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Research Article
Antimicrobial and Insecticidal Activities of n-Butanol Extracts from Some *Streptomyces* Isolates

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Abstract

Background: Increasing antibiotic resistance and the appearance of multidrug-resistant pathogenic strains of bacteria and fungi are becoming a serious global problem. The use of biological insecticides instead of the chemical insecticides has been increased because of their safety, specificity and biodegradability. **Objectives:** The objectives of the study were to identify *Streptomyces* isolates screened from soil and to determine their antibacterial, antifungal and insecticidal activities. **Materials and Methods:** *Streptomyces* isolates collected from soils in various habitats of Jordan were cultured on Starch-Casein-Nitrate Agar (SCNA) medium and identified based on their morphological and cultural characteristics. Tryptone Soy Broth (TSB) cultures of isolates were extracted by n-butanol and screened for their antibacterial and antifungal activities using the agar-well diffusion method. Furthermore, the extracts were examined for their insecticidal activity against *Drosophila melanogaster* using conventional bioassay protocol. The LC_{50} values of extracts at 95% confidence interval were determined by probit analysis. **Results:** In total, 127 *Streptomyces* isolates were isolated from Jordanian soils with white aerial mycelia and rectus-flexous sporophores being dominant. It was found that the n-butanol extracts of 37 *Streptomyces* isolates prepared from cultures that were grown in TSB medium, which was considered as the most suitable medium in this study for the production of antimicrobial activity, exhibited antibacterial and/or antifungal activities on multidrug resistant microorganisms. Interestingly, it was observed that 11 isolates exhibited antibacterial activity on MRSA. It was also found that none of the isolates which displayed orange, red and yellow aerial mycelia produced antimicrobial activity. On the other hand, 19 isolates divided into seven color series exhibited insecticidal activity against *D. melanogaster*. The insecticidal activity of combined crudes of the most significantly toxic *Streptomyces* isolate S2 and *Bacillus thuringiensis* subsp. *israelensis* J63 was higher than that produced from either S2 or J63. Based on the interspacer region 16S-23S rRNA gene sequence analysis, the sequence alignment of the selected isolates had the greatest possible identity to *Streptomyces* and are grouped into two subclusters in the phylogenetic tree. **Conclusion:** *Streptomyces* cultures obtained from TSB medium have an increased and a promising antimicrobial activity against multidrug-resistant pathogens. Combined crudes of *Streptomyces* and *B. thuringiensis* produced competitive insecticidal activity.

Key words: *Streptomyces*, antibacterial, antifungal, methicillin-resistant, insecticidal, *thuringiensis*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Increasing antibiotic resistance among human pathogens, particularly pathogenic bacteria and fungi, is becoming a serious challenge and a cause for global concern. The appearance of multidrug-resistant pathogenic strains of bacteria and fungi has caused a considerably high rate of morbidity and mortality among patients. In response, there is a need to renew the interest in discovering novel classes of antibiotics that have different mechanisms of action, especially from *Streptomyces* which are known to be the major source of antibiotics[1-3]. The *Streptomyces* species produces about three-fourth of commercially and medically useful antibiotics[4].

Streptomyces is the largest genus among prokaryotes with more than 560 species[5]. It is a Gram-positive soil bacterium that belongs to the family *Streptomycetaceae* and to the phylum Actinomycetes[6]. *Streptomyces* is an interesting and an unusual microorganism among bacteria because it has a complex developmental life cycle, involving the growth of mycelia and formation of spores. Many compounds produced by *Streptomyces* species have a high commercial value as antimicrobial, anticancer or immunosuppressant agents[7]. *Streptomyces* species are of great industrial importance because of their ability to produce many clinically useful antibiotics and has been the subject of many studies in the quest for novel antibiotics[8,9]. The importance of discovering new antimicrobial metabolites from *Streptomyces* is needed due to the increase in resistant pathogens as well as the evolution of novel diseases[10].

On the other hand, the use of biological insecticides in the place of chemical insecticides is safer for invertebrates and vertebrates, host specific and biodegradable. As a result, the demand for the use of biological insecticides instead of chemical insecticides for crop and forest protection and in insect vector control has been increased in the last years. The great success recorded in biological control came from the use of *Bacillus thuringiensis* products. However, little is known about the use of *Streptomyces* products in biological control. There are few recent studies[11-15] which illustrated the importance of *Streptomyces* antibiotics in the biological control of insects.

Streptomycetes are well known and successfully exploited as a source of secondary metabolites. It has been estimated that most of the naturally occurring antibiotics have been isolated from streptomycetes. Therefore, the objectives of the current study were to isolate and identify *Streptomyces* from Jordanian habitats and to determine their antibacterial and antifungal activities against multidrug-resistant pathogens as well as the assessment of their insecticidal activity against larvae of the model insect, *Drosophila melanogaster*.

MATERIALS AND METHODS

Collection of samples: A total of 150 soil samples were collected from the 12 governorates of Jordan; Ajloun, Amman, Aqaba (including, Red Sea shore), Balqa (including, Shoaib Valley and Dead Sea area), Jerash, Irbid (including, Yarmouk river and Jordan river areas), Karak, Ma'an, Madaba, Mafraq, Tafilah and Zarqa. The samples were taken 5 cm beneath the soil surface and placed in tightly closed bags.

Isolation of *Streptomyces*: For each collected sample, 1 g of soil was added to 9 mL sterile distilled water, mixed vigorously and allowed to stand. Three ten fold serial dilutions were prepared using sterile distilled water with a total volume of 10 mL. An aliquot of 100 µL from each dilution was plated on Starch-Casein-Nitrate Agar (SCNA) medium supplemented with antifungal agents (50 mg L^{-1} cyclohexamide and 50 mg L^{-1} Nystatin); the plates were incubated for 3 weeks at 30°C[16]. After incubation, typically pigmented, dry, powdery colonies were selected from a mixed plate culture, subcultured on new SCNA plates and incubated at 30°C for three weeks[17]. The colonial diversity of total bacteria and *Streptomyces* were estimated at the end of the incubation period.

The mass color of mature sporulating aerial mycelium was observed after growth on SCNA plates. The aerial mass was classified into different color series. Distinctive colors of the substrate mycelium and the production of soluble pigments was also recorded[16]. According to the shape of the sporophores, the isolates were grouped into different categories[18].

