Efficacy of Extracellular Bioactive Metabolites Produced by *Streptomyceslevis* Strain LX-65 as a Potential Antimicrobial and Herbicidal Agent

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Abstract: Weeds are known to cause enormous losses due to their interference in agroecosystems. Because of environmental and human health concerns, worldwide efforts are being made to reduce the heavy reliance on synthetic herbicides that are used to control weeds. In recent years there has been a rising interest in the discovery of environment friendly bioherbicidalcompounds and biocontrol agents for weed control in sustainable agriculture. In this regard an actinomycete strain LX-65isolated from cultivated soil of Luxor governorate, Egypt was found to produce extracellular metabolites with promising activities. The culture filtrate of this isolate exhibited strong broad spectrum antibacterial activity with mean diameter of inhibition zone ranged from 19.0 to 28.0 mm and strong antifungal activity with mean diameter of inhibition zone ranged from 15.0 to 31.0 mm. Also it showed herbicidal activity against some weeds associated with some of the economic crops in Egypt; winter wheat (*Triticumaestivum L.*) and maize (*Zea mays*). The results of herbicidal activity were represented by reduction of seeds germination from 100 to 0%, the root length from 100 to 8% and the shoot length from 100 to 10% for the weeds;*Eruca sativa*, *Raphanussativus*, *Loliummultiflorum* and *Echinochloacrusgalli*. At the same time the tested filtrate recorded insignificant effect on germination and growth of *Triticumsativum* while it exhibited stimulatory effect for growth of *Zea mays*.Morphological, cultural, physiological and biochemical characteristics, along with the cell wall analysis suggested that this isolate is belonging to the genus *Streptomyces*.The 16S ribosomal RNA gene sequencing revealed that the isolate was highly related to *Streptomyces levis*(~84 %),so it is designated as *Streptomyces levis*strainLX-65. The present study revealed that the bioactive metabolites produced by*Streptomyces levis* strain LX-65 are useful for applications in the different fields and especially as a potential biocontrol and plant growth promotion agents.

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1. Introduction

The word weed means any wild plant that grows at an unwanted place especially in fields or in gardens where it interferes with the growth of cultivated plants. Weeds may be defined as plants with little economic value and possessing the potential to colonize disturbed habitats or those modified by human activities **[1].**

Interference of weeds with agricultural crops causes huge economic losses to farmers in two ways. Firstly, it reduces crop quality and quantity, and secondly it increases cost of labour and herbicides to control them **[2].**

Herbicides, as the main weed control tool, play a very important role in modern agriculture. Chemical herbicides used on most weeds cause many long-term problems **[3]**, such as water and soil pollution, pesticide residue, and the development of herbicide resistance in many weed biotypes **[4, 5, 6].**

With increasing awareness about environmental protection and demand for sustainable agricultural development, the development of non-polluting, safe, and environment-friendly herbicides has become imperative **[3, 7].** There has been a rising interest in the discovery of environment friendly bioherbicidal compounds and biocontrol agent (BCA) for weed control in sustainable agriculture. Approaches to the biological control of weeds in arable crops and integration of biological weed control with other methods of weed management have been broadly made **[8].**

Natural products such as secondary metabolites of certain microbes have been investigated as biological pesticides and herbicides which are used as alternative for agrochemicals. Bioherbicidal metabolites are being studied as possible herbicides or herbicidal adjuvants to develop biological agents that are selective, easily degradable and environment friendly. Studies on the use of microbes with small amount of herbicides have shown that combinations of these agents can improve biocontrol efficacy **[9].**

Weed control by using specific microorganisms is an accepted weed management strategy **[10, 11].**

Soil Actinomycetes are prokaryotes with extremely various metabolic possibilities. These are prolific producers of various bioactive compounds,

and have provided over two third of naturally occurring antibiotics discovered and continue to be major source of novel and useful compounds such as antibiotics, enzymes, pigments, herbicides, insecticides and immunomodulatorsetc**[12-15].**

Soil actinomycetes, namely the *Streptomyces* spp., produce herbicidal compounds that have been commercially developed for weed control **[16].***Streptomyces* species have also been used directly as biological control agents, as an alternative to agrochemicals, for the control of plant disease**[17-19].**

In the course of our screening forbioherbicidal agent, an actinomycete strain, *Streptomyceslevis*LX-65 has potential a biocontrol agent (BCA) for control of problematic weeds associated with some of economic crops in Egypt. On account of the possible use of actinomycete metabolites as naturally occurring and safe herbicides, or in combination with actinomycete isolates in weed control, it is of interest to certain the production of phytotoxic metabolite by active strain.The main aim of the present work was to evaluate in vitro herbicidal activity of the bioactive metabolites produced by *Streptomyces levis*LX-65 against some of pathogenic microorganismsand crops associated weeds.

