placed in each 5cm diameter Petri dish. EM of *S. sp. 11*, *S. sp. 22*, *S. sp. 47*, *S. sp. 76* and *S. sp. 154* are diluted with distilled water for stock solutions and fresh water is used as a control. Each treatment was replicated three times. Petri dishes are kept in room temperature (28-30°C).

## RESULTS

Some strains of *Streptomyces* isolated from the soils of Republic of Moldova possess capacity to synthesize soluble pigment and accumulate biomass. These strains are different in aerial and substrate mycelium and also do not synthesized soluble pigment coloring culture medium. Table 1 show the culturing of strains on the mediums Chapek, SAA (starch-ammonia agar), potato glucose agar (PGA) and oatmeal agar the size of colonies ranged from 2.0 to 6.0 mm. According to the description, it is round colony with convex (strain 11) or flat center (strains 22, 47, 76) and smooth edges.

Table 1. Cultural characteristics of streptomycetes in growth on different mediums Chapek, SAA, PGA, oatmeal agar).

No. strains	Medium	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Grown activity
11	SAA	Pale green	Yellow	-	++
	Czapek	Pale gray	Colorless	-	++
	oatmeal agar	Pale gray	Pale honey	-	++++
	PGA	Pale sand	Pale brown	-	++
22	SAA	White	Grayish	-	+
	Czapek	Mauve	Colorless	-	+++
	oatmeal agar	Mauve	Colorless	-	++++
	PGA	Pale pink	Colorless	-	+
47	SAA	Light gray	Colorless	-	++
	Czapek	Grayish-purple	Colorless	-	++++
	oatmeal agar	Gray	Terracotta	-	++++
	PGA	Palely grayish purple	Colorless	-	+++
76	PGA	Cherry	Colorless	-	++
	Czapek	Pale turquoise	Colorless	-	+++
	oatmeal agar	Taupe	Colorless	-	++
	PGA	Gray	Colorless	-	++

Remark: very good growth (++++); good growth (+++); weak growth (++); very weak growth (+)

The studied strains have not synthesized the soluble pigment on Czapek’s medium with glucose: substrate mycelium was colorless, aerial mycelium was different such as pale gray (*S. sp. 11*), mauve (*S. sp. 22*), gray-violet (*S. sp. 47*) and pale turquoise (*S. sp. 76*). Moreover, they also did not synthesized the soluble pigment on oatmeal agar substrate mycelium in *S. sp. 11* had honey color, *S. sp. 47*- terracotta color and *S. sp. 22*- mauve. Furthermore, studied strains on SAA medium also did not release any coloring pigment, substrate mycelium - colorless and aerial mycelium has different colors in all strains. The difference in activity of the growth of all strains on these mediums is noticed, the high growth on oatmeal agar. All strains were compared in their ability to accumulate biomass after growth on a complex medium M-I (corn flow - the main source of carbon). The ranges of amount from 5.38 g/l to 12.5 g/l air-dry biomass present in figure 1.

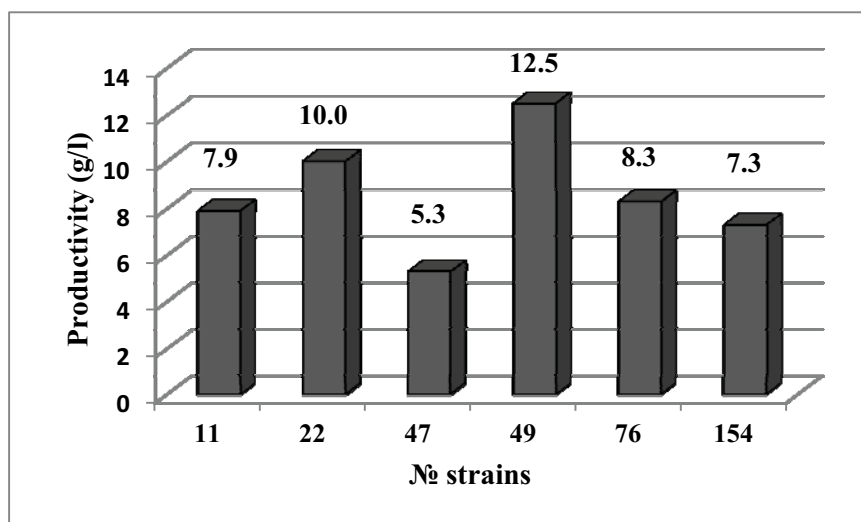


Figure 1. Biomass productivity of streptomycetes on liquid medium M-I.