Antimicrobial activity

Test microorganisms: A total of 11 reference bacterial species (two Gram positive; *Staphylococcus aureus* ATCC 25923 and Methicillin resistant *Staphylococcus aureus* ATCC 95047 [MRSA] and nine Gram negative; *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27253, *Klebsiella pneumonia* ATCC 7700, *Klebsiella oxytoca* ATCC 13182, *Enterobacter aerogenes* ATCC 35029, *Proteus vulgaris* ATCC 33420 and *Proteus mirabillis* ATCC 12453) and seven fungal species (two reference species: *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 and five clinical

species: *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium marneffei* and *Trichoderma hamatum*) were used in this study to test the antibacterial and antifungal activities, respectively.

Resistance of test microorganisms to some standard antibiotics: Seven standard antibiotics (Ampicillin 10 µg, Chloramphenicol 30 µg, Erythromycin 15 µg, Nalidixic acid 30 µg, Penicillin G (10 units), Streptomycin 10 µg and Vancomycin 30 µg) were examined for multidrug-resistance against the test bacteria and two standard antibiotics (Cycloheximide 250 µg, Nystatin 10 µg) were investigated in resistance to the test fungi. Aliquots of 50 µL from each test bacteria were swabbed uniformly on a Nutrient Agar (NA) medium and allowed to dry for 5 min. Thereafter, one disc of each disc type of standard antibiotics was placed on NA medium surface and incubated for 24 h at 37°C. The same regimen was performed for the test fungi using Potato Dextrose Agar (PDA) medium and an incubation period of 48 h at 28°C. The antimicrobial activities were determined by measuring the diameter of generated inhibition zones[19].

Preparation of inoculums: The test bacteria and fungi were cultured in Nutrient Broth (NB) at 37°C for 24 h and Sabouraud Dextrose Broth (SDB) at 28°C for 48 h, respectively. The cultures were adjusted to achieve 2×10^6 CFU mL^{-1} for bacteria and 2×10^5 spore mL^{-1} for fungi[20].

Medium determination for antimicrobial activity production from *Streptomyces:* For antimicrobial activity of isolated *Streptomyces*, five types of broth media were prepared, namely: Tryptone Soy Broth (TSB), Starch-Casein-Nitrate Broth (SCNB), Glycerol-Arginine Broth (GAB), Tryptone-Yeast Extract-Glucose Broth (TYEGB) and Actinomycete Isolation Broth (AIB), to determine the best broth medium type for the production of active antimicrobial agents. The pH was adjusted to 7.0-7.2 with 1 M NaOH before autoclaving at 121°C for 20 min[21]. A loopfull from pure *Streptomyces* SCNA-culture was inoculated into 250 mL of each sterile broth media and incubated for two weeks at 30°C on 200 rpm rotary shaker. *Streptomyces* cultures were centrifuged at 13,000 rpm for 10 min and the supernatant was extracted with n-butanol. Thereafter, the extracts were filtered through 0.45 µm membrane syringe filter. The filtrate was evaporated at 40°C in a water bath. After evaporation, the residues were resuspended in Phosphate Buffer Saline (PBS) to achieve a concentration of 200 mg mL^{-1} which is used for testing the antimicrobial activities.

The antimicrobial activities were determined using the agar-well diffusion method. Aliquots of 50 µL from each test microorganism were swabbed uniformly on NA medium for bacteria and on PDA medium for fungi and allowed to dry for 5 min. Sterilecork borer (6 mm in diameter) was used to make wells in the seeded agar. Then, 50 µL from each *Streptomyces* extract was added into each well and allowed to stand on the bench for 1 h for proper diffusion and thereafter, incubated for 24 h at 37°C for test bacteria and for 48 h at 28°C for test fungi. The antimicrobial activities were determined by measuring the diameter of generated inhibition zones[20]. Negative controls using 50 µL broth media were also run in the same manner which are parallel to the treatments. These studies were performed in triplicates. *Streptomyces* crude extracts that were obtained from the best growth medium were examined for their antimicrobial activities as described above using the agar-well diffusion method.

Insecticidal activity: Insecticidal activity of *Streptomyces* crude extracts obtained from the best growth medium is determined against the third instar larvae of *Drosophila melanogaster.* Different crude concentrations (60 , 40, 20, 10 and 5 mg mL^{-1}) were prepared for each isolate. After 2 weeks of incubation at 25°C of ten *D. melanogaster* flies (five males and five virgin females) in a flask containing 15 g artificial diet, the adults were cleared and the diet containing larvae was kept afloat by tap water, then the floated larvae were collected and kept in sterile petri dishes. Ten third instar larvae were placed into each well of 24 well-plates containing 700 µL *D. melanogaster* broth media and 300 µL *Streptomyces* crude extract. The toxicity of each crude was assayed in triplicates. The larvicidal effect of *Streptomyces* crudes was determined by counting the number of dead larvae after 24 h incubation at 25°C. Dead larvae were identified when they failed to move after probing with a needle, they also have a brown midgut in the middle region of the larvae[14]. The mortality rate was scored in comparison to parallel control, using 300 µL PBS instead of *Streptomyces* crude. The scored mortalities were corrected according to Abbott's formula[22] and the lethal concentration values of *Streptomyces* crude extract that killed 50% (LC_{50}) of larvae at 95% confidence interval were determined using probit analysis.

The insecticidal activity of the most active *Streptomyces* isolate was compared with that of *Bacillus thuringiensis* subsp. *israelensis* (Bt strain J63, LC_{50} = 0.17 ng mL^{-1}), which was previously reported[23] to exhibit the most significant insecticidal activity against *D. melanogaster* larvae. The mortalities were assayed in triplicates and each crude was

scored every 12 h. The combined insecticidal activity of *Streptomyces* and Bt crudes against *D. melanogaster* was also tested by preparing a mixture (1:1) from both bacterial crudes.

Molecular characterization of insecticidal *Streptomyces*

DNA extraction: Pellets of local *Streptomyces* isolates which exhibited insecticidal activity were harvested by centrifugation at 14,000 rpm for 5 min from TSB broth, cell pellets were washed twice with distilled water, then the DNA was isolated based on the manufacturer's instructions using the Wizard Genomic DNA purification kit (Promega, USA, part no. A1120).

