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Antibacterial and insecticidal activity of actinomycetes isolated from sandy soil of (Cairo-Egypt).

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ABESTRACT

The present study was undertaken to screen antibacterial and insecticidal activities of crude extract of twenty actinomycetes isolates from desert soil samples collected from different locations of Cairo- Egypt. Isolate No. A11 was found to be the most active actinomycetes isolate against Gram positive, Gram negative bacteria and 2nd instar larvae of cotton leaf worm *Spodoptera littoralis* (Boisd.). The most active isolate was selected for further studies concerning their identification. Morphological, physiological and phylogenetic analysis (16S rRNA); in addition to biochemical studies and cultural characteristics as well as chemical analysis of the cell wall, were carried out for the isolate under study. Based on the phenotypic and genotypic accumulated characteristics of the selected isolate and recommended International Key's of Bergey's Manual for identification of actinomycetes, it was found that this isolate matched with *Streptomyces bikiniensis* and was given the name *Streptomyces bikiniensis*A11. The active metabolite of selected isolate was extracted by chloroform. Fraction No. 17 was the most active fraction against bacteria and insect species under study. The separation, purification and characterization of the active metabolite(s) were performed using thin layer chromatography. Based on the physicochemical studies for the purified compound; it was found that the purified compound may be belonged to Aminoglycosidic antibiotic.

Keywards: *Streptomyces bikiniensis*, antimicrobial, cotton leafwarm, aminoglycoside.

INTRODUCTION

Several antibiotic substances obtained from bacteria and fungi have been applied in medicine, out of which about 75% are produced from gram positive actinomycetes bacteria such as *Streptomyces* sp. (Miyadoh, 1993). Actinomycete species are well-known saprophytic bacteria that decompose organic matter, especially biopolymers such as lignocellulose, starch, and chitinin soil (Crawford *et al*., 1993).Various antimicrobial substances from *streptomyces* sp.and actinomycetes bacteria have been isolated and characterized including aminoglycosides , anthracyclins, glycopeptides, B-lactams, macrolides, peptides, polyenes , polyester , polyketides, actinomycins and tetracyclines (Goodfellow *et al*.,1988; Okami and Hotta, 1988; Baltz,1998). Also, about 60% of the new insecticides and herbicides reported in the past 5 years originate from *Streptomyces* (Tanaka and Omura, 1993).

The problems of drug resistance, patient's sensitivity and inability to control certain infectious diseases have given an impetus for continuous search of new antibiotic all over the world. To combat the multidrug resistant organisms, production of new antimicrobial compound or antibiotics from new source is essential.

Cotton leafworm (*Spodoptera littoralis* (Boisd.)), is considered one of the most injurious and destructive polyphagous lepidopterous insect pests attacking crops, vegetables and fruit trees all over the world (Berlinger *et al*, 1997;

Kandil *et al*., 2003).It has the ability to develop relatively quick resistance to most conventional insecticides. (Temerak, 2002).

As the environmental contamination by toxic chemicals increases, alternative approaches for controlling pest populations have become research priorities. These have included biological or ecological control methods for limiting the destructive impacts of pest populations, especially in agriculture (Nakas & Hagedorn, 1990; Canaday, 1995; Hokkanen & Lynch, 1995). Egypt lost 50% of the national yield of cotton due to country wide resistance to Toxaphene in 1961 (EL-Sebae *et al*., 1993).

Many reports indicated that actinomycetes play an important role in the biological control against insect including the cotton leaf worm *spodopetra liltoralis* (Bream *et al*., 2001), house fly *Musca domestica* (Hussain *et al*., 2002), *Culex quinquefasciatus* (Sundrapandian *et al.,* 2002), *Drosophila melanogaster* (Gadelhak *et al*., 2005), *Helicoverpa armigera* (Osman *et al*., 2007), *Anopheles mosquito* larvae (Dhanasekaran *et al.,* 2010) and *Culex pippins* (Elkhawaga, *et al*, 2011).

Bream *et al*., 2001, investigate the biological activity of secondary metabolites of some actinomycetes isolates on last instar larvae of the cotton leaf worm *Spodoptera littoralis* through the food plant (Castor leaves) .They showed that Streptomyces and Streptoverticillum were the most potent actinomycetes, which cause larval and pupal mortality. In 2007 Osman *et al*., reported that the pellets of some strepyomyces isolates were more active against cotton leafworm than culture filtrate.

Kenneth, (1995) reported that the direct introduction of parasites, pathogen and predators to target insect is very effective method of biological control. Also, using of dead spores of varieties of the natural soil bacterium and actinomycetes show interfere in the digestion systems of larvae. These spores are no longer effective after the larvae turn into pupae because they stop eating (Walker and lynch, 2007). *Streptomyces* strains isolated from sea water and sea sediments from Beidiahe and Dagang of the east coast of China, screened for their insecticidal activities using bioassay against *Helicoverpa armigera*. (Xiong *et al*., 2004). Many of bioproduct which produced from actinomycetes showed different mode of action against different insect. Avermectin produced by the soil microorganism, *Streptomyces avermitilis* act on GABA- and glutamate-gated chloride channels of insect. (White *et al*., 1997). Spinosad is a neurotoxin mixture produced during fermentation of a soil actinomycete that has high activity towards Lepidoptera. It blocking the chloride channel associated with GABA receptor of the insect. (Hainzl *et al*., 1998).Spinosad and indoxacarb were introduced at the same time against insect pests of cotton in Pakistan which cause paralysis in the larvae on eating them (Tomlin, 2001).

The aim of this study was to determine the antibacterial and insecticidal activities of twenty actinomycetes isolates against some pathogenic bacteria and cotton leafworm.