Formation of biomass of studied strains of streptomycetes on a liquid complex medium M-I showed the maximum amount of biomass for strains 49 (12.5 grams of dry biomass) and 22 (10.0 grams of dry biomass); the remaining strains (11, 47, 154) – 5.3 -7.9 grams of dry biomass. Thus the complex media M-I was more active for the growing of strain 49 with the optimal accumulation of biomass, however for the remaining strains should be selected other compositions of medium (Fig. 1).

Streptomycetes possess the greatest ability to synthesize antibiotics, compared with all other actinomycetes. The most important aspect in agriculture is to reveal the biological active compounds that can protect plants from different diseases without pollution of environment. The advantage of use of antibiotics is to prevent contamination by different chemicals in the soil. Furthermore, the use of antibiotics increased plant production and decreased costs of treatment (VOBIS, 1992; EGOROV, 2004). Use of antibiotics in plant breeding has the different purposes such as the stimulation of plant growth and development, prevention and treatment of plant diseases (TULEMISOVA & CHORMONOVA, 1990; HOJAMURATOVA, 1997; PETRUK, 2006 etc.). Some of studied strains showed the antifungal abilities to delay the growth of *Botrytis cinerea* diameter of delayed zones of growth – 11.0-16.0 mm (Photo 1) and *Fusarium solani* (Photo 2) delay of growth was caused only by *S. sp. 11* (zone 19.0 mm).

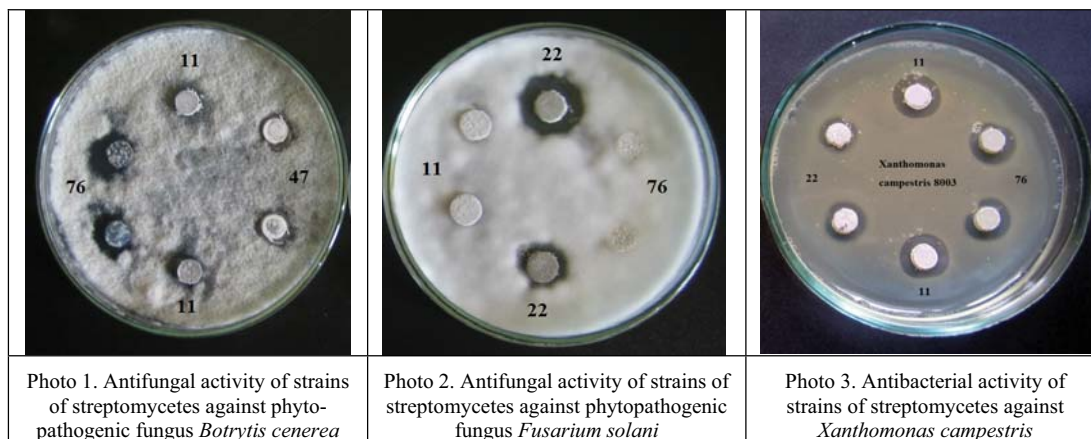


Photo 1-3. Antifungal activity of strains of streptomycetes against phytopathogenic fungus *Botrytis cinerea*, *Fusarium solani*, *Xanthomonas campestris*. (original).

All studied strains showed the ability to possess the various antibacterial properties (Table 2) and demonstrated it in different ways (Photo 4). Such as only strain *S. sp.* 22 delayed growth of *Erwinia carotovora* (delayed zone of 10.0 mm in diameter) however other strains didn't show this ability. All strains had the weak effect on *Agrobacterium tumefaciens* (zone 9.0 – 11.0 mm). However, the most antibacterial activities of these strains were against *Xanthomonas campestris* (Photo 3) and *Clavibacter michiganensis* (delayed zone of growth 13.0-16.0 mm and 13.5-15.0 mm).

Table 2. Antibacterial activity of streptomycetes from soils of Republic of Moldova.

No. Soil sample	No. strains of <i>Streptomyces</i>	The diameter of delayed zones-bacteria (mm)			
		<i>Clavibacter michiganensis</i>	<i>Agrobacterium tumefaciens</i>	<i>Erwinia carotovora</i>	<i>Xanthomonas campestris</i>
1	11	15,0 ± 0,74	9,0 ± 0,88	0	16,0 ± 0,74
	22	13,5 ± 0,74	11,0 ± 0,94	10,0 ± 0,66	14,5 ± 0,9
2	47	0	9,5 ± 0,95	0	13,5 ± 0,66
	76	14,0 ± 0,57	0	0	13,0 ± 0,68

These strains well grew and sporulated at three common microbiological media (mostly oatmeal agar, Czapek's medium with glucose and partly CAA). The result of determination of antibacterial activities of SM *Streptomyces sp.* 11, 22, 76 cultivated on Czapek's medium with glucose (photo 4) showed the different inhibition level of pathogenic bacteria growth. For example, areas of growth retardation *Xanthomonas campestris* 8003, causing vascular bacteriosis of cabbage, ranging from 9.0 mm (*S. sp.* 123) to 17.0 mm (*S. sp.* 52).

