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Insecticidal Activity of Actinomycetes Isolated from Salt Range, Pakistan against Mosquitoes and Red Flour Beetle

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Abstract.- A total of fifty-one actinomycetes strains were selectively isolated on glycerol casein KNO_3 agar and actinomycetes isolation agar (AIA). The isolates were screened for the production of insecticidal and larvicidal compounds against 3rd instar larvae of mosquitoes *Culex quinquefasciatus* and *Tribolium castaneum* (Red flour bettle). The biological screening revealed the metabolites of three isolates exhibit 100% mortality of the tested larvae. In chemical screening by thin layer chromatography (TLC) and HPLC-UV, the crude extracts obtained from the culture broths of these isolates, exhibited an impressive diversity of the chemical constituents. The selected isolates SA-10BC, SA-9K and SA-9L were characterized for their morphological, biochemical, physiological and cultural characteristics and by 16S rRNA gene sequencing. The the selected isolates show maximum genetic similarity with *Streptomyces rochei* (99%), *Streptomyces minutiscleroticus* (98%) *and Streptomyces phaeoluteigrisseus* (92%) respectively.

Key words: Insecticidal activity, larvicidal activity, saline actinomycetes, 16S rRNA gene sequencing,

INTRODUCTION

Mosquitoes are the most important single medically important group of insects acting as arthropod vector of diseases such as dengue fever, yellow fever, malaria, filariasis, japanese encephalitis and others etc. causing millions of deaths every year (Borah *et al.*, 2010; Kekuda *et al.*, 2010) and are major threat for over 2 billion people in the tropics (Odalo *et al*., 2005). *Culex quinquefasciatus* is a major causative agent for the transmission of filariasis. Lymphatic filariasis is an extremely painful, debilitating and disfiguring disease, affecting more than 120 million people in 80 countries worldwide and 18% of the world's population is at risk for contracting elephantiasis (WHO, 1998). *Tribolium castaneum* (Herbst) is one of the major and serious pests of cereal grains and causes great economic losses to the stored grain. Due to severe infestation caused by *T. castaneum*, the flour decays and turns yellowish, gives a pungent, unpleasant odor, negatively affects baking quality of flour and ultimately becomes unhealthy for human use (Li *et al.*, 2005).

Extensive application of chemical pesticides in agriculture and public health has lead to numerous undesirable effects. In order to search for environmentally safe substitute, biopesticides are more appropriate compared to synthetic pesticides. *Streptomyces* spp. have been acknowledged as prolific producer of valuable bioactive metabolite with broad spectrum of activities which has antibacterial, antifungal, antibiotic, anti- parasitic antitumor, antiviral, insecticide, herbicide, immuno-modulators, antithrombatic agents (Baltz, 2008; Reddy *et al.*, 2011; Huamei *et al*., 2008; Muzzamal *et al.*, 2012). Halophilic microorganisms can be easily grouped according to their requirements for NaCl for growth (Larsen, 1986). The occurrence of actinomycetes in high saline environments and the tolerance of these organisms to high concentrations of salts have been described by Tresner *et al.* (1968) and Gottlieb (1973).

The salt range of Pakistan is well known for its rich and unique assemblage of flora and fauna. To the best of our knowledge the actinomycetes flora of salt range has never been investigated for its insecticidal and larvicidal activity. The present study deals with the isolation of bioactive actinomycetes strains from saline soils of this unique ecological niche of Pakistan. The selected strains were cultivated on a small scale as 150 ml shaking culture and the obtained bioactive extracts were used to screen for insecticidal and larvicidal activity. About fifty one bioactive actinomycetes

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were recovered from the ten collected soil samples. Among them, three bioactive isolates were selected based on their larvicidal and insecticidal activity and were identified as species belonging to the genus *Streptomyces* by analyzing their morphological characteristics (Locci, 1989) and by preliminary physiological testing. The final taxonomic status of these strains was determined by 16S rRNA gene sequencing.