2. Subjects and methods

2.1. Isolation of actinomycete strain

Soil samples were collected at a place near Mina Coast, Luxor governorate, Egypt. Sample serially diluted to isolate a pure culture on a starch casein agar (soluble starch, 10.0 g; vitamin free casein, 0.3 g; KNO_3, 2.0 g; NaCl, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4.7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4.7H_2O$, 0.01 g; agar, 20.0 g; distilled water, 1.0 L; pH, 7.2; supplemented with rifampicin 25 µg/ml and cycloheximide 75 µg/ml to inhibit bacterial and fungal contamination, respectively) plate, which had been seeded with a soil sample suspension and incubated at 30°C for 7 days **[20].**

2.2. Screening for antimicrobial activity

2.2.1.Test organisms

The test organisms used in present study were the Gram positive bacteria;*Bacillus subtilis* (NCTC 1040) and *Staphylococcus aureus* (NCTC 7447) and Gram-negative bacteria; *Pseudomonas aeruginosa* (NCIB-9016) and *Escherichia coli* (NCTC10416) which were used for studying the antibacterial activity. *Fusariumoxysporum*(RCMB008002),*Aspergillusniger* (IMI31276), *Aspergillusflavus*(IMI111023),*Pencilliumchrysogenum*(RCMB 001015), *Trichodermviride*(RCMB008002)and *Candida albicans*(IMRU3669)were used to determine the antifungal activity of the actinomycete isolates.

2.2.2.Screening for antibacterial activity

The disc diffusion method was used for determination the level of antibacterial potentialityof the isolated actinomycetes as suggested byKaushik and Kishore**[21].**

2.2.3.Screening for antifungal activity

The most active actinomycete isolate, LX-65 was separately cultivated on the basal salt starch nitrate broth medium **[22]** adjusted to pH 7.0. The medium contained the following components (g/L): 20.0, starch; 2.0, KNO_3; 1.0, K_2HPO_4; 0.5, $MgSO_4.7H_2O$; 0.5, NaCl; 3.0, $CaCO_3$ and 0.01, $FeSO_4.7H_2O$. Moulds were cultivated on agar media of malt-extract.Erlenmeyer flasks (250 ml) containing 50 ml of the liquid basal salts medium **[22]** were inoculated with a disk of 0.4 cm diameter taken from 7 days old culture plates of the studied isolate. The flasks were incubated on a rotary shaker (200 r.p.m) at 30°C and initial pH 7.0 for 5 days. The culture broth was centrifuged at 5000 r.p.m at 4°C in order to separate the microbial cells and the supernatant was used to test the antifungal potentiality. The antifungal potentiality was expressed as the diameter of the inhibition zones according to the agar plate diffusion method **[23].**

2.3. Screening for bioherbicidal activity

2.3.1. Plant material

Seeds of the plants; bread wheat (*Triticumsativum*) and maize (*Zea mays*), and their problematic weeds; radish (*Raphanussativus*), Arugula (*Eruca sativa*), Barnyard grass (*Echinochloacrusgalli*) and Rya grass (*Loliummultiflorum*) were obtained from Agriculture Research Center, El-Dokki,Giza, Egypt.

2.3.2. Screening for bioherbicidal activity.

Bioassays used Petri dishes (90 mm diameter) with one sheet of Whatman no.1 filter paper as substrate. The filtrate of *Streptomyces levis*, LX-65 was diluted to different concentrations 0, 25, 50, 75, 100% by using distilled water. Seeds of the plants under study were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water, then ten seeds were placed on the filter papers Whatman no.1.

Aliquot (10 ml) from each concentration were used for herbicidal bioassay and sterile water was used as control. After the addition of seeds and aqueous solutions, Petri-dishes were sealed with Parafilm and incubated in the dark at 25°C. After growth plants were frozen at -10°C for 24 h to avoid subsequent growth during the measurement process **[24].** Germination percentage (G %) was recorded after 7 days of incubation, the experiment was terminated then stem and root lengths of weed seedlings were measured according to Chung and Miller **[25].**

2.4. Statistical Analysis.

Data were statistically analyzed by ANOVA, according toSnedecor and Cochran**[26]** and treatment means were compared by LSD test at 5% level of probability.

2.5. Taxonomic characterization of the most active actinomycete isolate, LX-65.

2.5.1.Conventional taxonomy

The characterization of isolated actinomycete, LX-65 followed the guidelines adopted by International *Streptomyces* Project **[27].** The cultural characteristics were studied according to the guidelines established by the ISP **[27]**,colours wereassessed on the scale adopted by Kornerup and Wanscher**[28]**.Micro-morphological studies were carried out using light and scanning electron microscope (JEOL JSM 5300, JEOL Technics Ltd., Japan) **[29]** according to the method of Tresner*et al.***[30].** Diaminopimelic acid isomers in the cell-wall and whole cell sugar pattern were analyzed using the method of Becker *et al.***[31].** The physiological characteristics; melanin pigment production, carbon and nitrogen sourcesutlization, enzymatic activities and other physiological and biochemical characters were also studied **[27, 32-34].**

