ORIGINAL RESEARCH

EVALUATION OF ANTIMUTAGENIC, ANTIMICROBIAL AND PLANT GROWTH PROMOTION ACTIVITIES OF *STREPTOMYCES* SPP.

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ABSTRACT

The present study is concentrated on the evaluation of antimutagenic, antimicrobial and plant growth promotion potential of *Streptomyces* spp. *viz. Streptomyces violascens* strain OS-6 and *Streptomyces* sp. strain TES-25. The chloroform and ethyl acetate extracts of both the strains were prepared. In the UHPLC analysis, the extracts revealed the presence of various polyphenolic compounds. The extracts exhibited potent antimutagenic potential in Ames assay against two tester strains of *Salmonella typhimurium* TA98 and TA100 in case of pre incubation mode with metabolic activation. The extracts showed considerable antibacterial and antifungal activities against the tested bacteria and fungal phytopathogens respectively. The treatment of wheat seeds with both strains resulted in the enhancement of growth parameters of wheat such as root length, shoot length, number of plants, fresh weight per plant, dry weight per plant, fresh weight per tiller, dry weight per tiller, number of tillers per plant and number of seeds per plant. The present work recommends the use of strains in the pharmaceutical drug formulations for the treatment of different diseases and as bio-fertilizer for enhancing the growth and yield of plants.

KEYWORDS: Actinobacteria, *Streptomyces*, antibiotics, polyphenolic, antimutagenic, ames assay, antimicrobial, gram positive bacteria, fungal phytopathogens, plant growth promotion

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INTRODUCTION

Environment is composed of large number of mutagenic components. These components are a major cause of several diseases including cancer. It is beneficial to explore substances or chemicals which directly suppress the action of mutagens i.e. desmutagen or the substances which indirectly inhibit their action i.e. antimutagen. There are reports of natural compounds acting as antimutagens.[1,2] The phytochemicals act as antimutagens by inactivating the dietary mutagens by method of binding or adsorption, cease the process of replication of damaged DNA as they act as bioantimutagenic agents or desmutagens by indirectly inactivating the mutagens.[3,4,5] The strains of actinobacteria produce numerous bioactive metabolites like antibiotics and antitumor compounds. These bioactive metabolites are also able to suppress mutagenicity. Actinobacteria are defined as gram positive bacteria belonging to order Actinomycetales.[6] *Streptomyces* is their dominant genus that is the producers of maximum number of natural products.[7] About 7600 of the bioactive compounds are synthesized by *Streptomyces*. The bioactive secondary metabolites possess various activities *viz.* anti-cancer, antioxidant, antibacterial, antifungal, anti-malarial, neuritogenic, anti-inflammatory, anti-algal and anti-helmintic.[8,9] *Streptomyces* produce about 80% of the antibiotics of natural origin. *Streptomyces* have proved to be the best source of novel bioactive compounds to tackle the problem of multidrug resistance for treating the infectious diseases. In addition to pharmaceutical industry, actinobacteria is also useful in agro industry as antifungal agents, plant growth regulators, antiparasitic agents, cell wall degrading agents and insecticidal agents.[10,11] They are used as biocontrol agents by inhibiting the growth of plant pathogens and aid in plant growth promotion activity.[12,13,14,15,16,17] *Streptomyces* are known to exhibit plant growth promoting potential in bean,[18] tomato,[19] pea,[20] wheat[21] and rice.[22,23] *Streptomyces* produce indole-3-acetic acid[24] or siderophores[20] that are responsible for enhancing plant growth or suppressing the growth of fungal pathogens residing in soil and air.[20, 24, 25, 26, 27, 28, 29, 30, 31] Most of the species of *Streptomyces* are still to be identified for their different beneficial bioactivities. Therefore, the objective of this study was to evaluate the antimutagenic, antimicrobial and plant growth promoting activities of *Streptomyces* spp. *viz. Streptomyces violascens* strain OS-6 (accession number: KY522799) and *Streptomyces* sp. strain TES-25 (accession number: KY522797) procured from Department of Microbiology, Guru Nanak Dev University (India). Starch Casein Nitrate Agar (SCNA) slants of the strains were maintained, and they were stored at -70ºC in the form of mycelia fragments and suspensions of spores in 20% v/v glycerol.