MATERIALS AND METHODS

Collection of samples:

Twenty samples used in this study were collected from different areas of sandy soil from Cairo-Egypt. The actinomycetes isolates were isolated from soil samples by dilute plating using starch nitrate agar medium (El-Nakeeb and Lechevalier, 1963) and then incubated at 30°C, for four days. All isolates were purified by repeated streaking on starch nitrate agar medium.

Isolates propagation and preparation:

Standard inoculums for each applied isolates were prepared by scraping the heavy spores from the surface of the growth of starch nitrate slant in the presence of 5 ml sterilized H_2O. An aliquot of 2 ml standard inoculums was transferred aseptically to 50 ml of a broth medium modified from starch nitrate broth medium as reported by Lechvalier and Waksman (1962) in a 250ml conical flask. Inoculated flasks were incubated at 28±2°C for five days on a rotary shaker (150 rpm/min).Thereafter; growth was centrifuged at 10000 rpm at four °C for 5minutes. The supernatant was then subjected for the evaluation of its antagonistic or insecticidal activities.

Antibacterial activities of isolates:

Screening of actinomycetes isolates was carried out according to their antibacterial activity against eight pathogenic bacteria including (*Sreptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteous vulgaris, Salmonella typhymurium, Klebsilla pneumoniae* and *Escherichia coli)*. Bacteria were grown on nutrient agar medium. The antibacterial activity was estimated by measuring the diameter of inhibition zone (Perez *et al.,* 1990).

Insect maintenance:

Larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.), were reared on fresh castor leaves, *Ricinus communis* L., in the Entomology Laboratory, Plant Protection Department, Faculty of Agriculture, Al -Azher University. This strain was not previously exposed to any insecticides. The colony was reared under constant conditions at 25 ± 2°C, 65 ±5 % R.H. and photoperiod 12:12 L: D, as described by El-Defrawi *et al*., (1964).

Insecticidal activities of isolates:

The filtrate of the 20 actinomycetes isolates of this study were used to determine their insecticidal activities against the 2nd instar larvae of *Spodoptera littoralis*. For each treatment, leaves of caster bean were washed, dried and immersed in the filtrate of tested isolates for 20 seconds, then allowed to dry under laboratory conditions and offered to 2nd instar larvae in clean jar, each jar contained 10 larvae. Three replicates were used for each treatment. Leaves dipped in water served as control. Mortality (%) was recorded after 3-5 days according to Finny (1962).

Larval mortality percent: was estimated by using the following equation: Larval mortality % = A – B / A × 100, where A = number of tested larvae and B = number of tested pupa (Briggs, 1960).

Pupation rate: The pupation percent was estimated by using the following equation:

Pupation % = A / B × 100, where A = number of pupae and B = number of tested larvae.

Pupal mortality: The pupal mortality percent was estimated by using the Following equation:

Pupal mortality % = A – B / A × 100, where A = number of produced pupae and B = number of observed adults.

Adult emergence: The emerged males and females adults were counted and the adult emergence percent was calculated by using the following equation:

Adult emergence % = A / B × 100, where A = number of emerged adults and B = number of tested pupae.

Statistical analysis: Data were analyzed by Analysis with SASS system for Windows v6.12 software. The means were compared by Least Significant Difference test, Finny (1971).

Identification of selected isolates:

The cultural, morphological and physiological characteristics of the selected isolates were studied using all media and methods of international Streptomyces Project (ISP) as described by Shirling and Gottlieb (1966, 1968a and 1969). Identification was carried out by using the keys suggested by Buchanan

and Gibbson (1974), Williams *et al*., (1989) and Hensyl (1994).

Studies concerning the phylogenetic characteristic:

The locally isolated actinomycetes strain was grown for 7 days on starch agar slant at 30 ± 2 ^{o}C. Two ml of a spore suspension were inoculated into starch – nitrate broth and incubated for 3 days on incubator shaker at 200rpm and 30± 2 ^{o}C to form a pellet of vegetative cells (pre – sporulation). The preparation of total genomic DNA was conducted in accordance with the methods described by (Sambrook *et al*., 1989).PCR amplification of 16S rDNA gene of the local actinomycete strain was conducted using two primers which were F27 with the sequence 5-AGAGTTTGATCMTGGCTCAG-3 and R1492 with the sequence 5-TACGGGYTACCTTGTTACGACTT-3, in accordance with the method described by Edwards *et al*., (1989). Purification of PCR products and sequencing of PCR products for the isolate under study; were performed in the Sigma company of scientific service, Cairo-Egypt.

Sequence similarities and phylogenetic analysis:

Sequence data was analyzed in the Gene Bank database by using the BLAST program available on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).The unknown sequence was compared to all of the sequences in the database to assess the DNA similarities. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software (Hall, 1999)

Fermentation:

A slant culture of Actinomyces isolate grown on starch nitrate agar was used to inoculate a 250 ml Erlenmeyer flask containing 100ml of the same medium in its liquid form. The cultivated flask were incubated at 30± 2 ^{o}C for four days on a rotary shaker (200 rpm) to give a first seed culture. Approximately, 2 ml portions of the seed culture were inoculated into each of twenty 250 ml conical flasks containing 100 ml of the above liquid medium and incubated at 30 ± 2 ^{o}C on a rotary shaker (200 rpm) for 10 days. The samples were taken daily for determination of antimicrobial activity. Antimicrobial activity was determined using the classical agar well diffusion (Perez *et al.,* 1990).

Extraction:

The extraction process was carried out to the clear filtrate using different solvents including, diethyl ether, ethyl acetate, chloroform, aceton, butanol, methyl alcohol, petroleum ether and water. Each extract was dried and concentrated under vacuum using a rotary evaporator at a temperature not exceeding 50 ^{o}C. All extracts were tested for their antimicrobial and insecticidal activity.

Purification by TLC:

Separation of the antimicrobial and insecticidal compound into its individual components was conducted by thin layer chromatography using chloroform and methanol (24:1.v/v) as a solvent system.