2.5.2.Molecular and phylogenetic identification

The nucleotide sequence of partial 16S rRNA gene of the local actinomycete strain LX-65 was performed through inoculation of LX-65 spores on 50 ml of starch nitrate broth and the culture was incubated at 200 rpm and 28 °C for 72 hours. The total genomic DNA was extracted according to the method of Sambrook*et al.***[35]**. The 16S rRNA of the strain was amplified by PCR using a GeneAMP PCR System 9700 from PE Applied Biosystems (Perkin Elmer, Ohio, USA). The following primers were used: F27, 5'-AGAGTTTGATCMTGGCTCAG-3'and R1492 5'-TACGGYTACCTTGTTACGACTT-3' using Biolegio BV software (Biolegio, Nijmegen, Netherlands) **[36]**, PCR mixture conditions were performed according to El-Naggar**[37]**. The PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were detected using a gel documentation system, (Alpha-Imager 2200, CA, USA). The PCR products were sequenced using gene analysis unit in genetics laboratories of the Holding Company for Biological Products and Vaccines (VACSERA), El-Dokki, Giza, Egypt using an ABI PRISM 377 DNA sequencer and ABI PRISM BigDye Terminator Cycle Sequencing (Perkin Elmer, Ohio, U.S.). BLAST (www.ncbi.nlm.gov) was used to assess the DNA similarities. A multiple sequence alignment and molecular phylogenetic analyses were performed using BioEdit software **[38]**. The phylogenetic tree was constructed using the TreeView program **[39]**.

3. Results and discussion

3.1. Antibacterial activity

Antibacterial activity (Table 1) of 12 different actinomycete isolates was tested against five different pathogenic organisms. 68% of isolates were active against one or more of the tested organisms, of that 54% exhibited activity against Gram negative and 79% exhibited activity against Gram positive bacteria.

The results of antibacterial activity revealed that majority of the isolates were active against Gram positive bacteria than Gram negative bacteria. In many previous reports **[40, 41]** high percentage of inhibition was recorded against Gram positive bacteria while Gram negative bacteria were less inhibited. Isolate LX-65 metabolite showed activity against all the tested organisms. Its inhibition zone diameter was also high (Table 1).

3.2. Antifungal activity

The antifungal activity of the most active actinomycete isolates, LX-65 (Table 2) showed that the degree of antifungal activity varied greatly among the fungal pathogens, the inhibitive effect of obtained active metabolite on the growth of *Fusariumoxysporum*and *Candida albicans*26.0 and 31.0mm respectively, The LX-65 isolate metabolite had a moderate effect on *Aspergillusniger* and *Aspergillusflavus*22.0 and 19.0 mm respectively, while *Trichodermviride* was comparatively less affected 15.0 mm and not active against *Pencilliumchrysogenum*.

Several researchers have already reported similar antimicrobial activity of actinomycetes against pathogenic fungi. Mukherjee and Nandi **[42]** found that out of 170 actinomycete 21.1% exhibited antifungal activity. Basilo*et al*. **[43]** reported 335 isolates, of these 230 isolates were active against bacteria, fungi and yeast. Augustine *et al.***[44]** obtained 312 actinomycete strains from different regions, of which, 22% exhibited antifungal activity against fungi. Jain and Jain *et al.***[45]** screened 287 isolates from various habitats and recorded 166, 164, 134, and 132 actinomycetes isolate active against *C. albicans*, *A. niger, M. gypseum* and *T. rubrum* respectively.

3.3. Bioherbicidal activity

The pre-emergence activity of the isolate, LX-65 filtrate with four concentrations (25, 50, 75, and 100) was tested to determine their inhibitory effects against the selected weed germination and seedling growth.

The highest reduction effect achieved in the case of *Eruca sativa*, in which all dilutions ofLX-65 metabolite,significantly (p<0.05) reduce *Eruca sativa*germination by 47, 100, 100 and 100%, shoot

length by 41, 100, 100 and 100%, respectively, and reduced root length significantly by 65, 100, 100 and 100%, respectively as compared with its control (Table 3).

The metabolite ofLX-65 with its all dilutions inhibited *Loliummultiflorum*germination by 30, 33, 37, and 40%, shoot length by 21, 51, 61 and 79%, respectively, and root length significantly inhibited by 43, 45, 72 and 84%, respectively as compared with its control. While only 75 and 100% reduced germination significantly by20 and 33% in the case of *Raphanussativus,* butall dilutions inhibited shoot lengthby 41, 60, 63, and 78%, and root length by 43, 50, 79 and 82%, respectively as compared with its control.

While results showed that a significant reduction achieved in the case of *Echinochloacrusgalli* from metabolite with concentrations 50, 75 and 100 by 17, 37 and 45% (germination), 58, 79 and 93% (shoot length),79, 71 and 98% (root length), respectively, than the control. It was noted that *Triticumsativum* are less response to metabolite, in which 50, 75 and 100% reduced *Triticumsativum* germination by 23, 30 and 33 %, shoot length by 45, 59 and 70 % and root length by 38, 54 and 64%, respectively than untreated control.

Finally, in the case of *Zea mays* only 100 % of *Streptomyces levis* LX-65 metabolite reduce the germination by 17%, and from the results shoot length reduce with 75 and 100% by 25 and 70, while at 25 and 50% they give stimulatory effect and shoot length increased by 74 and 82% respectively, while only 50, 75 and100% reduce root length by 17, 43 and 32%, respectively as compared with its control. Also, it was noted that the degree of inhibition increased with the filtrate concentration, and the root growth was always inhibited at lower concentrations than shoot growth or germination.