MATERIALS AND METHODS

Sample collection

Ten soil samples were collected from various sites in salt range, Kalar Kahar, Pakistan, in labeled sterile polythene bags, and were processed further by physical (50-55°C for 2-16 h) and chemical treatment ($CaCO_3$ in ratio10:1 w/w) methods described by Hayakawa and Nonomura (1989) for enrichment of streptomycetes.

Selective isolation of actinomycetes

One gram of each soil sample was suspended in 10 ml of sterile water and vortexed for 45 sec. Casein KNO_3 agar (glycerol 10 g, KNO_3 2 g, casein 0.3 g, NaCl 2 g, K_2HPO_4 2 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_20$ 0.01 g, agar 18 g in one liter) was prepared and sterilized at 121 $^{\circ}$C in 15 lbs pressure for 15 min. The media was supplemented with 50 µg/ml of nystatin to prevent the growth of fungal contaminants. The soil samples were serially diluted and 0.1 ml of the dilution 10^{-3} was spreaded on the surface of the Casein KNO_3 agar. The plates were incubated at 28±2 ºC for 7-21 days, the presumptive actinomycetes colonies were selected and transferred to the commonly used growth medium for actinomycetes *i.e.* GYM agar (10 g malt extract, 5 g yeast extract, 5 g glucose, 15 g agar in one liter of distilled water) (Shirling and Gottlieb, 1966). The selected colonies were purified by repeated sub culturing on GYM agar until pure cultures were obtained.

Identification of the selected actinomycetes

The selected actinomycetes strains were identified by morphological, biochemical and physiological characterization and by 16S rRNA gene sequencing. The morphological characteristics including colony morphology, aerial and substrate mycelia, spore morphology, and pigmentation were studied after cultivating the individual isolates on GYM agar at 28ºC for at least 7 days by the methods as described by (Hucker and Conn, 1923). The selected isolates were investigated for various biochemical and physiological characteristics including, melanin production, utilization of nine different sugars as carbon source, formation of organic acids, utilization of organic acids and oxalate, hydrolysis of esculin, urease, amylase, lipase, hemolysin, tyrosine hydrolysis, gelatinase and indole production. For 16S rRNA gene sequencing, high molecular weight chromosomal DNA of selected *Streptomyces* strains was prepared from GYM grown mycelia according to Felnagle *et al*. (2007). PCR amplification of 16S rRNA gene was done by using two primers (27f: AGAGTTTGATCCTGGCTCAG) and (1522r: AAGGAGGTGATCCARCCGCA). The PCR products were purified by gel extraction kit (Fermentas) and the purified product was sequenced using dye terminator chemistry on an automated sequencer. The sequence data obtained was analyzed using the advanced BLAST search program at the NCBI website: http://www.ncbi.nlm.nih.gov/BLAST, to determine the genetic similarity of the isolates with those already reported in the gene bank.

Determination of antimicrobial activity

Agar diffusion method was used for the determination of the antimicrobial activity of selected strains against a panel of six test organism including *Bacillus subtilis, Staphylococcus aureus,* methicillin resistant *S. aureus, E. coli, Proteus vulgaris, Klebsiella pneumoniae*. The isolates were inoculated in 30 ml of glucose yeast extract malt extract (GYM) broth (Shirling and Gottlieb, 1966) and were incubated at 28°C on a shaker at 95 rpm for 5-7 days. 1 ml of cell free supernatant was centrifuged at 10,000 rpm for few minutes. The plates for the test organisms were prepared by pouring 14 ml of Luria Bertani agar (LB-agar) as base layer, after solidifying this was overlaid with 4ml of inoculated seed layer. By using sterile cork borer, agar wells (5 mm diameter) were made and 60µl of the supernatant was loaded to these wells.

The plates were then left at room temperature for 2 h for the supernatant to diffuse and were incubated at 37°C for 18-24 h. After incubation the zone of inhibition was measured in mm.

Rearing Tribolium castaneum

Adults of *T. castaneum* were mass produced in glass jars (250 ml) covered with a piece of fine cloth, starting with insects found in grains free from pesticides three months before. Insects were reared on a diet of wheat flour. 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). Both the larvae and adult insects of the reared *T. castaneum* were used after two weeks for the experiments.