Purification by Column Chromatography:

The purification of the antimicrobial and insecticidal compound was carried out using silica gel column (2.5x50) chromatography. Chloroform and methanol 95:5 (v/v) (Guangying *et al.,* 2005) was used an eluting solvent. The column was left overnight until the silica gel was completely settled. One-ml crude extract to be fractionated was added on the silica gel column surface and the extract was adsorbed on the silica gel. Forty fractions were collected (each of 5ml) and tested for their antimicrobial and insecticidal activity.

Physicochemical properties and spectroscopic analysis:

Physical and chemical properties of the purified active substance were studied. The elemental analysis, IR, H-NMR and ^{13}C-NMR spectrum were

determined at the micro-analytical center, Cairo University, Egypt. Mass spectrum was determined at Regional Center for Mycology and Biotechnology (RCMB) at AL-Azhar University.

Determination of Minimum Inhibitory Concentration (MIC):

Minimum Inhibitory Concentration (MIC) of the antimicrobial agent has been determined by using the agar plate dilution technique (Betina, 1983).

RESULTS

Antagonistic activities of isolates:

Twenty actinomycetes isolates differ in their cultural and morphological characters were isolated in pure form from sand soil from different site of Cairo, Egypt, using starch nitrate agar medium. Among these isolates five isolates only exhibited antibacterial activity against tested organisms as illustrated in Table 1. The most potent isolate against all tested organisms was No. (11).

Table 1: Antibacterial activities of most potent actinomycetes filtrates (100 µl).

Isolates code / Tested bacteria	A8	A9	A11	A13	A20
Streptococcus pyogenes	20	NA	27	20	9
Bacillus subtilis	22	10	24	20	11
Staphylococcus aureus	12	NA	26	25	12
Pseudomonas aeruginosa	18	NA	19	16	NA
Proteous vulgaris	13	6	22	18	9
Salmonella typhymurium	15	NA	28	17	11
Klebsilla pneumoniae	12	NA	19	19	9
Escherichia coli	21	11	24	13	12

-NA: No activity.

-The data expressed as mean diameter of inhibition zone of pathogenic microbial growth.

Insecticidal Activities of Isolates against Cotton Leafworm Larvae:

In this investigation, the insecticidal activities of culture filtrates had been tested against 2nd instar larvae of cotton leaf worm. Results in Table 2 revealed that the culture filtrates of some isolates were more active against cotton leaf worm than other culture filtrates. Isolates No.A10 andA.11 were recorded as the most effective as they showed 100.0 % mortality, while isolates No.A12 caused 60.0% mortality. On the other hand, two isolates namely A5 and A16, showed percentages of mortalities about 36.7%, while the other isolates cause mortalities ranged from 33.3 to 13.3 % in comparison with 10 % mortalities in control. Also the lethal effect was extended to the pupal stage by all the metabolites of isolates for example, isolates A 1 & A12 which cause 33.3 and 26.7% mortalities in the resulted pupae from the treated larvae, respectively, no mortalities were observed in the adult stage. On the other hand, the percentage of adult emergence was decreased in some isolates to 0, 0, 13.3, 36.7, 40.0 & 46.7 % at A10, A11, A12, A1, A5 &A 9, respectively, vs. 90 % of adult emergence in control.

With regard to the development, the larval duration was high significantly prolonged with some isolates A5, A10, A11 and A12 and significantly with other isolates A1, A6, A9, A16 andA18 in comparison with that of control.

Statistical analysis showed that there is a significant difference of pupal duration between some isolates A3, A4, A 7, A12, A 14, A17 and A19 when compared with control.

Table 2: Screening of the biological activity of filtrate of actinomycetes isolates against 2nd instar larvae of *Spodoptera littoralis* under laboratory conditions.

Isolates code	Larval duration (day)	Larval mortality %	Pupal duration (day)	% of Pupation	Pupal mortality %	Emergency %	Longevity (day)	Total mortality %
A1	20.4 *	30.0	12.9	70.0 **	33.3	36.7**	8.9 **	63.3
A2	21.0	26.7	12.6	73.3 *	23.3	50.0**	9.8 *	50.0
A3	21.3	20.0	13.4 *	80.0	13.3	66.7**	10.4	33.3
A4	21.2	23.3	13.3 *	76.7	10.0	66.7**	10.6	33.3
A5	20.1 **	36.7	12.7	66.7 **	23.3	40.0**	9.2 **	60.0
A6	20.4 *	30.0	12.6	70.0 **	13.3	56.7**	10.3	43.3
A7	21.3	16.7	13.4 *	83.3	10.0	73.3**	11.0	26.7
A8	21.7	20.0	12.8	80.0	13.3	66.7**	10.3	33.3
A9	20.2 *	33.3	13.1	66.7 **	20.0	46.7**	9.7 *	53.3
A10	19.3 **	100.0	0.0	0.0 **	0.0	0.0**	0.0	100.0
A11	18.6 **	100.0	0.0	0.0 **	0.0	0.0**	0.0	100.0
A12	19.5 **	60.0	13.2 *	40.0 **	26.7	13.3**	8.2 **	86.7
A13	21.4	20.0	12.7	80.0	20.0	60.0**	10.2	40.0
A14	21.2	23.3	13.2 *	76.7	6.7	70.0**	10.7	30.0
A15	21.3	23.3	13.1	76.7	6.7	70.0**	10.2	30.0
A16	20.3 *	36.7	12.7	63.3 **	10.0	53.3**	10.1	46.7
A17	21.5	26.7	13.3 *	73.3 *	6.7	66.7**	10.9	33.3
A18	20.3 *	30.0	13.1	70.0 **	13.3	56.7**	10.0	43.3
A19	21.1	16.7	13.3 *	83.3	6.7	76.7**	10.7	23.3
A20	21.2	13.3	12.9	86.7	3.3	83.3**	10.8	16.7
cont	21.1	10.0	12.4	90.0	0.0	90.0	11.2	10.0
L S D 5%	0.70		0.8	14.9		1.2	1.4	
L S D 1%	0.94		1.1	20		1.7	1.8	

*= significant **= highly significant

From the results showed in Table (1) and (2) the isolate No. (11), is the most potent against tested bacteria and also cotton leafworm larvae. Therefore, it was subjected to further studies.