These inhibitory effects of microbial metabolite toward target weeds are due to presence of the phytotoxic compounds. Natural herbicidal products are present in the culture broth from aerobic shake cultures of the Rhizosphere *Streptomyceslevis* LX-65, *Streptomyces spp* are known to synthesize an array of biologically active metabolites, phytotoxic in nature from liquid culture filtrates **[46, 47].**

Also the observed result have less significant effect on *Triticumsativum* and *Zea mays* growth and germination(p<0.05) with some stimulatory effect in the case of *Zea mays*, regardless of the concentration of the tested metabolite.

3.4. Conventional taxonomy

After 7 days of incubation at 30˚C, pure colonies were isolated on starch casein agar medium and subcultured on ISP4 agar medium.

Cultural characteristics of strain LX-65 are presented in Table (4). It exhibited excellent growth on ISP-3 and ISP-4 and good growth on ISP-7 media. The color of the aerial mycelium appeared very pale green to moderate bluish green, while that of substrate mycelium range from moderate pink to pinkish white. The strain produced pinkish white pigment on ISP-3 and ISP-4 and exhibited melanin pigmentation on ISP-6 and ISP-7 media. The strain exhibited superior growth on ISP-4 and ISP3, good growth on ISP 6, 7 and poor growth on ISP 1, 2, 5 media. Diffusible pigment or melanin on any of the tested media was noticed on ISP 6 and ISP 7 media.

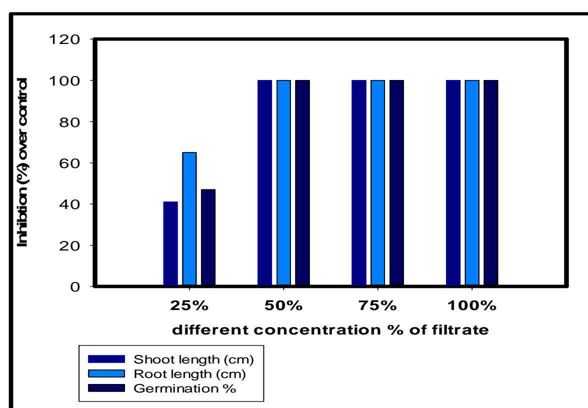
Micro-morphological characteristics of actinomycete isolate, LX-65 grown on inorganic salts-starch agar (ISP-4) under light microscopy figure (5) exhibited spiral shaped mycelium that further differentiated into hairy surfaced spores and special morphological characteristics were seen under scanning electron microscope figure (6).

Table (1): Antibacterial activity of the most activeactinomycetes isolates

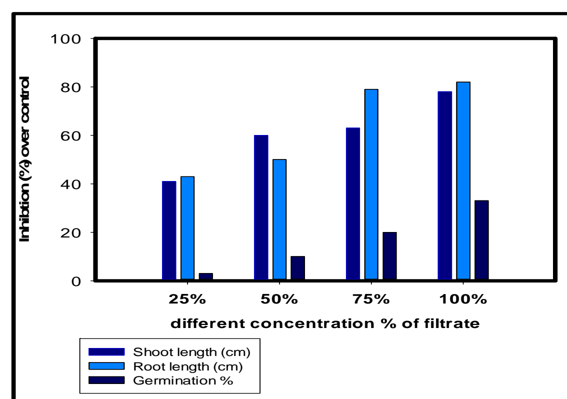
Isolates	**Mean diameter of inhibition zone (mm) of the tested bacterial strains**			
	B. subtilis ATCC 6633	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 7839
DK-2	13.0	16.0	0.0	21.0
FA-25	16.0	30.0	0.0	23.0
FA-34	20.0	17.0	0.0	0.0
FA-35	16.0	17.0	0.0	0.0
CA-45	0.0	18.0	14.0	15.0
LX-60	16.0	0.0	0.0	17.0
LX-65	**19.0**	**35.0**	**16.0**	**28.0**
LX-66	19.0	0.0	0.0	20.0
LX-78	0.0	16.0	14.0	0.0
LX-105	0.0	18.0	10.0	18.0
LX-118	20.0	21.0	13.0	0.0
LX-120	16.0	20.0	0.0	15.0

Table (2):Antifungal activity of the selected most potent isolate, LX-65.

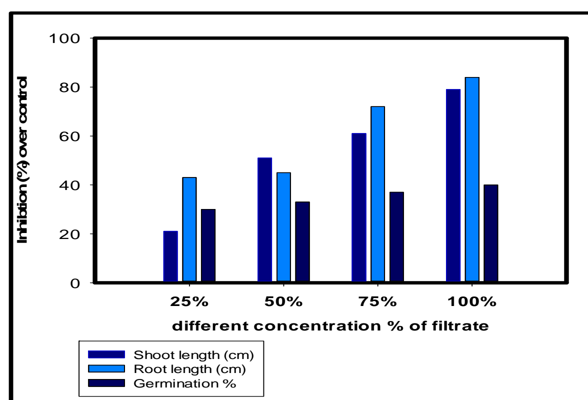
Isolate	Mean diameter of inhibition zone (mm) of the tested fungal strains					
	Candidaalbicans (IMRU3669)	*Fusariumoxysporum* (RCMB008002)	*Aspergillusniger*(IMI31276)	*Aspergillusflavus*(IMI111023)	*Trichodermviride*(RCMB008002)	*Pencilliumchrysogenum (RCMB 001015)*
LX-65	31.0	26.0	22.0	19.0	15.0	0.0