SM of the studied strains of streptomycetes showed the different effects EM on the growth of tomato rootlets (Photo 4). Thus, the increasing of length of tomato rootlets under the influence SM of *Streptomyces sp.* 154 and *Streptomyces sp.* 11 were observed in comparison with the control. Seeds applied with *Streptomyces sp.* 11 produced the longest rootlets (3-5 cm).

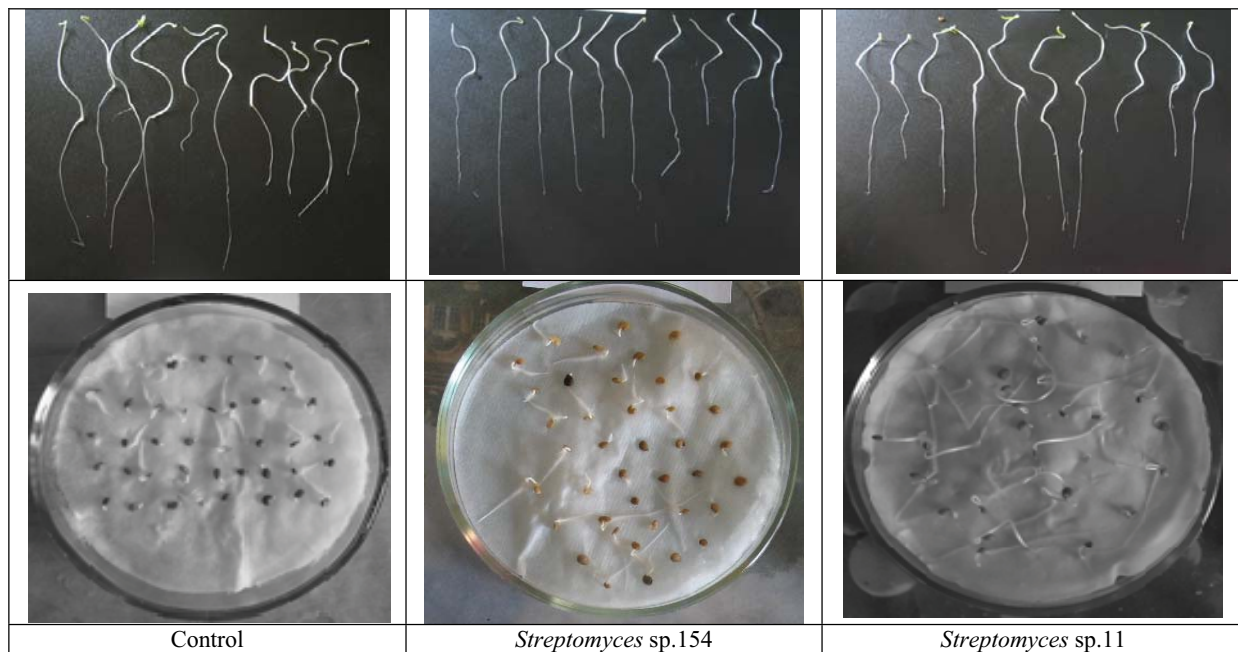


Photo 4. Influence of EM strains *Streptomyces sp.* 11 and *Streptomyces sp.* 154 on rootlet lengths of tomato grade "Leana" compared with the control. (original).

Exometabolites of *Streptomyces* possess also the nematicidal effect against the dangerous plant parasitic nematodes of greenhouses such as root-knot nematode *Meloidogyne incognita*. Studies have been done by DISKLOW et al., 1996, JAYAKUMAR, 2009, BELYAVSKAYA et al., 2009, GALAGAN et al., 2009, POIRAS et al., 2010, POIRAS et al., 2011, POIRAS N. et al., 2012 showed that streptomycetes isolates demonstrated the high pathogenicity against hatching and nematicidal effect on invasive juveniles (J2) of *M. incognita* *in vitro* tests. Among seventeen strains of *Streptomyces* spp. isolated from Moldavian soils (National Collection of non-pathogen micro-organisms IMB ASM) six strains (*S. sp.* 11, *S. sp.* 22, *S. sp.* 47, *S. sp.* 49, *S. sp.* 76, *S. sp.* 154) exhibited the nematicidal properties on mortality of J2 *M. incognita* *in vitro* in different degrees. Observation of nematode mortality was calculated after 1, 2, 4, 6, 12, 24 hours, by counting live and dead invasive juveniles of *M. incognita* after the influence of studied SM.