Morphological and cultural characteristics of the selected isolates:

The isolate No. A11. The spore chains of isolate A11, composed of oval to elongate shaped spores with smooth surface in Fig.1 and grows well on various standard media including the ISP media which were recommended by Shirling and Gottlieb (1966, 1968a, 1969and 1972). Waksman (1961), Buchanan and Gibbson's (1974), Williams *et al*., (1989) and Hensyl (1994)

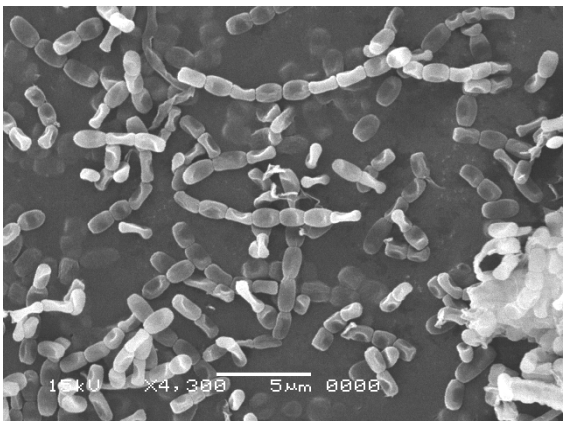

Fig. 1: Scanning electron micrograph of *Streptomyces bikiniensis* A11, grown on starch nitrate agar media at 30± 2 °C for 14 days. (X4.300 -5µm).

Table (3) shows the results regarding the rate of growth, spore mass color, reverse side growth color and production of soluble pigment by the isolated organism on nine different media. The aerial mycelia are gray, substrate mycelia are deep brown, no diffusible pigment produced on starch nitrate media. Formation of melanoid pigments is positive on ISP No. 1and ISP No. 6.

Table 3: Cultural and physiological characteristics of the actinomycete isolate No. A11.

No	Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
1	Starch nitrate agar	good	Gray 264	deep brown 72	No
2	Yeast extract –Malt extract agar (ISP 2)	good	White 263	White 263	No
3	Oat meal agar (ISP3)	moderate	White 263	White 263	No
4	Inorganic salts-starch agar (ISP4)	good	Gray 264	Red 17	No
5	Glycerol-asparagine agar (ISP5)	good	Gray 264	brown 59	No
6	**Melanin pigment media** 1-Peptone yeast extract iron agar (ISP6)	good	Gray 264	Black 276	Deep brown 72
7	2-Tyrosine agar (ISP7)	week	Gray 264	White 263	No
8	3-Trepton yeast extract broth iron agar (ISP 1)	good	Gray 264	Brown 59	Light brown
9	Dox agar	moderate	White 263	White 263	No

Physiological characteristics of the selected actinomycete isolateNo.A11:

Physiological properties of the selected isolate are summarized in Table 4. The isolate was found to be able to utilize, D-lactose, meso-lnositol, L-Rhamnose, D- glucose, D-Melibiose, Dextran as carbon sources, and also can utilize L-Histidine, D-alanine, and L-serine as nitrogen source. The isolate could hydrolyze starch, Elastin, Xanthine and lipid. Whole cell hydrolyzates of the isolate contained LL-diaminopimilic acid.

Table 4: Morphological and biochemical characteristics of the actinomycetes isolate No. A11.

Characteristics	Results	Characteristics	Results
Chemotaxonomic analysis: **-Cell wall hydrolysis for:** Diaminopimelic acid (DAP) Sugar pattern	LL-DAP ND	Thallous acetate 0.001	+
Morphological characteristics: Spore chains	Rectiflexibiles	**Utilization of nitrogen source:-** DL-α-Amino-n-butyric acid	-
Spore surface	smooth	L-Cysteine	-
Spore mass color	gray	L-Valine	-
Substrate mycelial color	brown	L-Phenylalanine	-
Diffusible pigment produced	-	L-Histidine	+
Melanin on peptone yeast iron agar	+	L-Hydroxproline	-
Melanin on tyrosine agar	-	D-alanine	+
Biochemical characteristics: Lecithinase activity	+	L-asparagin	-
Lipolysis	+	L-serine	+
Pectin hydrolysis	-	L-leucine	_
Nitrate reduction	-	L-prolin	-
H2S production	+	L-aspartic acid	-
Starch hydrolysis	+	**Utilization of carbon source** :- arabinose	-
Elastin degradation	+	cellobiose	-
Xanthine degradation	+	D-lactose	+
Arbutin degradation	-	Sucrose	-
Sensitivity assay: Neomycin (50µg/ml)	+	meso-lnositol	+
Rifampicin (50µg/ml)	+	Mannitol	-
Oleandomycin (100µg/ml)	-	L-Rhamnose	+
Penicillin G (10 i. u.)	+	D- glucose	+
Growth at 45C^{o}	-	fructose	-
Growth with (%,w/v): NaCl 7%	+	D-Melibiose	+
NaN3 0.01	-	Raffinose	-
Phenol 0.1	+	Dextran	+
Potassium tellurite 0.001	-	Xylitol	-

-ND: not determined

16S rRNA gene sequencing:

The 16S rRNA gene sequence was defined to the isolate No. A11 at 1517 bits (fig.2). Table 5 shows the multiple sequences alignment which, showed that this isolate close to *Streptomyces bikiniensis* by 96%.