PCR amplification of the interspacer region 16, 23 rRNA gene: Amplification of the interspacer region16S-23S rRNA gene from each genomic DNA extract was carried out in a DNA Thermal Cycler for 35 reaction cycles as described previously[24] with some modifications. Each reaction was carried out in 25 µL; 3 µL of template DNA (1 ng) was mixed with 1X reaction buffer (5 µL), 1.5 mM $MgCl_2$ (3 µL), 200 µM deoxynucleoside triphosphates (dNTPs; 0.5 µL), 0.4 µM forward primer GP1 5´-GCGATTGGGACGAAGTCG-3´ (0.5 µL), 0.4 µM reverse primer GP2 5´-TATCGTGGC CTCCCACGTCC-3´ and 1 U of *Taq* DNA polymerase (1 µL). The PCR program used was an initial denaturation (96°C for 5 min), 35 cycles of denaturation (95°C for 5 min), annealing (55°C for 1 min) and extension (72°C for 1 min), as well as the final extension (72°C for 10 min). Products were analyzed by electrophoresis in 1% agarose gel. A 500 bp DNA ladder marker (Genedirex, USA) was used to estimate the approximate molecular weight of the amplified interspacer region 16S and 23S rRNA gene products (Predicted size is 300-400 bp). Generated bands were visualized and photographed by UV Trans-illumination.

Sequencing and phylogenetic analysis: The sequences of the interspacer region 16S-23S rRNA gene from PCR products of *Streptomyces* isolates were determined with an Applied Biosystems model 373A DNA sequencer using the ABI PRISM cycle sequencing kit (Macrogen, Korea). The sequences were compared with those contained within the GenBank[25] using a BLAST search[26]. The most closely related 16S-23S rRNA gene sequences to the isolates of this study were retrieved from the database. Retrieved sequences were then aligned and the phylogenetic tree was constructed by the use of DNAMAN 5.2.9 sequence analysis software. The phylogenetic tree was built by the neighbor-joining method, using maximum likelihood parameter distance from the partial 16S-23S rDNA sequences. The reference strain, *S. griseus* ATCC 10137 was used for comparison and *E. coli* ATCC 25922 was used as the outgroup.

RESULTS

Phenotypic and microscopic characterization of isolates: A total of 150 soil samples were collected from cultivated and non-cultivated lands in 12 locations of Jordan (Table 1). From the screened soil samples, 574 diverse bacterial colonies were obtained. Out of them, 127 (22.13%) met the criteria of the genus *Streptomyces*. The *Streptomyces* isolates, cultured on SCNA were classified according to the color of aerial mycelium (Table 2), including; black, brown, gray, green, orange, pink, red, white and yellow. It was observed that the white color series (49 isolates), followed by the green color series (38 isolates) were the most common among the isolates.

Reverse (substrate mycelium) and soluble (diffusible) pigments as well as melanin pigment production from *Streptomyces* isolates were identified (Table 2). The majority of isolates (83%) produced soluble pigment and more than 60% of the isolates produced reverse pigment. For melanin pigmentation, about one-third of the isolates were melanin producers. The isolates that displayed orange, pink and red aerial mycelia did not produce melanin. In addition, the isolates with orange and pink aerial mycelia gave no reverse and soluble pigments. It was observed that all isolates producing black, brown, green and yellow aerial mycelia produced pigments for both soluble and reverse sides.

Based on the shape of sporophore, the *Streptomyces* isolates were divided into four major groups: Rectus-Flexous (RF), Monoverticillate (MV), Biverticillate (BIV) and Spira (S) (Table 2). The isolates that produced RF sporophores were the most common (94 isolates, 74%). Eighteen isolates produced MV sporophores. Only four isolates produced BIV sporophores and the remaining eleven isolates produced S-shaped sporophores.

Antimicrobial activities: For determination of the nutritionally suitable medium for the production of bioactive metabolites

Table 1: Isolation of *Streptomyces* from Jordanian soils

Location	No. of samples	No. of diverse colonies	No. of diverse *Streptomyces* (%)
Ajloun	14	44	9 (20.45)
Amman	19	93	19 (20.43)
Aqaba	12	47	9 (19.15)
Balqa	25	106	25 (29.07)
Irbid	25	113	34 (30.09)
Jerash	8	32	8 (25.00)
Karak	7	17	4 (23.53)
Ma'an	8	18	3 (16.67)
Madaba	10	29	7 (24.14)
Mafraq	7	25	2 (8.00)
Tafilah	8	24	5 (20.83)
Zarqa	7	26	2 (7.69)
Total	150	574	127 (22.13)

Table 2: Classification of *Streptomyces* isolates based on morphological and cultural characteristics

	Color series of aerial mycelium									
	Black	Brown	Gray	Green	Orange	Pink	Red	White	Yellow	Total No.
No. of isolates	2	9	17	38	3	2	2	49	5	127
Pigment production										
Melanin	2	9	5	10	0	0	0	16	2	44
Reverse	2	9	5	38	0	0	2	16	5	77
Soluble	2	9	14	38	0	0	1	36	5	105
Sporophore morphology										
Rectus-Flexous (RF)										
RF-flexous	0	4	4	20	0	0	0	16	3	47
RF-fascicled	0	0	1	6	0	0	2	12	2	23
RF-straight	2	0	6	8	2	0	0	6	0	24
Monoverticillate (MV)										
MV with no spirals	0	2	0	2	0	0	0	4	0	8
MV with spirals (MV-S)	0	0	2	0	0	2	0	6	0	10
Biverticillate (BIV)	0	2	0	0	0	0	0	2	0	4
Spiral (S)										
S-closed	0	1	4	2	1	0	0	2	0	10
S-opened	0	0	0	0	0	0	0	1	0	1

Table 3: Resistance of test microorganisms to some standard antibiotics

*Test microorganisms	**Resistance patterns
Bacteria	
Escherichia coli ATCC 8739	AMP10, ERY15, VA30
Escherichia coli ATCC 25922	AMP10, P10, VA30
Enterobacter aerogenes ATCC 35029	AMP10, ERY15, P10, VA30
Klebsiella oxytoca ATCC 13182	CHL30, ERY15, NA30, P10, S10, VA30
Klebsiella pneumonia ATCC 7700	AMP10, P10, VA30
MRSA ATCC 95047	AMP10, P10
Pseudomonas aeruginosa ATCC 27253	AMP10, CHL30, ERY15, P10, S10
Proteus mirabillis ATCC 12453	AMP10, P10
Proteus vulgaris ATCC 33420	AMP10, P10, VA30
Salmonella typhimurium ATCC 14028	AMP10, NA30, P10, VA30
Staphylococcus aureus ATCC 25923	AMP10, ERY15, P10, VA30
Fungi	
Aspergillus brasiliensis ATCC 16404	CYX250, NYS10
Candida albicans ATCC 10231	NYS10
Alternaria alternata	CYX250
Aspergillus niger	CYX250, NYS10
Fusarium oxysporum	NYS10
Penicillium marneffei	CYX250
Trichoderma hamatum	NYS10