Collection of Culex quinquefasciatus *Larvae*

The mosquito used in this study was *C. quinquefasciatus*. They were collected in platic boxes in and around the Botanical Garden, Punjab University, Lahore and reared for several generations 3rd instar larvae were collected from a water reservoir in and around the Botanical Garden, Punjab University, Lahore. The mosquitoes were kept in insectariums, under the controlled conditions at temperature of 27±2ºC, relative humidity 70±10% and 12-12 light-dark regime.

Cultivation and extraction of the bioactive compounds from the selected actinomycetes

The larvicidal activity was determined by the method of WHO (1996) with some modification and as per the method of Rahuman *et al.* (2000). The selected strains mainly *Streptomyces* were inoculated in 250 ml conical flask containing 150 ml GYM broth (the pH was adjusted to 7.8 before sterilization) and were incubated as shaker culture (95 rev/min) for 7 days at 28 ºC. After incubation, bioactive extracts were recovered from cell free culture filtrate by solvent extraction method following the methods described by Liu *et al.* (2008). Ethyl acetate was added in a ratio of 1:1 (v/v) and sonicated for 15 min. Later, the mixture was separated by separating funnel and filtrate was evaporated on a rotary evaporator to obtain the crude extracts. The extracts were subjected to insecticidal and larvicidal screening and were analyzed by thin layer chromatography (TLC) and by high performance liquid chromatography (HPLC-UV) for the characterization and partial identification of bioactive compounds.

Determination of larvicidal and insecticidal activity of the culture filtrates against T. castaneum

Insecticidal activity of the culture supernatant of the selected actinomycete isolates was determined against the wheat flour insects and larvae. Insects (5-6) and their larvae were placed separately in each of the sterile and labeled petri-plates containing Whatmann's filter paper soaked with culture supernatant. A control was kept without adding any culture supernatant to the filter paper. The insecticidal effect of the culture supernatant was determined by counting the number of dead insects after 0 h, 12 h and 24 h. Dead insects were identified when they failed to move after probing with a needle in the siphon or cervical region. The test was repeated thrice and insects and larval mortality was calculated (Fernandes *et al.,* 2005).

Determination of larvicidal activity of the methanolic extracts against C. quinquefasciatus

Insecticidal activity of the methanolic extracts was determined against the third instars larvae of the *C. quinquefasciatus.* Ten larvae were placed separately in each of the sterile and labeled beaker containing methanolic extract. The control beakers were kept without adding any extract. The larvicidal activity was observed for over 30 minutes and death of the larvae confirmed the larvicidal activity. The test was repeated thrice and larval mortality for each concentration of the extracts was calculated (Khanna and Kannabiran, 2007).

Thin layer chromatography (TLC)

TLC was carried out as described previously by Sajid *et al.* (2009). A line was drawn about one inch from the base of TLC plate (TLC Aluminum sheets 20×20 Silica Gel 60 F254 Merck, Germany) and sample numbers were marked on it. By using capillary tube each sample extract was spotted on the TLC plate drop wise, with allowing the former spot to dry before super imposing it. The TLC plate was air dried after loading the appropriate quantity (2-5 µg), and was developed with 10% MeOH/

CH_2Cl_2 solvent system. The developed and air dried TLC plates were visualized under UV (366 nm and 254 nm). The components showing UV absorbance and fluorescence were marked and were scanned. The TLC plate was stained by spraying it with Anisaldehyde/H_2SO_4 and Ehrlich's reagent separately and was dried under a hot air drier to analyze the color bands produced.

High performance liquid chromatography (HPLC-UV)

The extracts were analyzed by HPLC-UV using the software clarity. HPLC system consisted of two pressure pumps (Syknm S1122 delivery system), an injection port with a 20 µl loop (Syknm S 5111 injector valve bracket), and a UV detector (Syknm S 3210 UV/Vis detector). The column used was RP C18 (Thermo Hypersil Keystone, 250 x 4.6 mm 5µm Hypersil). Mobile phase used was methanol and water (95:5) and the flow rate was adjusted to 1ml/min. The crude extracts were dissolved in 200 µl methanol and 20µl was injected through a micro syringe. The sample was run for 15 minutes and UV absorbance was determined at 254 nm. The peaks of each sample were analyzed and were compared at different retention times (t_R) with standard UV absorption data of secondary metabolites.