Table 5: Sequence producing significant alignment for isolate No.11.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
NR_043345.1	Streptomyces cinereoruber subsp. cinereoruber strain JCM 4205 16	1917	1917	91%	0.0	96%
NR_041114.1	Streptomyces violaceorectus strain NBRC 13102 16S ribosomal RN	1886	1886	91%	0.0	96%
NR_041129.1	Streptomyces showdoensis strain NBRC 13417 16S ribosomal RNA	1884	1884	91%	0.0	96%
NR_042308.1	Streptomyces viridobrunneus strain :LMG 20317 16S ribosomal RN	1884	1884	91%	0.0	96%
NR_043819.1	Streptomyces hirsutus strain NRRL B-2713 16S ribosomal RNA, pa	1879	1879	91%	0.0	96%
NR_043846.1	Streptomyces werraensis strain NRRL B-5317 16S ribosomal RNA,	1869	1869	90%	0.0	96%
NR_041106.1	Streptomyces nashvillensis strain NBRC 13064 16S ribosomal RNA	1864	1864	91%	0.0	96%
NR_026177.1	Streptomyces bikiniensis strain DSM 40581 16S ribosomal RNA, co	1860	1860	91%	0.0	96%
NR_041076.1	Streptomyces roseolus strain NBRC 12816 16S ribosomal RNA, par	1857	1857	91%	0.0	95%

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Query  8     ACATGC AGTCGAACGATGAAGCCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACA  66
             |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27    ACATGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACA  86
Query  67    CGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA  126
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  87    CGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA  146
Query  127   CGACCTGCCGAGGCATCTCGGCGGGTGGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGC  186
             |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  147   CGACCTGCCGAGGCATCTCGGTGGGTGGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGC  206
Query  187   CTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGA  246
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207   CTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGA  266
Query  247   GGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGG  306
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267   GGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGG  326
Query  307   GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCT  366
             |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  327   GGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCT  386
Query  367   TCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAG  426
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387   TCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAG  446
Query  427   CGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAA  486
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447   CGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAA  506
Query  487   TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTGAAAGCCCGGGGCTT  546
             |||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  507   TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTT  566
Query  547   AACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCC  606
             |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567   AACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCC  626
Query  607   TGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCT  666
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  627   TGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCT  686
Query  667   GGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG  726
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  687   GGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG  746
Query  727   TAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCG  786
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  747   TAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCG  806
Query  787   CAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGA  846
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  807   CAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGA  866
Query  847   ATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAA  906
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  867   ATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAA  926
Query  907   CCTTATCAAGGCTTGACATATACCGGACAGCATCAGAGATGGT-GCCCTCCTTGATGTCG  965
             ||||| |||||||||||||||| |    |||||||| |||| |||||  ||||
Sbjct  927   CCTTACCAAGGCTTGACATATACCGGAAACGGCCAGAGATGGTCGCCC-CCTTGTGGTCG  985
Query  966   GTATACATGTG-TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT-A-GTCC-G  1021
             |||||| ||| |||||||||||||||||||||||||||||||| | |||| |
Sbjct  986   GTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG  1045
Query  1022  CA-CGAGCGCA-CCCTTGT-CTGAGTGGC-AGCATGCC-TTCCGCGTGATGGG-ACTG-C  1074
             || ||||||| |||||| ||| || || ||||||| ||| | ||||||| |||  |
Sbjct  1046  CAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCAC  1105
Query  1075  AG-AGAC-GTCGG--TCAACTCGGAGTA-GGTGGG-ACGACGTCA-GTCATCATGCC--T  1125
             || |||| | |||  ||||||||||| | |||||| |||||||| ||||||||||  |
Sbjct  1106  AGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCT  1165
Query  1126  TATGTCT-GG-CTGCAC-C-TGCTACA  1148
             |||||| || |||||| | |||||||
Sbjct  1166  TATGTCTTGGGCTGCACACGTGCTACA  1192
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Fig. 2: 16rRNA gene sequencing for isolate No.A11
Complete sequence Length=1517- Score = 1860 bits (1007) Identities = 1127/1180

Fermentation, Extraction and purification:

The fermentation process was carried out for seven days at 30 ^{o}C using liquid starch nitrate as production medium. Twenty-liter total volume filtered was conducted followed by centrifugation at 5000rpm for 20

minutes. The most suitable solvent for extraction was chloroform so it was used for the extraction of the metabolite(s) from the broth of a culture of the selected organism. The chloroform extract was concentrated and subjected to thin layer chromatography by using solvent system chloroform: methanol (4:1).The metabolite was eluted solvent in the form of eight bands which fluctuate in their color between red, orange and yellow. The purification process was carried out through a column chromatography packed with silica gel. Fraction number 17 was found to exhibit antimicrobial and insecticidal activity.

Spectroscopic characteristic:

Mass spectrometry analysis of the active substance (Mass spectrum) showed the following fragments: 56.80 (100), 77 (87.32), 104 (72.43), 115 (36.24), 129 (29.73), 145 (21.91), 257 (7.86), 330 (6.19), 389 (4.00), 464 (3.33), 491 (3.73) and 626 (3.91) gave molecular weight of 626 atomic mass unit.

The infrared absorption spectrum (IR) of the active compound showed the following absorption beaks: abroad peak at 3448 cm^{-1} for OH group (Fig. 3). The peaks at 2927 cm^{-1} and 2862 cm^{-1} for CH_3 asym. and CH_2 asym. respectively. The peaks 1326 cm^{-1} for - C H_2 bend. The peak at 1639 cm^{-1} indicate the presence of =C=C=, while peak at 1045 cm^{-1} for C-N stret. and peak at 1523 cm^{-1} for NH bending.

Further, evidence for its characterization came from its $^{+1}$H-NMR (Fig. 4) which exhibited a rest protons appeared in high field region in between 0 .9-1.8 for terminal C H_3 and C H_2 groups. Existence of proton at δ 3.6-3.7 and multiple at 4.1-4.2 corresponding to olefinic proton and H for hydroxyl groups and δ at 7.2 (NH).