*MRSA: Methicillin resistant *Staphylococcus aureus*, **AMP10: Ampicillin 10 μg, CHL30: Chloramphenicol 30 μg, ERY15: Erythromycin 15 μg, NA30: Nalidixic acid 30 μg, P10: Penicillin G (10 units), S10: Streptomycin10 μg, VA30: Vancomycin 30 μg, CYX250: Cycloheximide 250 μg, NYS10: Nystatin 10 μg. CYX250 and NYS10 activities were not determined for bacteria. The resistance for AMP10, P10 and S10 when inhibition zone (IZ) $\leq$11 mm; for CHL30 when IZ $\leq$12 mm, for ERY15, NA30 and VA30 when IZ$\leq$13 mm; CYX250 and NYS10 when IZ$\leq$8 mm

from *Streptomyces* isolates, five broth media (TSB, SCNB, GAB, TYEGB and AIB) were used. Antimicrobial activity was screened against the test microorganisms which exhibited multidrug resistance (Table 3). It was found that the highest number of active *Streptomyces* isolates with antimicrobial activity against each test microorganism was obtained from TSB cultures (Table 4). Therefore, the TSB broth medium was chosen for the production of active antimicrobial agents.

Antimicrobial activities were estimated by measuring the diameters of inhibition zone. The inhibitory effects of *Streptomyces* crudes prepared from TSB cultures were investigated against 18 test microorganisms, including 11 bacterial species resistant to at least two antibiotics especially AMP10 and P10 and seven fungal species, exhibiting resistance against either CYX250 or NYS10 or both (Table 3). Out of the 127 *Streptomyces* isolates subjected to antimicrobial screening process (Table 5), only 37 isolates were found to exhibit antibacterial and/or antifungal activities. Most of the active isolates were found to belong to white color series. It was observed that at least two isolates belonging to

Table 4: Determination of the suitable growth medium for production of bioactive products from 127 *Streptomyces* isolates when screening of antimicrobial activity

	No. of active isolates				
Growth medium	TSB	SCNB	GAB	TYEGB	AIB
Escherichia coli ATCC 8739	25	10	8	8	4
Escherichia coli ATCC 25922	28	12	9	10	6
Enterobacter aerogenes ATCC 35029	18	7	5	6	4
Klebsiella oxytoca ATCC 13182	18	9	8	12	7
Klebsiella pneumonia ATCC 7700	18	12	9	11	5
MRSA ATCC 95047	11	6	4	7	8
Pseudomonas aeruginosa ATCC 27253	17	8	11	9	13
Proteus mirabillis ATCC 12453	17	8	8	9	11
Proteus vulgaris ATCC 33420	24	10	6	8	10
Salmonella typhimurium ATCC 14028	18	11	5	4	12
Staphylococcus aureus ATCC 25923	23	16	14	14	17
Aspergillus brasiliensis ATCC 16404	20	8	6	9	9
Candida albicans ATCC 10231	20	7	7	9	9
Alternaria alternata	15	6	8	6	8
Aspergillus niger	14	7	7	5	6
Fusarium oxysporum	16	6	6	6	5
Penicillium marneffei	17	5	4	8	7
Trichoderma hamatum	15	6	5	6	3

TSB: Tryptone soy broth, SCNB: Starch-casein-nitrate broth, GAB: Glycerol-arginine broth, TYEGB: Tryptone-yeast extract-glucose broth, AIB: Actinomycete isolation broth. MRSA: Methicillin resistant *Staphylococcus aureus*

Table 5: Antimicrobial activity of *Streptomyces* isolates against test microorganisms

	Color series of aerial mycelium									
	Black	Brown	Gray	Green	Orange	Pink	Red	White	Yellow	Total (%)
No. of isolates	2	9	17	38	3	2	2	49	5	127
No. of active isolates	1	1	5	12	0	1	0	17	0	37 (29.13)
Escherichia coli ATCC 8739	0	1	5	7	0	1	0	11	0	25 (67.57)
Escherichia coli ATCC 25922	1	1	4	10	0	1	0	11	0	28 (75.68)
Enterobacter aerogenes ATCC 35029	1	1	3	7	0	1	0	5	0	18 (48.65)
Klebsiella oxytoca ATCC 13182	0	1	3	5	0	1	0	8	0	18 (48.65)
Klebsiella pneumonia ATCC 7700	1	1	3	2	0	0	0	11	0	18 (48.65)
Pseudomonas aeruginosa ATCC 27253	1	0	3	5	0	0	0	8	0	17 (45.95)
Proteus mirabillis ATCC 12453	0	0	5	3	0	1	0	8	0	17 (45.95)
Proteus vulgaris ATCC 33420	1	0	5	4	0	1	0	13	0	24 (64.86)
Salmonella typhimurium ATCC 14028	0	0	2	7	0	1	0	8	0	18 (48.65)
Staphylococcus aureus ATCC 25923	0	1	4	7	0	1	0	10	0	23 (62.16)
MRSA ATCC 95047	1	0	2	2	0	1	0	5	0	11 (29.73)
Aspergillus brasiliensis ATCC 16404	0	0	4	4	0	1	0	11	0	20 (54.05)
Candida albicans ATCC 10231	0	0	4	4	0	1	0	11	0	20 (54.05)
Alternaria alternate	0	0	3	4	0	1	0	7	0	15 (40.54)
Aspergillus niger	0	0	3	3	0	0	0	8	0	14 (37.84)
Fusarium oxysporum	0	0	3	4	0	0	0	9	0	16 (43.24)
Penicillium marneffei	0	0	4	4	0	1	0	8	0	17 (45.95)
Trichoderma hamatum	0	0	4	2	0	1	0	8	0	15 (40.54)

MRSA: Methicillin resistant *Staphylococcus aureus*. Percentage represents the number of active isolates against test microorganisms out of total number of isolates

gray, green, or white color series produced antimicrobial activity against each test microorganism investigated in this study, including; the reference species of bacteria and fungi as well as the clinical strains of fungi. Eleven isolates showed antibacterial activity toward MRSA. However, none of the isolates which displayed orange, red and yellow aerial mycelia produced antimicrobial activity. Moreover, none of the isolates which displayed black and brown aerial mycelia produced antifungal activity.