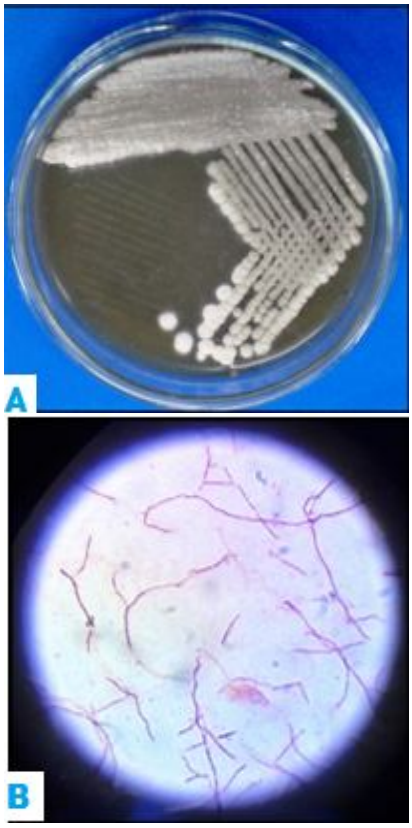

Fig. 1 *Streptomyces* isolate SA-10BC on GYM agar (pure culture), b: Microscopic appearance of the *Streptomyces* isolate SA-9K (100X)

RESULTS

Characteristics of the selected actinomycetes

The selected actinomycete isolates produce colored aerial and substrate mycelia ranging from light grey to dark brown. All the strains are gram positive with characteristic branching substrate mycelia (Fig. 1). The optimum temperature for the growth was found to be 28ºC and the optimum pH for growth was in the range of 7.5- 8.5, along with NaCl tolerance up to 7%. All the isolates were able to efficiently utilize D-glucose, D-lactose and D-fructose as sole source of carbon, more over majority of the isolates exhibited growth on D-raffinose, L-rhamnose, L-arabinose, D-galactose, sucrose and D-xylose. D-mannose was the least utilized sugar by the isolates as carbon source. Among the 51 strains characterized only 19 isolates exhibited production of melanin, while 74.5% exhibited organic acids utilization and 54.9% showed formation of organic acids. Similarly 72.5% isolates exhibited esculin hydrolysis, while starch hydrolysis was shown by 70% of the strains. The lipase, tyrosinase and gelatinase activity was shown by 50% of isolates and formation of clear zones around growth on blood agar were exhibited by 75% of isolates more over 35% of the isolates gave positive results for nitrate reduction test. None of the strain was found to be able to utilize phosphates (Table I). In 16S rRNA gene sequencing of SA-10BC SA-9K and SA-9L, the most active isolates in the whole collection, the isolates exhibited maximum genetic similarity with *Streptomyces rochei* (99%), *Streptomyces minutiscleroticus* (98%) and *Streptomyces phaeoluteigriseus* (92%).

Table I.- Taxonomic characteristics of the selected *Streptomyces* strains exhibiting significant larvicidal and insecticidal activity.

Characteristics	SA-10BC	SA-9K	SA-9L
Morphological			
Colony appearance	Grey with dark brown colony	Dark grey with yellowish colony	White with light brown colony
Gram staining	Positive	Positive	Positive
Biochemical			
Utilization of organic acids	+	+	+
Formation of organic acids	+	+	-
Utilization of oxalate	+	+	-
Hydrolysis of esculin	+	+	+
Utilization of			
D-Glucose	+	+	+
D-Lactose	+	+	+
D-Raffinose	-	+	+
L-Rhamnose	+	+	+
L-Arabinose	-	+	+
D-Mannose	+	+	+
D-Galactose	+	+	+
Succinate	+	+	+
Ribose	-	+	+
Enzyme potential			
Urease	+	-	-
Amylase	+	+	+
Lipase	+	+	+
Hemolysin	-	+	-
Tyrosine hydrolysis	+	-	-
Gelatinase	-	-	+
Protease	-	-	-
Phosphatase	-	-	-
Nitrate reduction	+	+	+
Pectinase	+	+	+
Indole production	-	-	-
Growth at temperature			
10 ºC	-	+	+
28 ºC	++	+++	+++
37 ºC	++	++	++
45 ºC	-	+	-
pH			
7	++	++	++
8	+++	+++	++
9	+	+++	+++
NaCl tolerance			
0%	++	+++	+
4%	+++	+++	+
7%	+++	+++	+
10%	+	+	-
13%	-	-	-