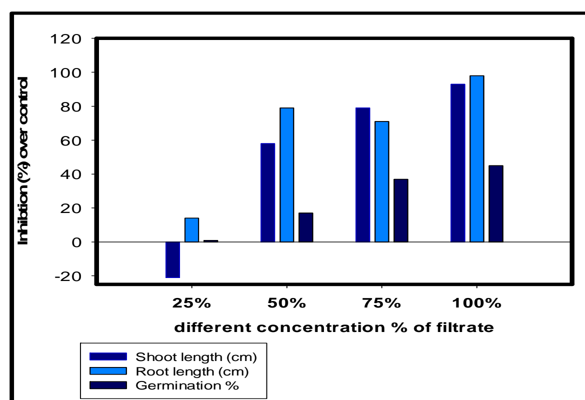
(1) *Erucda sativa*



(2) *Raphanussativus*

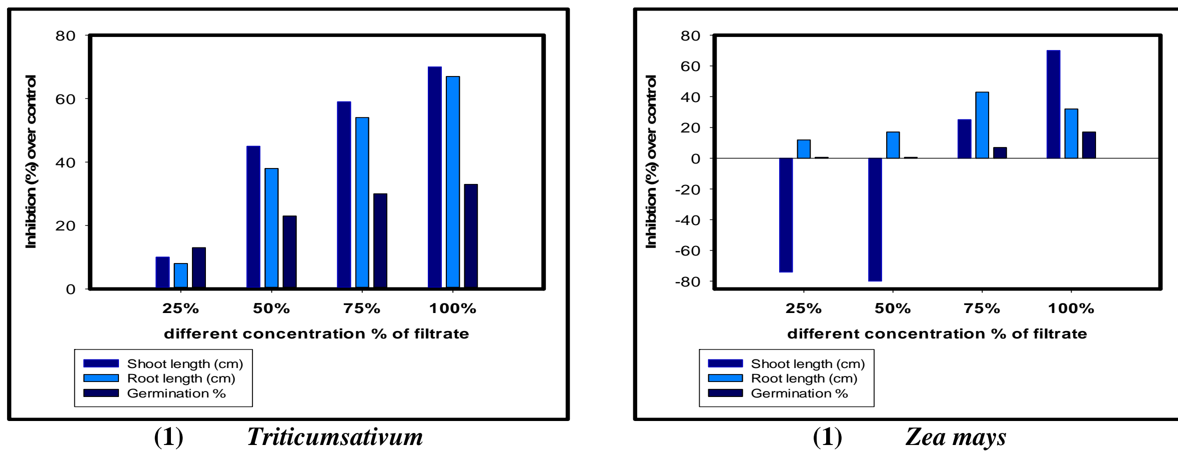


(3) *Loliummultiflorum*



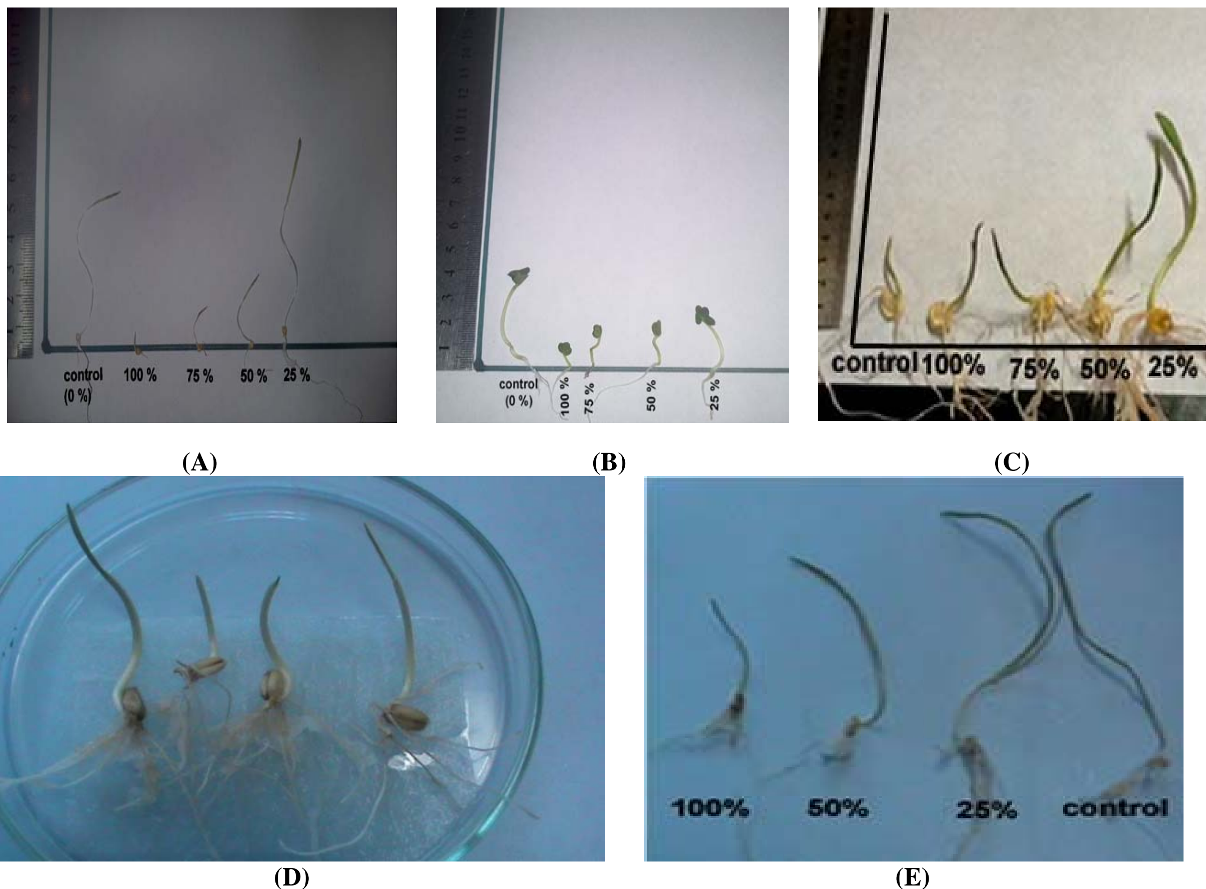
(4) *Echinochloacrusgalli*

Figure (1): The percentage of inhibition seed germination, shoot length and root length of seed weeds treated with the different concentrations of the culture filtrate.



(1) ***Triticumsativum*** (1) ***Zea mays***

Figure (2): The percentage of inhibition seed germination, shoot length and root length of seeds treated with the different concentrations of the culture filtrate.



(A) **(B)** **(C)**

(D) **(E)**

Figure (3): (A)*Echinochloacrusgalli*seedstreatedwith the culture filtrate of different concentration of LX-65 isolate, (B)*Raphanussativus* seedstreatedwith the culture filtrate of different concentration of LX-65 isolate, (C)) *Zea mays* seedstreatedwith the culture filtrate of different concetration of LX-65 isolate.(D) And (E)*Triticumsativum*seedstreatedwith the culture filtrate of different concetration of LX-65 isolate and **Taxonomic characterization of actinomycete isolate, LX-65**.

Table (3): % Reduction of target weed seedling growth and germination as response to LX-65 isolate metabolites.

LX-65 isolate							
% Reduction	Conc.	*Eruca sativa*	*Raphanussativus*	*Loliummultiflorum*	*Echinochloacrusgalli*	*Triticumsativum*	*Zea mays*
Shoot length (cm)	25%	41	41	21	-21	10	-74
	50%	100	60	51	58	45	-80
	75%	100	63	61	79	59	25