Insecticidal activities: The larvicidal activity of the 127 *Streptomyces* isolates was investigated against the third instar larvae of *D. melanogaster* and the LC_{50} values were determined for toxic crudes. Nineteen isolates were found to exhibit larvicidal activity against *D. melanogaster* with LC_{50} values ranging from 4.04-19.56 mg mL^{-1} (Table 6). Those isolates belonged to seven color series, including; black, brown, gray, green, orange, pink and white. The most significantly toxic isolate to *D. melanogaster* larvae was

Table 6: Insecticidal activity of n-butanol extracts of local *Streptomyces* isolates against *Drosophila melanogaster*

Color series	Isolate	Sporophore morphology	LC_{50} (mg mL^{-1})	Regression equation
Black	S1	RF-Straight	8.52 (8.18-8.87)	y = 13.114Ln(x) + 21.904
Brown	S2*	BIV	4.04 (3.79-4.20)	y = 52.028Ln(x) - 142.49
	S6*	RF-Flexous	13.57 (13.21-13.78)	y = 31.866Ln(x) - 33.101
Gray	S13	RF-Straight	5.93 (5.85-6.29)	y = 17.832Ln(x) + 18.246
	S14	RF-Flexous	4.93 (4.60-5.23)	y = 20.196Ln(x) + 17.799
Green	S19	RF-Straight	4.93 (4.61-5.20)	y = 20.196Ln(x) + 17.799
	S21	RF-Flexous	13.57 (13.15-13.76)	y = 31.866Ln(x) - 33.101
	S25	RF-Fascicled	12.35 (12.06-12.54)	y = 30.74Ln(x) - 27.282
	S29*	RF-Flexous	14.52 (14.17-14.79)	y = 12.194Ln(x) + 17.377
Orange	S35*	RF-Straight	8.86 (8.49-9.20)	y = 16.224Ln(x) + 14.613
Pink	S37	MV-S	19.56 (19.12-19.94)	y = 19.938Ln(x) - 9.282
White	S39	RF-Straight	6.82 (6.49-7.27)	y = 7.1677Ln(x) + 36.241
	S44	RF-Flexous	8.84 (8.57-9.15)	y = 25.308Ln(x) - 5.1638
	S45	RF-Fascicled	10.28 (9.89-10.68)	y = 30.482Ln(x) - 21.029
	S46*	BIV	8.99 (8.61-9.31)	y = 22.279Ln(x) + 1.0746
	S50	RF-Flexous	10.25 (9.90-10.65)	y = 19.458Ln(x) + 4.7114
	S53	MV-S	13.57 (13.25-13.85)	y = 31.866Ln(x) - 33.101
	S54	MV	9.50 (9.16-9.77)	y = 31.336Ln(x) - 20.553
	S62	RF-Fascicled	13.97 (13.51-14.20)	y = 18.751Ln(x) + 0.5508

*Isolates which were not exhibited antimicrobial activity. LC_{50}: Median lethal concentration. The confidence limit at the 95% level is given in parentheses

Table 7: Comparison of the 16S-23S rRNA gene sequences of insecticidal *Streptomyces* isolates representing seven color series with that in the GenBank

Color series	Isolate	Sporophore morphology	Sequence No. of nucleotides[a]	Closest phylogenetic relative (GenBank accession No.)	Identity (%)[b]
Black	S1	RF-Straight	373	*S. griseus* subsp. *griseus* (NC010572)	87
Brown	S6	RF-Flexous	348	*S. coelicolor* (NC003888)	82
Gray	S13	RF-Straight	372	*S. griseoflavus* (ACFA01000783)	83
Green	S25	RF-Fascicled	346	*S. griseus* subsp. *griseus* (NC010572)	93
Orange	S35	RF-Straight	428	*S. griseus* subsp. *griseus* (NC010572)	95
Pink	S37	RF-Flexous	207	*S. roseosporus* (ABYX01000136)	85
White	S54	MV	359	*S. griseus* subsp. *griseus* (NC010572)	89

[a]Number of 16S-23S rRNA gene nucleotides used for the alignment. [b]Percentage identity with the 16S-23S rRNA gene sequence of the closest phylogenetic relative of *Streptomyces*

isolate S2 (LC_{50} = 4.04 mg mL^{-1}), which belonged to brown color series and produced BIV sporophores. Whereas, the least toxic isolate to *D. melanogaster* larvae was isolate S37 (LC_{50} = 19.56 mg mL^{-1}), which belonged to pink color series and produced MV-S sporophores. Interestingly, most isolates that exhibited larvicidal activity against *D. melanogaster* (14 isolates) were found to produce RF sporophores (Table 6). None of the isolates producing S-Closed or S-Open sporophores exhibited toxicity against *D. melanogaster* larvae. It was found that 15 isolates, out of the 19 isolates with larvicidal activity, also had antimicrobial activities. Isolate S2 was selected because it has the most significant insecticidal activity and this was compared with that of Bt strain J63 (Fig. 1). It was also observed that the crude of Bt strain J63 produced higher mortalities than that produced from S2. Unexpectedly, it was observed that the combined insecticidal activity of J63 and S2 crudes against *D. melanogaster* was higher than that observed in a single crude.

Molecular characterization of insecticidal isolates: To confirm the classification of local *Streptomyces* isolates, genomic DNA was extracted from seven insecticidal isolates which represent the seven color series. The 16S-23S rRNA gene sequence was analyzed by amplification with GP1 and GP2 primers. The amplified genomic DNA of the isolates produced a single PCR band of about 500 bp in size (Fig. 2). The obtained 16S-23S rRNA gene sequences were aligned by BLAST alignment of GenBank sequences. Based on the BLAST alignment, all seven isolates were allocated to the phylum actinobacteria which contains the genus *Streptomyces* with 82-95% identity (Table 7). Isolate S35 was found to have the highest identity (95%). Furthermore, the phylogenetic analysis of the 16S-23S rDNA sequences reflected the clustering of four isolates (S1, S25, S35 and S54) together with the reference strain *S. griseus* ATCC 10137 with 98% bootstrap value at the node (Fig. 3). Whereas, the remaining three isolates were clustered together with a high bootstrap value (96%) at the node.