+++= best growth, ++= good growth, +=growth, -=no growth

Antimicrobial activity of the selected isolates

The selected actinomycetes strains exhibited potent antimicrobial activity against the test organisms including: *B. subtilis, S. aureus, K. pneumoniae,* methicillin resistant *S. aureus* (MRSA), *P. vulgaris*, and *E. coli*. Among all the strains 20 isolates were detected as the promising strains for the production of active compounds as they exhibited significant growth inhibition of the test organisms. Among them the extracts of 19 strains were active against *B. subtilis*, 17 against *S. aureus*, 16 against *E. coli*, 16 against *MRSA*, 9 against *P. vulgaris* and 8 were found to be active against *K. pneumoniae* by cross streak method. The extracts of these 20 isolates were further investigated by agar diffusion method for antimicrobial activity, in this screening 9 were active against *S. aureus*, 12 against *MRSA*, 3 against *E. coli*, 8 against *B. subtilis*, 7 against *P. vulgaris* and 7 against *K. pneumoniae*. A zone of inhibition up to 22 mm was recorded in case of the extract of isolate SA-12b against *S. aureus,* and *B. subtilis,* similarly a zone of inhibition up to 20 mm was observed in case of the extract of isolate SA-8BB against *E. coli* by agar diffusion method. The strain SA-10BC extract showed maximum zone of inhibition 22 mm against *B. subtilis* and 16mm both against *S. aureus* and *MRSA*. The extracts of the remaining strains showed zones of inhibition between 9 mm to 22 mm. A detailed profile of the antimicrobial activity of the extracts of selected isolates is shown in Figure 2 and Table II.

Insecticidal and larvicidal activity of the selected isolates

The insecticidal and larvicidal activity of the bioactive compounds produced by the selected actinomycetes isolates was determined by *in vitro* screening against *T. castaneum* and 3rd instar larvae of *C. quinquefasciatus.* Among all the isolates, seventeen were found to possess significant larvicidal and insecticidal activity. In case of *T. castaneum*, the isolates SA-10BC, SA-9K, SA-9L exhibited 100% larval and adult mortality, while the isolate SA-26n exhibited 80% mortality, isolates SA-2A, SA-27b 60%, isolates SA-8BB, SA-10C^{2}, SA-12, SA-31, SA-53, SA-80C and SA-91d 40%, isolates SA-100, SA-26p, SA-10n and SA-10AA