	100%	100	78	79	93	70	70
LSD (0.05)	1.133						
Root length (cm)	25%	65	43	43	14	8	12
	50%	100	50	45	79	38	17
	75%	100	79	72	71	54	43
	100%	100	82	84	98	67	32
LSD (0.05)	2.775						
Germination %	25%	47	3	30	0	13	0
	50%	100	10	33	17	23	0
	75%	100	20	37	37	30	7
	100%	100	33	40	45	33	17
LSD (0.05)	1.089						

Whole cell hydrolysate of this strain contained LL-diaminopimelic acid (LL-DAP) and glycine indicating that, the strain has a chemo-type I cell wall but no characteristic sugars could be detected. Cell-wall composition analysis is one of the main methods that can be employed to identify the chemotaxonomic characteristics of *Streptomyces*; the presence of LL-DAP in the cell wall also signifies that this strain is *Streptomyces* **[48].** The physiological and biochemical properties; carbon and nitrogen sources utilization; enzymatic activities, tolerance to NaCl; growth pH; growth temperature; growth inhibitors and resistance to antibiotics were presented in table (5).

Enzymes such as amylase, Lipase, Catalase, Nitrate reductase and Urease by the strain was noticed. The strain utilized D-galactose, D-glucose, D-xylose, L–Arabinose, Maltose, Lactose, Mannitol,Raffinose, cellulose, starch and sucrose as carbon sources indicating its wide pattern of carbon assimilation. It exhibited salt tolerance up to 6% that may be placed in the intermediate group of salt tolerance, as suggested by Tresner*et al.* (1968)**[49]**. It was found positive for biochemical tests like xanthine degradation.