## DISCUSSIONS

Strains of streptomycetes possess such activities as phytostimulating, antibacterial and antifungal, moreover nematicidal abilities. The studies have shown that the strains of *Streptomyces* isolated from various soil samples significantly affects the shape, size and color of the colonies, and most strains of the size of the colonies was more on Czapek's medium with glucose and oatmeal agar. Wider color range of the air and substrate mycelium of the cultures, also growing on Czapek's medium or oatmeal agar.

Analyzing and comparing the figure presented in the data, it was observed that only actively growing strain 49 on complex environment M-I had the optimal of biomass accumulation in the greatest number than the other strains, for which should choose a different medium composition, containing not only the corn flour as the sole source of carbon and nitrogen, but also soy, wheat or combination with corn supplemented with the additional glucose (GAUSE et al., 1983).

The experiments on the effect of culture medium on the antimicrobial activity of soil streptomycetes showed that as a result of growth on mineral medium Capek and complex organic medium - oatmeal agar studied strains *Streptomyces* soil synthesized metabolites that can delay the growth of pathogenic bacteria. Moreover, it was found that three strains of *Streptomyces* metabolite occurred in complex formation, when cultured on Czapek's medium and delaying the growth of selected test bacteria to a greater extent than the growth in the environment of "oatmeal agar."

All strains of *Streptomyces* isolated from soil of Moldova differ in cultural properties: color of aerial and substrate mycelium, the ability to form water-soluble pigments. New strains differ in the activity of growth and sporulation. *Streptomyces* studied on oatmeal agar medium gave best growth and sporulation. *Streptomyces* isolated from Moldova soils characterized by their ability to accumulate biomass at growth in an integrated environment, M-I: biomass ranges from 5.3 g/l (Strain 47) and 12.5 g/l (Strain 49).

*Streptomyces* isolated from different soil samples of Republic of Moldova exhibit antimicrobial properties against phytopathogenic bacteria: the outbreak strain, actively inhibit the growth *Clavibacter michiganensis* 10 (bacterial canker of tomato) - *Streptomyces* sp.151 and *Streptomyces* sp. 154 (4 soil sample). Also exometabolites of the studied streptomycetes' strains have shown the nematicidal properties on activity of invasive juveniles (J2) of root-knot nematodes *M. incognita in vitro*. All studied strains of *Streptomyces* spp. have shown the nematicidal effect on activity of invasive juveniles of root-knot nematodes in varying degrees. In the experiment, different concentrations of EM of strains *Streptomyces* sp.11 and *Streptomyces* sp.154 were used on second stage juveniles of root-knot nematodes (50 - 100 specimens); exposure time (0.5 - 24 hours and more). It was noticed that about 20-40% of second stage juveniles lost their activity by influence of EM *Streptomyces* sp. 11, 22, 47, 49, 76 and 154 at high concentration 1:2 during 0.5 -2 hours. Further studies are needed in this scientific field.

## CONCLUSIONS

All investigated strains of streptomycetes isolated from the different soil types of R. Moldova significantly effect on the shape, size and color of the colonies, and most strains increase the size of the colonies on Capek's medium and oatmeal agar. Maximum amount of biomass on a complex medium M-1 among the surveyed strains of streptomycetes showed strains *S. sp. 47* and *S. sp. 49* (10.0 -12.5 g/l dry biomass). The effect of culture medium on the antimicrobial and antifungal activities of soil streptomycetes showed in Capek and oatmeal agar mediums to synthase the exometabolites with abilities to delay the growth of pathogenic bacteria and fungus. Such as the strains *S. sp. 11*, *S. sp. 22* and *S. sp. 76* showed to varying digress the antibacterial activities against *Xanthomonas campestris*, *Clavibacter michiganensis*, less *Agrobacterium tumefaciens* and partly antifungal activities *S. sp. 22*, *S. sp. 76* against phytopathogenic fungus *Fusarium solani* and *Botrytis cinerea*. Exometabolites of *S. sp. 11* and *S. sp. 154* increase the growth of tomato rootlets compared with control and showed some nematicidal activities against *Meloidogyne incognita in vitro*.

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