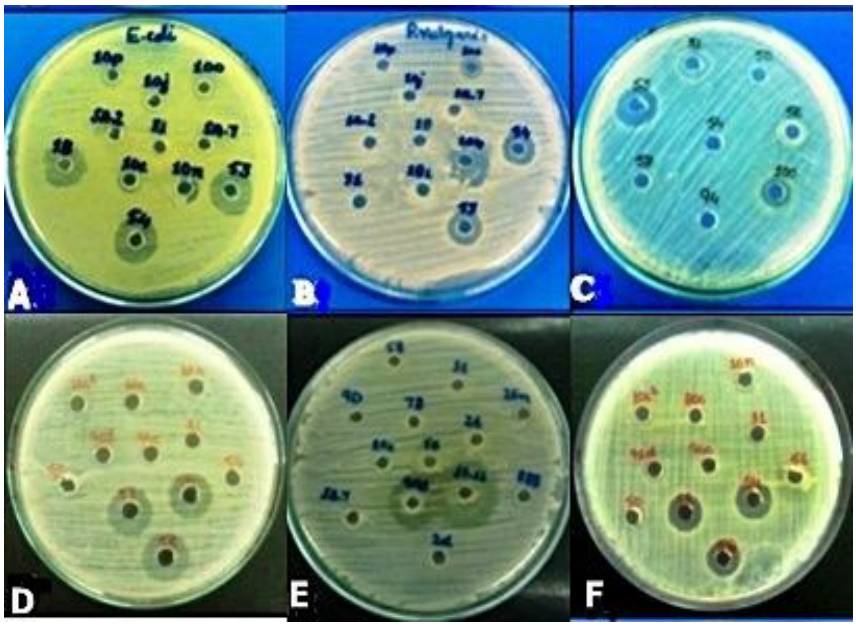

Fig. 2. Antimicrobial activity of the selected *Streptomyces* against clinical isolates A) Activity of SA-1B, SA-53, SA-53 and SA-10n against *E.coli*. **B)** Activity of SA-53, SA-54, SA-10AA and SA-80c against *P.vulgaris*. **C)** Activity of SA-53 and SA-10BC against *S.aureus*. **D)** Activity of SA-53, SA-9K and SA-9L against *MRSA.* **E)** Activity of SA-1B and SA-10BC against *K.pneumoniae.* **F)** Activity of SA-53, SA-54 and SA-10AA against *B.subtilis.*

Table II.- Antimicrobial activity of crude extracts of the selected *Streptomyces* isolates (Zone of inhibition in mm).

S. No.	Strains	*Staphylococcus aureus*	*MRSA*	*E. coli*	*Bacillus subtilis*	*Proteus vulgaris*	*Klebsiella pneumonia*
1	SA-2A	0	5	0	9	0	5
2	SA-9D	8	7	0	-	0	9
3	SA-9L	6	10	0	14	0	6
4	SA-9Q	12	11	12	11	11	11
5	SA-10	19	7	0	-	10	0
6	SA-10BC	16	16	5	22	17	10
7	SA-10C[2]	12	12	0	20	18	6
8	SA-12b	11	10	0	-	13	0
9	SA-53	11	13	0	16	0	0
10	SA-54	12	17	0	-	11	0
11	SA-78	0	8	6	11	12	5
12	SA-100	0	9	0	8	0	0

exhibited 20% larval and adult mortality after 24 h at concentration of 5mg/ml (Table II). Likewise in case of 3rd instar larvae of *C. quinquefasciatus* the isolates SA-10BC, SA-9K, SA-9L 100% larval mortality after 24 h. While the methanolic extracts of the isolates SA-94c, SA-53 and SA-10B exhibited 80% larval mortality, isolate SA-10n 60%, isolates SA-9AA, SA-12, SA-31 and SA-80c 40%, the isolates SA-2A, SA-8BB, SA-9P, SA-26n and SA-56 exhibited 20% larval mortality at different time intervals. Overall the screening revealed that the isolates 10BC, SA-9K and SA-9L are potent producers of insecticidal and larvicidal compounds which can be effectively used against *T. castaneum*

and larvae of *C. quinquefasciatus.*

TLC and HPLC-UV profile of the selected strains

The extracts of the selected *Streptomyces* strains SA-10BC, SA-9K and SA-9L gave prominent bands both under short (254 nm) and long (366 nm) UV as well as with the spraying reagents. The crude extract of the strain SA-9K gave pink, yellow, brown and chocolate brown bands. SA-10BC also gave pink, yellow and light brown bands but less prominent than SA-9K. The prominent presence of these bands indicates that these saline soil *Streptomyces* might possess diverse bioactive compounds that show effective larvicidal and insecticidal activity (Fig.3). The HPLC-UV chromatograms were used in order to find out peaks of unidentified bioactive compounds at different retention times (t_R). HPLC-UV chromatogram of crude extract of strains SA-9K, SA-9L showed major peaks at retention time 3.33, 3.38 min with % age peak area 92.1% and 72.3% respectively. SA-10C was very fascinating because its HPLC-UV chromatogram shows 4 peaks at retention times 2.18, 2.58, 3.52 and 3.70 min with (%) peak area 20.3, 16.3, 1.5, and 42.1 respectively. The isolate SA-10BC also gave four major peaks at retention time 2.31, 2.62, 3.37 and 3.66 with (%) peak area 19.9, 11.1, 3.9, 46.6, respectively (Fig.4).