^{13}C- NMR (400 MH_Z, DMSO, TMS) of this separated compound showed δ (ppm) at range 20-40 for CH_2 (alkyl chain), δ (60) for –C H_2 N, δ (100-160) for=C=C= and δ (152-154) for–C (OH). Fig. (5).

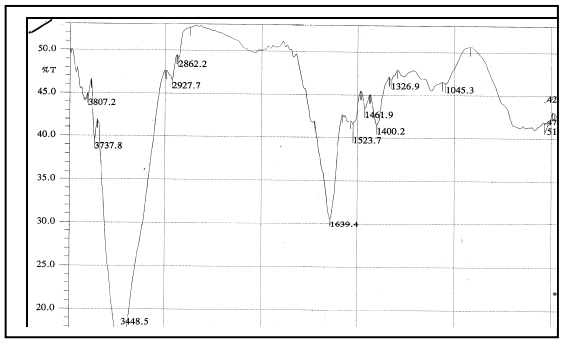

Fig. 3: IR Spectrum in DMSO of active substance

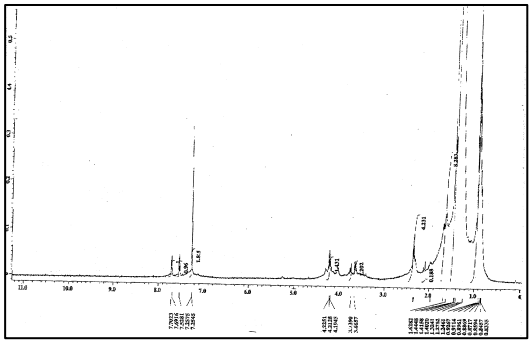

Fig. 4: +1H-NMR spectra of active substance

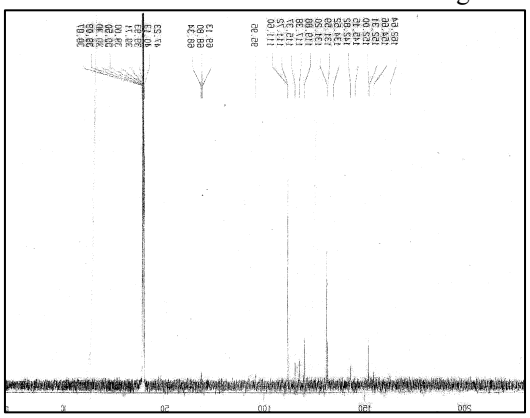

Fig. 5: C^{13} NMR Spectra of active substance

Elemental analysis:

The elemental analysis data showed the following: C=48.14%, H= 7.64%, N=13.437% and O= 30.73 % from which the empirical formula could be calculated for $C_{25}H_{48}N_6O_{12}$.From the fragmentation pattern of the MS, $^{+1}$H -NMR, ^{13}C -NMR, IR and elemental analysis it was found that, the active purified metabolite may be related to aminoglycosidic antibiotic.

Determination of Minimum Inhibitory Concentration (MIC):

The results recorded in Table 6. Showed the MIC of the active compound, the results indicated that the active compound produced from *Streptomyces bikiniensis* A11, has antibacterial activity against both Gram-positive and Gram-negative bacteria.

Table 6: In vitro antimicrobial activity as MICS (μg/ml) of the purified metabolite produced by *Streptomycs bikiniensis* A11

Tested microorganisms	MIC (μg/ml)
Streptococcus pyogenes	9.8
Staphylococcus aureus	39
Bacillus subtilis	19.5
Pseudomonas aeruginosa	78.1
Proteous vulgaris	39
Klebsiella pneumonia	9.8
Salmonella typhymurium	78.1
Escherichia coli	19.5

DISCUSSION

The filamentous actinomycetes are well known as prolific producers of biologically active secondary metabolites of economic significance to the chemical, pharmaceutical and agricultural industries. Genus *streptomyces* considered the most antibiotic producing genus of actinomycetes, where it produce about 45-55% of known antibiotics. For this reasons an attempt to isolate *streptomyces* species having the capacity for producing active agents from natural soil habitat of microorganisms was achieved. The selected actinomycetes isolate was subjected to trial to identification using international keys (Shirling and Gottlieb, 1966, 1968a, 1969 and 1972; Buchanan and Gibbson's, 1974; Williams, 1989 and Hensyl, 1994). The morphological and physiological characteristics of selected strain indicated similarity to *streptomyces bikiniensis*. Also phylogenetic analysis demonstrates the isolate shared a sequence identity of 96% with known *S. bikiniensis*. It similar to the isolate sporophores and it is monopodically branched with oval to elongate rectiflexibiles spore. The growth on ISP medium give an aerial mycelium colour fluctuate from gray to white while substrate mycelium give brown, white, red and black colors.

S. bikiniensis discovered by Jhonstone and Waskman (1947), they indicated that this species was capable of elaborated an antibiotic that proved to be similar to streptomycin. Also, Wang, *et al*., 2009 showed that the *S. bikiniensis* has antimicrobial activity against the gram- positive and gram–negative bacteria and fungi. Development of resistance to antibiotics and insecticides represented an increasing problem in veterinary and human medicine. This required development of antibiotics and search of new antibiotic used for treatment of resistant strains. From the fragmentation pattern of the MS, ^{13}C-NMR, IR , ^{1}H NMR and elemental analysis , it was found that active purified metabolite may be is consider as a member of aminoglycosidic antibiotic. Aminoglycosides are an important class

of antibiotics that are active against both gram-positive and gram negative bacterial infections, and they have found widespread use in both human and veterinary medicine. They act by creating assures in the outer membrane of the bacterial cell (Gonza and Spencer 1998). Aminoglycosides, including G418 and streptomycin, inhibit bacterial protein synthesis and are used widely to treat infections caused by Gram-negative bacteria. These antibiotics are nephrotoxic, and the mechanisms underlying this phenomenon are under investigation. Cellular toxicity of aminoglycosides is thought to be initiated by interaction of the compounds with negatively charged phospholipids in biological membranes (Mingeot, *et al.*, 1995).