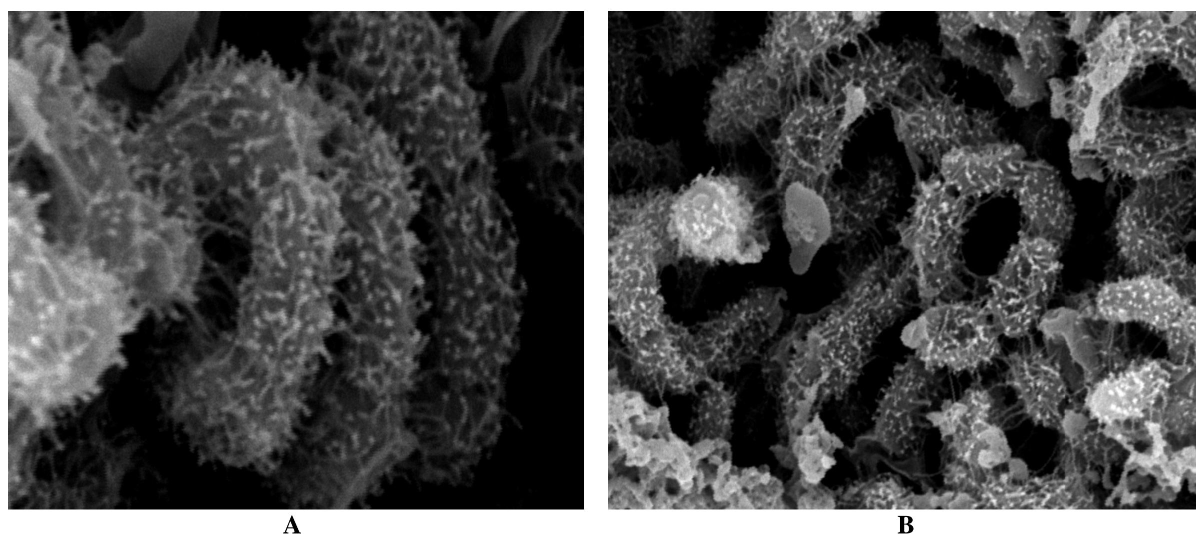
It showed sensitivity to a variety of antibiotics, but was resistant to Penicillin, and norfloxacin. Kampfer*et al*. (1991) **[34]** suggested that all these tests are indispensable tools for classification of actinobacteria and suggesting that bioactive compounds produced by the strain may be responsible for the resistance of the strain to the antibiotics.

Table (4): Cultural characteristics of actinomycete isolate LX-65 grown on different ISP media.

Culture media	Growth	Substrate mycelium	AerialMycelium	DiffusiblePigments
Tryptone yeast extract broth (ISP–1)	Weak	pinkish white (ISCC-NBS 9)	light reddish purple (ISCC-NBS 240)	None
Yeast -malt extract agar(ISP–2)	Weak	pinkish white (ISCC-NBS 9)	-ve	None
Oatmeal agar(ISP–3)	Excellent	moderate pink (ISCC-NBS 5)	very pale green(ISCC-NBS 148)	pinkish white (ISCC-NBS 9)
Inorganic-trace salt-starch agar (ISP–4)	Excellent	dark pink(ISCC-NBS 6)	moderate bluish green (ISCC-NBS 164)	pinkish white (ISCC-NBS 9)
Glycerol asparagine agar(ISP–5)	Weak	white (ISCC-NBS 263)	-ve	None
Peptone yeast extract iron agar (ISP–6)	Good	greenish white (ISCC-NBS 153)	light gray (ISCC-NBS 264)	dark gray (ISCC-NBS 266)
Tyrosine agar(ISP–7)	Good	dark gray (ISCC-NBS 266)	pale green (ISCC-NBS 149)	dark gray (ISCC-NBS 266)

A B

Figure (5): A and B Phase-contrast micrograph of actinomycete LX-65 showing spiral shaped mycelium.



A B

Figure (6): A and B Scanning electron microscopy (SEM) showing spiral shaped mycelium that further differentiated into hairy surfaced spores.

3.4.1. 16S rRNA gene sequencing and phylogenetic analysis

To confirm the identification of the isolated strain LX-65, 16S rRNA gene sequence of the local isolate was compared to sequences of 9 *Streptomyces* spp. Experimental analysis of the PCR amplification was studied through the agarose gel electrophoresis exhibited specific 16S rRNA band. The phylogenetic tree Figure (7) showed that the locally isolated strain is closely related to *Streptomyces levis,* was constructed using the neighbour-joining method with aid of geneius pro 7.1.5 tree builder program. Bar 0.003 substitutions per nucleotide position. Multiple sequence alignment was done between the sequences of the 16S rRNA genes of *Streptomyces levis* and other eight *Streptomyces* spp and the local isolate. Computer assisted DNA similarly searches against bacterial database revealed that 16S rRNA sequence was 84% identical to *Streptomyces levis*. The nucleotide 16S rRNA was deposited in NCBI genbank under accession number KJ726667.1 (Fig. 8).

Table (6): Physiological and biochemical characteristics of actinomyceteisolateLX-65