DISCUSSION

Actinomycetes are filamentous, gram-positive bacteria and are the major producers of biologically active secondary metabolites of economic importance in agricultural, chemical and pharmaceutical industry. Among them, *Streptomyces* is by far the most prolific producer and has provided about 10000 known antibiotics which is 45-55% of the total known antibiotics (Berdy, 2005). Actinomycetes play a significant role in the biological control of insects by producing active secondary metabolites and are being extensively screened worldwide for their agricultural and industrial importance (Gadelhak *et al.,* 2005). In this study, from 10 soil samples collected from saline soils a total of 51 actinomycetes were isolated by using the effective enrichment methods including physical treatment at 50-55ºC for 2-16 h and chemical treatment with $CaCO_3$ (10:1). Glycerol casein KNO_3 agar supplemented with Nystatin (50µg/ml) as antifungal agent was found to be most suitable medium for the isolation of saline actinomycetes because it yielded maximum actinomycetes colonies in the crowding plate technique in comparison to the actinomycetes isolation agar (AIA). The morphological, biochemical and physiological characteristics of the selected isolates and comparison with those mentioned in Bergy's Manual of Systematic Bacteriology (Lechevalier and Lechevalier, 1967) clearly suggested that these isolates belong to the genus *Streptomyces*. However, the species level identification of the actinomycetes is not possible merely by morphological, biochemical and physiological characterization; it needs intensive genetic level identification such as comparison of the 16S rRNA gene sequences with the type strains (Kutzner, 1981). In this study our major focus was to identify some potential actinomycetes candidates for the production of larvicidal and insecticidal compounds and not as such the diversity of saline actinomycetes in the target area (salt range), so all the isolates were identified up to genus level by conventional microbiological approach and only those strains were identified up to species level by 16S rRNA gene sequencing which exhibited promising larvicidal and insecticidal activity.

The selected 51 active strains were then screened for larvicidal and insecticidal activity where 17 isolates were found to be active producers of insecticidal and larvicidal compounds. Among them it was found that culture filtrate and methanolic extracts of *Streptomyces rochei* SA-10BC, *Streptomyces minutiscleroticus* SA-9K and *Streptomyces phaeoluteigriseus* SA-9L exhibit 100% mortality against *T. castaneum* and 3rd instar larva of *C. quinquefasciatus* and could be used as an effective natural insecticidal and larvicidal agents. Jiang and Mulla (2009) reported *Streptomyces spinosa,* the soil actinomycete which exhibit elevated activity against phytophagous insects and insects affecting human and animal health. Natural control of *Culex* mosquitoes for 14 days or more have been obtained by using this formulation in outdoor ponds at concentration 0.025 to 0.1 mg (AI) L^{-1}. Spinosid has been considered effective for