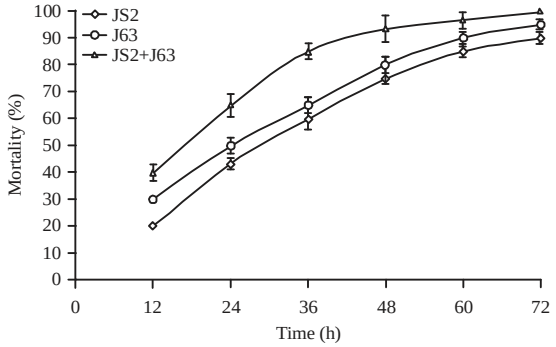

Fig. 1: Insecticidal activity of combined n-butanol extract of *Streptomyces* (isolate S2; LC_{50} = 4.04 µg mL^{-1}) and δ-endotoxin from *Bacillus thuringiensis* subsp. *israelensis* (Bt strain J63; LC_{50} = 0.17 ng mL^{-1}) against *Drosophila melanogaster* larvae
Error bars represent standard deviation of the mean

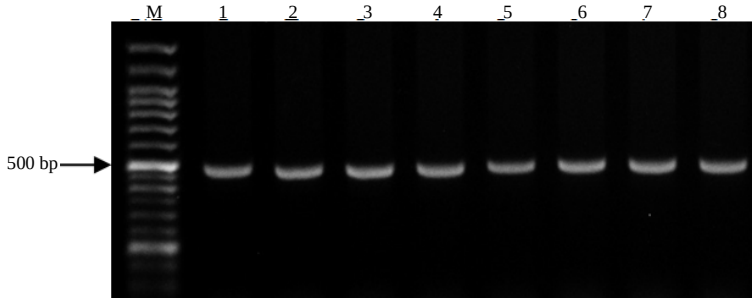

Fig. 2: Agarose gel (1%) electrophoresis of PCR amplification of 16S-23S rRNA gene fragments with oligonucleotide forward primer GP1 and reverse primer GP2 of 7 local *Streptomyces* isolates
Lanes 1-7: Isolates S1, S6, S13, S25, S35, S37 and S54, respectively, Lane 8: Reference strain *S. griseus* ATCC 10137, Lane M: 50 bp DNA ladder marker (Genedirex, USA). The left arrow indicated 500 bp bands

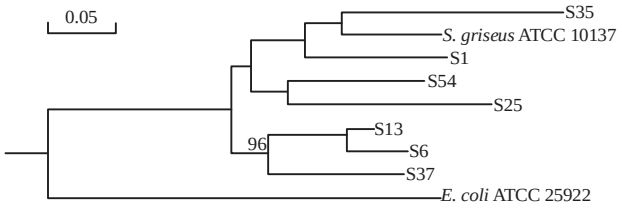

Fig. 3: Phylogenetic tree showing the relationships between the interspacer region 16S-23S rRNA gene sequences of the *Streptomyces* isolates and the reference strain *S. griseus* ATCC 10137
The reference strain *E. coli* ATCC 25922 was used as the out group. The numbers at the nodes are bootstrap confidence values and they are expressed as percentages of 1000 bootstrap replications

DISCUSSION

In the present study, the occurrence of local *Streptomyces* was investigated in 150 soil samples representing 12 different locations. A total of 127 *Streptomyces* isolates were recovered from the collected soil samples. The isolates were classified into nine color series based on the aerial mycelium color; isolates with white color series appeared to be the most common. Whereas, isolates with black, pink and red color series were the least common. This result is in agreement with findings of Msameh[27]. Isolates belonging to green color series were found to be the second most common among the obtained *Streptomyces* isolates. This finding disagrees with that of Saadoun and Gharaibeh[28], who proved that green color series were the least common in local Jordanian habitats. It was observed that most *Streptomyces* isolates were able to

produce RF sporophores (94 isolates) with 50% of them being RF-Flexous. This is in agreement with previous studies[28-30] which demonstrated that sporophores of RF-type were the most common among Jordanian isolates. It was also observed that sporophores of S-type has the lowest distribution among isolates. The differences in sporophores shape among *Streptomyces* isolates recovered from different locations could be related to the differences in climate, humidity, vegetation and soil type in the locations of isolation.

In the 21st century, most critical bacterial infections have become resistant to commonly used antibiotics. Thus, the bacterial resistance to conventional antibiotics has become a major healthcare problem[31]. For example, the bacterium *S. aureus* has developed resistance to most classes of antibiotics[20,32,33] and recently to vancomycin[34-36]. Many clinically useful antibiotics are derived from *Streptomyces*[37]. Therefore, there is an urgent need to discover new antimicrobial agents from *Streptomyces* which may produce secondary metabolites that can control serious bacterial and fungal infections.

It was found that TSB medium is the best medium for production of active antimicrobial agents from *Streptomyces*. The increased activity in TSB medium may be correlated with the fact that TSB medium compared to other media was the best in enhancement of growth, by increasing the amount of mycelium. This result is in agreement with findings of Suutari *et al*.[21] and Vijayakumari *et al*.[38].

About one-third (29.13%) of *Streptomyces* isolates, investigated in the current study, exhibited a wide range of antimicrobial activity against the test microorganisms. Results of the antibacterial activity of *Streptomyces* indicated that the Gram-negative bacterium, *E. coli* was the most susceptible (*E. coli* ATCC 8739 and *E. coli* ATCC 25922 were inhibited by 25 and 28 crude extracts of *Streptomyces* isolates, respectively), whereas, the Gram-positive bacterium MRSA was the least susceptible (inhibited by extracts of 11 isolates). In addition, the Gram-negative bacterium *K. oxytoca*, which was resistant to all antibiotics tested except for AMP10, was susceptible to 18 *Streptomyces* isolates. This result is in agreement with Ceylan *et al*.[20] and Saadoun *et al*.[30] who reported that Gram-negative bacteria were more susceptible to *Streptomyces* extracts when compared to Gram-positive bacteria. In contrast, other studies[39-41] demonstrated that Gram-positive bacteria were more susceptible to *Streptomyces* extract than the Gram negative bacteria. This might be due to differences in the type of extracting solvent. Regarding the antifungal activity, it was observed that 20 isolates exhibited antifungal activity against the reference strains, *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404. Interestingly, both fungal species were found to be susceptible to the same *Streptomyces* isolates. This similarity in susceptibility may be due to the fact that both species are eukaryotic cells.

In recent years, there has been an alarming increase in the resistance of crop and forest insects to chemical insecticides. The demand for using biological products instead of chemical products as an insecticide has led to an increase in the search for insecticidal agents from new sources, including microorganisms especially the bacterium, *Bacillus thuringiensis*. Few studies[11-15,42-46] have dealt with the insecticidal activity of *Streptomyces*. As an achievement of this study, 19 *Streptomyces* isolates were found to exhibit an insecticidal activity against the third instar larvae of *D. melanogaster* (with LC_{50} value of 4.04 mg) for the most toxic isolate S2. This is the first study to investigate the larvicidal activity of *Streptomyces* crudes against *D. melanogaster*.

The combined insecticidal activity of S2 and J63 (*B. thuringiensis* subsp. *israelensis*) was investigated in this study (Fig. 1). It was found that the combination of S2 and J63 crudes produced higher insecticidal activity than that produced from each crude alone. Thus, biological products of S2 and J63 can be used in the future for the development of Bt and *Streptomyces*-based formulations for the biocontrol of insects. Further studies are required to identify the bioactive compounds in the crude extracts of *Streptomyces* isolates that offer promising antimicrobial and insecticidal activities.