Character	Results	Character	Results
• **Melanin pigment:**		8. L-tyrosine	+
Tryptone-yeast extract broth	–[a]	9. Peptone	+++[e]
Peptone-yeast extract iron agar	–	10. Ammonium sulphate	+++
Tyrosine agar	++[b]	11. Yeast extract	+++
• **Enzymatic activities:**		12. Urea	+++
1. Amylase	++	• **Tolerance to NaCl concentrations:**	
2. Protease	–	1.0 - 5.0 %	+++
3. Lipase	++	6.0 %	+
4. Pectinase	–	7.0 %	–
5. Catalase	+[c]	• **Growth temperature °C:**	
6. Gelatinase	–	10.0	–
7. Nitrate reductase	+	25.0 – 30.0 °C	++
8. Urease	+	35.0 – 45.0 °C	+++
9. Xanthine degradation	+	50.0 °C	Wg
10. H_2S production	–	55.0 °C	–
• **Carbon sources utilization:**		• **Growth pH:**	
1. Starch	++	4.0	–
2. Maltose	+	5.0 –10.0	++
3. D–Glucose	++	11.0 –12.0	+++
4. L–Arabinose	+	13.0	–
5. D–XyLose	++	• **Tolerance to growth inhibitors:**	
6. Lactose	++	Sodium azide (0.01%)	+
7. Mannitol	++	Sodium azide (0.02%)	+
8. Sucrose	+	Phenol (0.1%)	+
9. D–Galactose	+	Crystal violet (0.0001%)	+
10. Raffinose	++	Thallus acetate (0.001%)	–
11. Cellulose	+	• **Resistance to antibiotics:**	
• **Nitrogen sources utilization:**		Erythromycin (15 µgm)	++
1. L-asparagine	++	Penicillin (25 µg/ml)	–
2. L-arginine	+	Ciprofloxacine (30 µgm)	++
3. L-histidine	++	Tetracycline (15 µgm)	+++
4. L-phenylalanine	++	Bacitracin (50 µgm)	++
5. L-threonine	Wg[d]	Chloramphinicol (30µgm)	++
6. L-cysten	Wg	Norfloxacin (30 µgm)	–
7. L-serine	++	Rifampicin(50 µg/ml)	++

[a](-) = negative or no growth, [b](++) = good growth, [c](+) = moderate, [d](wg) = weak growth, [e](+++) = abundant(very good growth).

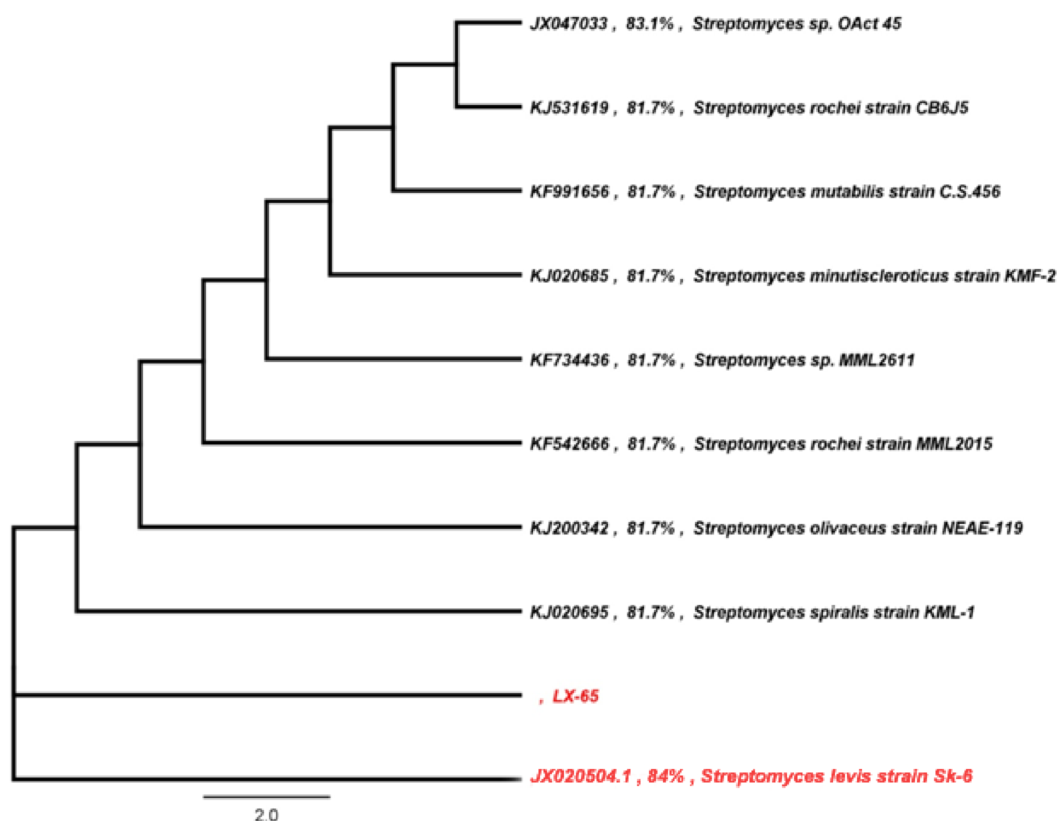


Figure (7):Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequence of *Streptomyces levis* strain LX-65 and phylogenetically related members of this genus

Conclusion

Conventional herbs management has been significantly influenced by bioactive natural products that are used directly, or in a derived form, as herbicides. Biobasedherbicides are commonly used as alternatives to synthetic compounds in organic agriculture. While some of these herbicidal and fungicidal compounds have transferred successfully in the more conventional crop production systems, good natural herbicides have been lacking.Actinomycetes and specially *Streptomyces* species have also been used directly as biological control agents, as an alternative to agrochemicals, for control of the plant diseases. The bioactive metabolities produced by the isolated soil *Streptomyces levis* strain LX-65 can be produced on a large scale as an agrochemical biocontrol agent.

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