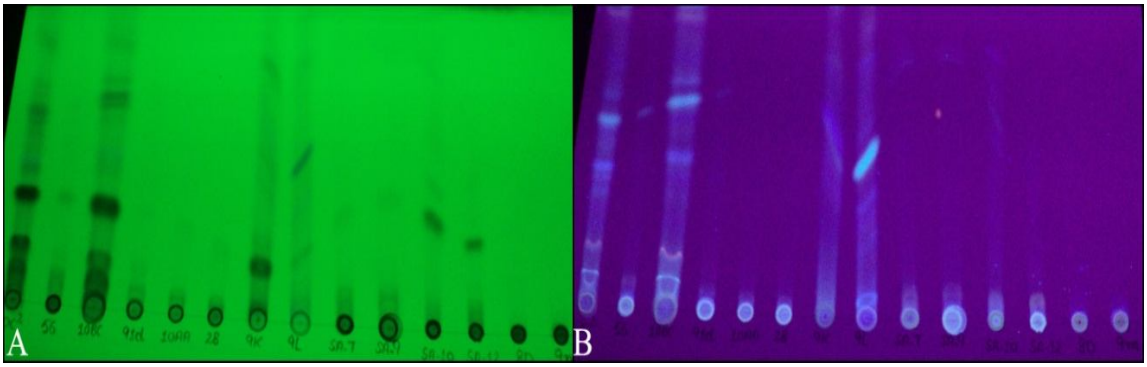

Fig. 3. Chemical screening using TLC A) under UV at 254 nm, B) under UV at 366 nm. Crude extracts of *Streptomyces* isolates from a-n are: a=SA10C[2], b=SA-53, c=SA-10BC, d=SA-91d, e=SA-10AA, f=SA-9K, g=SA-9L, h=SA-7, i=SA-9, j=SA-10, k=SA-12, l=SA-9D, m=SA-9m, n=SA-9Q

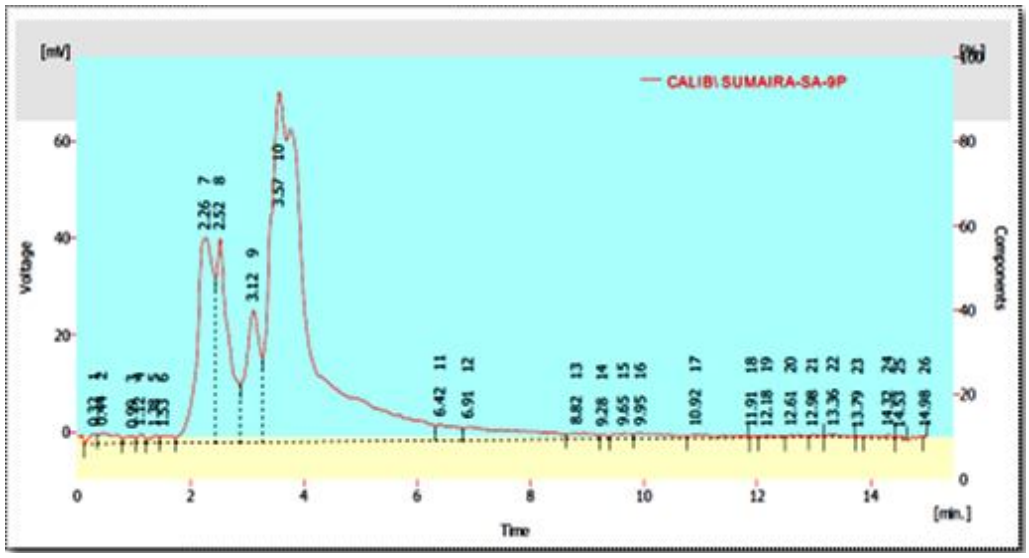

Fig. 4. HPLC-UV chromatogram of crude extract of the isolate SA-9P.

controlling mosquito larvae. Similarly Dhanasekaran *et al*. (2009) found some actinomycete isolates producing strong larvicidal activity against *Anopheles* mosquito larvae. Among them 4 isolates potentialy inhibits 100% growth of *Anopheles* mosquito larvae.

The chemical screening of the methanolic extracts by thin layer chromatography (TLC) and by HPLC-UV helped to determine the diversity of the chemical constituents produced by the active strains. The verity of colored bands on TLC after staining with anisaldehyde/H_2SO_4 and Eherlich's reagent represent secondary metabolites diversity in the methanolic crude extract of the respective actinomycete isolate. Similarly the number of peaks at different retention time (t_R) in HPLC-UV analysis represent the number of potentially active compounds in each of the crude extract. In most of the cases 2-5 significant peaks were observed between the retention time (t_R) of 3 to 10 minutes in HPLC-UV chromatograms. This shows that these isolates may be able to produce two, three or even up to five different compounds simultaneously with significant concentrations.

It can be conferred from the study that actinomycetes flora of salt range Pakistan harbor a

great potential for the production of larvicidal and insecticidal compounds. Moreover the *Streptomyces* isolates SA-10BC, SA-9K and SA-9L are the potential candidates which can be exploited commercially for the production of useful insecticidal compounds. Further studies for the identification and structure elucidation of the active components produced by these isolates can help to get some novel larvicidal and insecticidal compounds.

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