The 16S-23S rRNA genes of seven *Streptomyces* isolates, distributed among all color series of the aerial mycelium which produced insecticidal activity were sequenced. As a result, the sequences of the seven isolates showed 82-95% sequence identity to the phylum Actinobacteria containing the genus *Streptomyces*. The general cut-off value for genus *Streptomyces* is 83.5% for 16S-23S rRNA gene sequence identity[47]. Although, the sequences of isolates S6 (82% identity) and S13 (83% identity) have lower identities to the nearest relative than *Streptomyces* cut-off value, they were subclustered with the reference strain, *S. griseus* ATCC 10137, with high bootstrap values and they were closely related to the 16S-23S rRNA gene sequence of *Streptomyces* retrieved from the GenBank database, suggesting that they belong to the genus *Streptomyces*. In conclusion, the current study demonstrated that Jordan soils are rich sources for the isolation of *Streptomyces* producing antibacterial, antifungal and insecticidal activities. Therefore, extracts prepared from *Streptomyces* cultures can be used in pharmaceutical and medical fields, especially against multidrug resistant bacterial and fungal infections and together with *B. thuringiensis* can be used in biological control.

CONCLUSION

The n-butanol extracts of the *Streptomyces* isolates obtained from TSB medium displayed a promising antimicrobial activity against multidrug-resistant bacteria, such as MRSA and against fungi, such as *C. albicans*. Therefore, extracts of the isolated *Streptomyces* can be used for medical and pharmaceutical purposes or to develop and improve the current therapies of some diseases. This study is the first to show the insecticidal activity of combined crudes of *Streptomyces* and *B. thuringiensis* subsp. *israelensis*. The combined insecticidal activity of S2 and J63 crudes showed significant insecticidal activity compared to that produced from each crude alone. Thus, such combination can be used in the future for biological control strategies.

SIGNIFICANCE STATEMENT

The findings of this study will be beneficial in medical and pharmaceutical applications, particularly in the treatment of common human pathogens that display multidrug-resistance. The combined byproducts of *Streptomyces* and *B. thuringiensis* can be used in the future to improve the current methods used in biological control and to develop insecticidal formulations that are used for crop and forest protection and in insect vector control.

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REFERENCES

1. Atta, H.M., 2009. An antifungal agent produced by *Streptomyces olivaceiscleroticus*, AZ-SH514. World Applied Sci. J., 6: 1495-1505.
2. Atta, H.M., S.M. Dabour and S.G. Desoukey, 2009. Sparsomycin antibiotic production by *Streptomyces* sp. AZ-NIOFD1: Taxonomy, fermentation, purification and biological activities. Am.-Eur. J. Agric. Environ. Sci., 5: 368-377.
3. Manteca, A., R. Alvarez, N. Salazar, P. Yague and J. Sanchez, 2008. Mycelium differentiation and antibiotic production in submerged cultures of *Streptomyces coelicolor*. Applied Environ. Microbiol., 74: 3877-3886.
4. Watve, M.G., R. Tickoo, M.M. Jog and B.D. Bhole, 2001. How many antibiotics are produced by the genus *Streptomyces*? Arch. Microbiol., 176: 386-390.
5. Euzeby, J.P., 2016. Genus *Streptomyces*. List of Prokaryotic Names with Standing Nomenclature. http://www. bacterio. net/streptomyces.html.
6. Kampfer, P., 2012. Genus *Streptomyces*: The Actinobacteria. In: Bergey's Manual of Systematic Bacteriology, Goodfellow, M., P. Kampfer, H.J. Busse, M.E. Trujillo, K. Suzuki, W. Ludwig and W.B. Whitman (Eds.). 2nd Edn., Springer, New York, pp: 1455-1767.
7. Ravikumar, S., S.J. Inbaneson, M. Uthiraselvam, S.R. Priya, A. Ramu and M.B. Banerjee, 2011. Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. J. Pharm. Res., 4: 294-296.
8. Procopio, R.E.D.L., I.R. da Silva, M.K. Martins, J.L. Azevedo and J.M. de Araujo, 2012. Antibiotics produced by *Streptomyces*. Brazil. J. Infect. Dis., 16: 466-471.
9. Bosso, J.A., P.D. Mauldin and C.D. Salgado, 2010. The association between antibiotic use and resistance: The role of secondary antibiotics. Eur. J. Clin. Microbiol. Infect. Dis., 29: 1125-1129.
10. Nikaido, H., 2009. Multidrug resistance in bacteria. Annu. Rev. Biochem., 78: 119-146.
11. Dhanasekaran, D., V. Sakthi, N. Thajuddin and A. Panneerselvam, 2010. Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria. Int. J. Applied Biol. Pharm. Technol., 1: 374-381.
12. Liu, H., S. Qin, Y. Wang, W. Li and J. Zhang, 2008. Insecticidal action of Quinomycin A from *Streptomyces* sp. KN-0647, isolated from a forest soil. World J. Microbiol. Biotechnol., 24: 2243-2248.
13. Kaur, T., A. Vasudev, S.K. Sohal and R.K. Manhas, 2014. Insecticidal and growth inhibitory potential of *Streptomyces hydrogenans* DH16 on major pest of India, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). BMC Microbiol., Vol. 14. 10.1186/s12866-014-0227-1
14. Kekuda, T.R.P., K.S. Shobha and R. Onkarappa, 2010. Potent insecticidal activity of two *Streptomyces* species isolated from the soils of the western ghats of Agumbe, Karnataka. J. Nat. Pharm., 1: 30-32.
15. Usuki, H., T. Nitoda, M. Ichikawa, N. Yamaji, T. Iwashita, H. Komura and H. Kanzaki, 2008. TMG-chitotriomycin, an enzyme inhibitor specific for insect and fungal β-N-acetylglucosaminidases, produced by actinomycete *Streptomyces anulatus* NBRC 13369. J. Am. Chem. Soc., 130: 4146-4152.
16. Taddei, A., M.J. Rodriguez, E. Marquez-Vilchez and C. Castelli, 2006. Isolation and identification of *Streptomyces* spp. from Venezuelan soils: Morphological and biochemical studies. I. Microbiol. Res., 161: 222-231.
17. Dastager, S.G., W.J. Li, A. Dayanand, S.K. Tang and X.P. Tian *et al*., 2006. Seperation, identification and analysis of pigment (Melanin) production in *Streptomyces*. Afr. J. Biotech., 5: 1131-1134.