The first aminoglycoside, streptomycin, was isolated from *Streptomyces griseous* in 1943. Neomycin, isolated from *Streptomyces fradiae*, had better activity than streptomycin against aerobic gram-negative bacilli but, because of its formidable toxicity, could not safely be used systemically (Walsh, 2003). Gentamicin, isolated from *Micromonospora rhodorangea* in 1963, was a breakthrough in the treatment of gram-negative bacillary infections, including those caused by *Pseudomonas aeruginosa,* and it is useful in the treatment of infected skin cysts and other skin abscesses preceded by incision and drainage and infected insect bites and stings. It blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells (Davies and Jimenez, 1980). Quinomycin A from *Streptomyces sp*. KN-0647 displayed growth inhibition on the test pathogenetic insects such as *Spodoptera exigua, Dendrolimus punctatus, Plutella xylostella, Aphis glycines and Culex pipiens.*(Liu, *et al.*, 2008). The structure of these antibiotics including intermolecular hydrogen bonding interactions among the carboxyl, hydroxyl and amino groups stabilize the channel pore causing the cytoplasm contents to escape, so it may be toxic to some insect cell types (Walsh, 2004).

REFERENCES

Baltz, R.H. (1998): Genetic manipulation of antibiotic producing Streptomyces. Trends Microbial. 6: 76-83.

Berlinger, M.J.; Miller, M. Tal and Tamin M. (1997): Resistance mechanisms of *Lycopersicon pennellii* accessions to *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae). J. Econ. Entomol. 90: 1690-1696.

Betina, V. 1983. The Chemistry and Biology of Antibiotics. Elsevier Scientific Publishing Company Inc, Amesrdam, New York, USA.

Bream, A.S.; Ghazal, S. A.; Abd el-Aziz, Z.K. and Ibrahim, S.Y. (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. 66:503-12.

Briggs, J.N. (1960): Reduction of adult house fly emerge effective *Bacillus* sp. On the development of immature forms. J. Insect Pathol. 2: 418-432.

Buchanan, R.E. and Gibbson, N.E. (Ed) (1974): "Bergey's Manual of Determinative Bacteriology" 8th ed. The Williams& Wilkins Company, Baltimore.

Canaday, C.H. (1995): Biological and cultural tests for control of plant diseases. American Photopathological Society, St.Paul, MN.

Crawford, D.L.; Lynch, J.M.; Whipps, J.M. and Ousley, M.A. (1993): Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl.Environ. Microbiol. 59: 3899-3905

Davies, J. and Jimenez, A. (1980): A new selective agent for eukaryotic cloning vectors. Am J Trop Med yg 29 (5 Suppl):1089-92

Dhanasekaran, D.; Sakthi, V.; Thajuddin, N. and Panneerselvam, A. (2010): Preliminary evaluation of anopheles mosquito larvicidal efficacy of mangrove actinobacteria. Int.J. of Appl. Biol. and Pharmaceutical Technol., 1(2): 374 - 381.

Edwards, U.; Rogall, T.; Bocker, H.; Emade, M. and Bottger, E. (1989): Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal DNA. Nucleic Acid Res. 17:7843-7853.

El-Defrawi, M. E.; Toppozada, A. T.; Salama, A. and El-Kishen, S. A. (1964): Toxicological studies on the Egyptian cotton leafworm, *Prodenia litura* F. II. Reversion of Toxaphene resistance in the Egyptian cotton leafworm. J. Econ. Entomol. 57: 593-595.

El-Khawagh, M. A.; Hamadah, Kh. Sh. and El-Sheikh, T. M. (2011): The insecticidal activity of actinomycete metabolites, against the mosquito *Culex pipieins.* Egypt. Acad. J. biolog. Sci., 4 (1): 103- 113.

EL-Nakeeb, M. A. and Lechevalier, H. A. (1963): Selective isolation of aerobic actinomycetes.Appl. Microbiol. 11: 75.

El-Sebae, A.H.; Abo-zeid, M. and Salah, M.A.(1993): Status and environmental impact of toxaphene in the third world-a case of African Agriculture. Chemosphere, 27: 2063-2072.

Finny, D.J. (1962). Probit Analysis. Cambridge University Press, Cambridge.

Finny, D.J. **(**1971). Probit Analysis. Cambridge University Press, 3rd Edn. pp: 350.

Gadelhak, G. G.; EL-Tarabily, Kh. A. and AL-Kaabi, F. K. (2005): Insect control using chitinolytic soil actinomycetes as biocontrol agents. Int. J. Agri. Biol., 7 (4): 627–633.

Goodfellow, M.; Williams, S.T.and Mordarski, M. (1988): Actinomycetes in biotechnology. London, Academic Press.

Gonza,L. Z ; Spencer, J.P. (1998): Aminoglycosides: A Practical Review, Am. Family Physicial L.S. 15, 1811.

Guangying, C., Birun, L., Yongcheng, L., Fengchun, X., Wen, L. and Wang-Fun, F. (2005): A new Fungicide produced by a Streptomyces sp. GAAS7310. J. Antibiot. 58(8):519-522.

Hall, T.A. (1999): A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41:95-98.

Hensyl, W.R. (1994): "Bergey's Manual of Systematic Bacteriology" 9th ed. John. G. Holt and Stanley, T. Williams (Ed.) Williams and Wilkins, Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokyo.

Hainzl, D.; Cole, L. M. and Casida, J**.** E. (1998): Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem. Res. Toxicol. 11: 1529-1535.

Hokkanen, H.M.T. and Lynch, J.M. (1995): *Biological Control: Benefits andRisks*. Cambridge University Press, New York.