18. Pridham, T.G., C.W. Hesseltine and R.G. Benedict, 1958. A guide for the classification of streptomycetes according to selected groups; placement of strains in morphological sections. Applied Microbiol., 6: 52-79.
19. Morley, D.C., 1945. A simple method of testing the sensitivity of wound bacteria to penicillin and sulphathiazole by the use of impregnated blotting paper discs. J. Pathol., 57: 379-382.
20. Ceylan, O., G. Okmen and A. Ugur, 2008. Isolation of soil Streptomyces as source antibiotics active against antibiotic-resistant bacteria. EurAsia. J. BioSci., 2: 73-82.
21. Suutari, M., U. Lignell, A. Hyvarinen and A. Nevalainen, 2002. Media for cultivation of indoor *Streptomycetes*. J. Microbiol. Methods, 51: 411-416.
22. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
23. Obeidat, M., H. Khyami-Horani and F. Al-Momani, 2012. Toxicity of *Bacillus thuringiensis* β-exotoxins and δ-endotoxins to *Drosophila melanogaster*, *Ephestia kuhniella* and human erythrocytes. Afr. J. Biotechnol., 11: 10504-10512.
24. Atalan, E., 2001. Restriction fragment length polymorphism analysis (RFLP) of some *Streptomyces* strains from soil. Turk. J. Biol., 25: 397-404.
25. Benson, D.A., M.S. Boguski, D.J. Lipman, J. Ostell, B.F.F. Ouellette, B.A. Rapp and D.L. Wheeler, 1999. GenBank. Nucl. Acids Res., 27: 12-17.
26. Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman, 1990. Basic local alignment search tool. J. Mol. Biol., 215: 403-410.
27. Msameh, Y.M., 1992. *Streptomyces* in Jordan: Distribution and antibiotic activity. M.Sc. Thesis, Yarmouk University, Irbid, Jordan.
28. Saadoun, I. and R. Gharaibeh, 2002. The Streptomyces flora of Jordan and its' potential as a source of antibiotics active against antibiotic-resistant Gram-negative bacteria. World J. Microbiol. Biotechnol., 18: 465-470.
29. Saadoun, I., M.J. Mohammad, F. Al-Momani and M. Meqdam, 1998. Diversity of soil streptomycetes in Northern Jordan. Actinomycetes, 9: 52-60.
30. Saadoun, I., F. Al-Momani, H. Malkawi and M.J. Mohammad, 1999. Isolation, identification and analysis of antibacterial activity of soil streptomycetes isolates from North Jordan. Microbios, 100: 41-46.
31. Alanis, A.J., 2005. Resistance to antibiotics: Are we in the post-antibiotic era? Arch. Med. Res., 36: 697-705.
32. Enright, M.C., 2003. The evolution of a resistant pathogen-the case of MRSA. Curr. Opin. Pharmacol., 3: 474-479.
33. Ug, A. and O. Ceylan, 2003. Occurrence of resistance to antibiotics, metals and plasmids in clinical strains of *Staphylococcus* spp. Arch. Med. Res., 34: 130-136.
34. Bozdogan, B.U., D. Esel, C. Whitener, F.A. Browne and P.C. Appelbaum, 2003. Antibacterial susceptibility of a vancomycin-resistant *Staphylococcus aureus* strain isolated at the hershey medical center. J. Antimicrob. Chemother., 52: 864-868.
35. ZChang, S., D.M. Sievert, J.C. Hageman, M.L. Boulton and F.C. Tenover *et al*., 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. N. Engl. J. Med., 348: 1342-1347.
36. Kacica, M. and L.C. McDonald, 2004. Brief report: Vancomycin-resistant *Staphylococcus aureus*-New York. Morb. Mortal. Weekly Rep., 53: 322-323.
37. Hasani, A., A. Kariminik and K. Issazadeh, 2014. Streptomycetes: Characteristics and their antimicrobial activities. Int. J. Adv. Biol. Biomed. Res., 2: 63-75.
38. Vijayakumari, S.J., N.K. Sasidharannair, B. Nambisan and C. Mohandas, 2013. Optimization of media and temperature for enhanced antimicrobial production by bacteria associated with *Rhabditis* sp. Iran. J. Microbiol., 5: 136-141.
39. Kariminik, A. and F. Baniasadi, 2010. Pageantagonistic activity of actinomycetes on some gram negative and gram positive bacteria. World Applied Sci. J., 8: 828-832.
40. Kekuda, P.T.R., N. Dileep, S. Junaid, K.N. Rakesh, S.C. Mesta and R. Onkarappa, 2013. Biological activities of *Streptomyces* species SRDP-07 isolated from soil of Thirthahalli, Karnataka, India. Int. J. Drug Dev. Res., 5: 268-285.
41. Manasa, M., G. Poornima, V. Abhipsa, C. Rekha, K.T.R. Prashith, R. Onkarappa and S. Mukunda, 2012. Antimicrobial and antioxidant potential of *Streptomyces* sp. RAMPP-065 isolated from Kudremukh soil, Karnataka, India. Sci. Technol. Arts Res. J., 1: 39-44.
42. Bream, A.S., S.A. Ghazal, E.A.Z. Abd and S.Y. Ibrahim, 2001. Insecticidal activity of selected actinomycete strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). Meded. Rijksuniv. Gent. Fak. Landbouwkd. Toegep. Biol. Wet., 66: 503-512.
43. Gadelhak, G.G., K.A. El-Tarabily and F.K. AL-Kaabi, 2005. Insect control using chitinolytic soil actinomycetes as biocontrol agents. Int. J. Agric. Biol., 7: 627-633.
44. Hussain, A.A., S.A. Mostafa, S A. Ghazal and S.Y. Ibrahim, 2002. Studies on antifungal antibiotic and bioinsecticidal activities of some actinomycete isolates. Afr. J. Mycol. Biotechnol., 10: 63-80.
45. Liu, B. and C. Sengonca, 2003. Conjugation of δ endotoxin from *Bacillus thuringiensis* with abamectin of *Streptomyces avermitilis* as a new type of biocide, GCSC BtA, for control of agricultural insect pests. Anzeiger fur Schadlingskunde, 76: 44-49.
46. Sundarapandian, S., M.D. Sundaram, P. Tholkappian and V. Balasubramanian, 2002. Mosquitocidal properties of indigenous fungi and actinomycetes against *Culex quinquefasciatus* Say. J. Biol. Control, 16: 89-91.
47. Rossi-Tamisier, M., S. Benamar, D. Raoult and P.E. Fournier, 2015. Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. Int. J. Syst. Evolutionary Microbiol., 65: 1929-1934.