Hussain, A.A.; Mostafa, S.A.; Ghazal, S.A. and Ibrahim, S.Y. (2002): Studies on antifungal antibiotic and bioinsecticidal activities of some actinomycete isolates. African J.Mycol. Biotechnol., 10: 63-80.

Jhonstone,D. B., and Waskman, S.A. (1947): Streptomycin II, an antibiotic substance produced by a new species of *streptomyces.* Proc. Soc. Exptl. Biol. Med., 65:294-295.

Kandil, M. A.; Abdel-Aziz, N. F. and Sammour, E. A. (2003): Comparative toxicity of chlorfluazuron and leufenron against cotton leaf worm, *S. littoralis* (Boisd). Egypt. J. Agric. Res. NRC, 2: 645-661.

Kenneth T. Mackay. (1995). PART111:INTERACTION31. A Comparative Study of the ability of fish to Catch Mosquito Larvae". Rice-fish culture in china. International Development Research Center. ISBN 1-55250-313-5.

Lechevalier, H.A., and S.A. Waksman. (1962): The actinomycetes. III. Antibiotics of actinomycetes. Williams & Wilkins, Baltimore.430 pp.

Liu, H.; Qin, S.; Wang Y.; Li, W.and Zhang J.(2008): insecticidal action of Quinomycin A from *Streptomyces sp*. KN-0647, isolated from a forest soil. World Journal of Microbiology & Biotechnology,24(10):2243-2248.

Mingeot,L. M.P; Brasseur, R. and Schanck, A. (1995): Molecular parameters involved in aminoglycoside nephrotoxicity. J. Toxicol. Environ. Health 44(3): 263-300.

Miyadoh, S. (1993): Research on antibiotic screening in Japan over the last decade. A producing microorganism approach. Actinomycetologica 9: 100-106.

Nakas, J.P. and Hagedorn, C. (1990): *Biotechnology of Plant Microbe Interactions*. McGraw–Hill, New York.

Okami, Y.; Hotta, K. (1988): Search and discovery of new antibiotic, actinomyces in Biotechnology, London, Acadimic, pp., 33-36.

Osman G.; Mostafa, S. and Sonya, H. M. (2007): Antagonistic and insecticidal activities of some *streptomyces* isolate Pak. J. Biotechnol. 4 (1-2): 65-71.

Perez, C.; Pauli, M. and Bazerque, P. (1990). An antibacterial assay by agar well diffusion method. Acta Bio .ET. Med .Exp.15: 113-115.

Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning: a laboratory Manual (2^{nd}ed.). Cold Spring Harbor Laboratory, Cold Spring.

Shirling, E.B.; Gottlieb, D. (1966): Methods for characterization of *Stereptomyces* species. Int.J.Syst. Bacteriol. 16:313-340.

Shirling, E.B.; Gottlieb, D. (1968a): Comparative descriptions of type culture of S*tereptomyces* II, Species descriptions from first study. Intern. J. Syst. Bacteriol. 17: 69.

Shirling, E.B.; Gottlieb, D. (1969): Comparative descriptions of type culture of *Stereptomyces* IV, Species descriptions from second, third and fourth studies. Intern. J. Syst. Bacteriol.19: 391.

Shirling, E.B; Gottlieb, D. (1972): Comparative descriptions of type culture of *Stereptomyces* V, Additional description. Intern. J. Syst. Bacteriol. 22:265.

Sundarapandian, S.; Sundaram, M.D.; Tholkappian, P. and Balasubramanian, V. (2002): Mosquitocidal properties of indigenous fungi and actinomycetes against Culex quinquefasciatus Say. J. Biol. Control., 16: 89-91.

Tanaka, Y. and Omura. C. S. (1993): Agroactive compounds of microbial origin. Annu.Rev. Microbiol. 47: 57-87.

Temerak, S.A. (2002): Historical records of cotton leaf-warm (*Spodoptera littoralis)* resistance to conventional insecticides as influenced by the resistance programs in Egypt from 1950-2002. Resistant Pest Management, 12:33-36.

Tomlin, C.D.S. (2001): The Pesticide Manual (A world compendium). 12^{th} Ed. British Crop Protection Council, UK.

Walker, K.; Lynch, M. (2007): Contribution of An anopheles larvae control to malaria suppression in tropical Africa: review of achievements and potential". Med. Vet. Entomol. 21(1):2-21.

Walsh, C. (2003): Antibiotics: Actions, Origins and Resistance, ASM Press: Washington, D. C., pps.107-120:222-226

Walsh, C.T. (2004): Polyketide and nonribosomal peptide antibiotics: Modularity and versatility. Science 303:1805-1810.

Wang, L.; Lei, J.; Xiang, W.; Jiang, S.; Sun, J. and Yang, Z. (2009): Identification and characterization of purple-producing actinomycete strain. Chin J Appl Environ Biol. 15(1): 139-142.

Waskman, S.A. (1961): an Antibiotic of actinomycetes. In:"The Actinomycetes," Vol.111, the Williams and Wilkins Company, U.S.A.

White, S.M.; Dunbar, D.M.; Brown, R., Cartwright, B., Cox, D., Eckel, C.; Janson, R. K., Moorkerjee P. K.; Norton, J. A.; Peterson, R. F.; Starner, V. R. (1997): Emamectin benzoate: a novel Avermectin derivative for control of lepidopterous pests in cotton. Proc. Beltwide Cotton Conf., pp. 1078-1082.

Williams ST.; Goodfellow M.; Alderson G. (1989): Genus *stereptomyces.* In :Bergy's Manual of Systematic Bacteriology (ed), Williams ST. Sharope ME. Holt JM. Baltimore, 2452-2492.

Xiong, L., Li. J. and Kong, F. (2004): *Streptomyces* sp. 173, an insecticidal microorganism from marine. Lett. Appl. Microbiol.38 (1):32-37.