MATERIALS AND METHODS

Antimutagenic Activity

The crude extracts of *Streptomyces violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 were prepared as described in our previous paper.[32] The antimutagenic potential of the extracts was analyzed by *Salmonella* histidine point mutation assay of Maron and Ames[33] as proposed by Aqil et al. (2008)[34] with slight modifications. The Ames test along with the protocol for S9 mix [33]were carried on *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 to analyze the action of extracts on the direct acting mutagens (4-nitro-o-phenylenediamine and sodium azide) as well as indirect acting mutagens (2-Aminofluorene). The strains were procured from Prof. B.N. Ames, University of California, Berkeley, USA. The different concentrations of extracts were prepared *viz.*100, 250, 500 and 1000 µg/0.1 ml DMSO. Sodium azide and NPD act as direct acting mutagens which results in directly affecting the genetic material bringing about structural distortion while 2-AF affects the DNA in indirect way (Table 1).

Table 1. Direct and indirect acting mutagens used in the Ames assay

Mutagen	Strain	Mode of action
4-nitro-o-phenylenediamine(NPD)	TA98	TA98/direct acting frameshift mutagen
Sodium Azide	TA100	TA100/direct acting base pair substitution mutagen
2-Aminofluorene (2-AF)	TA98 and TA100	TA98 and TA100, S9 dependent mutagen

Spontaneous reversion

The spontaneous reversion frequency was analyzed by growing revertent colonies of bacteria spontaneously on minimal agar plates containing traces of histidine and biotin. In this method, 100 µl of bacterial culture that was prepared freshly was added to top agar (2 ml) containing 0.6% agar, 0.5% sodium chloride, 0.5 mM D-biotin and 0.5 mM L-histidine and poured on minimal agar plates. The incubation of the plates was done at 37°C for 48 hours. After incubation, the colonies (revertent) were counted.

Negative control

Negative control is used for determining the toxicity of extracts. All the non-toxic concentrations of extracts were mixed with top agar (2 ml) and 100 µl of bacterial culture and were spread on minimal agar plates. In case of indirect acting mutagens, reaction mixture was also supplemented by the addition of 500 µl of S9 mix. The plates were kept for incubation at a temperature of 37ºC for 48 hours before the counting of revertent colonies. In case of non-toxic extracts, the number of colonies will be same as in spontaneous reversion. Table 2 shows the procedure followed for determining the antimutagenic potential of extracts.

Positive control

The characteristic pattern of revertent colonies of both the strains was evaluated by dissolving the mutagens in their respective solvents. Bacterial culture (100 µl) was added to 100 µl of mutagen and spread over minimal agar plates. S9 mix (500 µl) was mixed along with other ingredients in case of 2-AF (indirect acting mutagen). After incubating the plates for 48 hours at 37°C, the counting of revertent colonies was done using PROTOCOL SR (Symbiosis, U.K.) colony counter.

Co-incubation

The co-incubation was carried out by mixing 100 µl of bacterial culture with 100 µl of NPD or sodium azide (direct acting mutagens). In case of indirect acting mutagens i.e. 2-Aminofluorene, 500 µl of S9 mix was mixed into the reaction mixture. This mixture was spread over minimal agar plates. The plates were incubated after solidification for 48 hours at 37°C. PROTOCOL SR (Symbiosis, U.K.) colony counter was used for counting the number of revertent colonies.

Pre-incubation

In pre-incubation mode of experimentation, the extract (100 µl) was added to 100 µl of NPD and Sodium azide (direct acting mutagens) and incubated for 20-30 minutes at 37°C. In case of indirect acting mutagens, extracts were incubated with 500 µl of S9 mix. The reaction mixture along with 100 µl of bacterial culture was mixed to 2 ml of top agar and was spread over minimal agar plates. The plates were kept for incubation at 37 °C for 48 hours in the upside down position for inhibiting the occurrence of contamination. The revertent colonies after incubation were counted using PROTOCOL SR (Symbiosis, U.K.) colony counter.

The mutagenic potential was calculated by following equation:

$$\text{Inhibition rate (\%)} = \frac{x-y}{x-z} \times 100$$

where,

x = number of revertents initiated by the action of mutagen i.e. positive control

y = number of revertents initiated by the action of mutagen along with the extract i.e. co-incubation or pre-incubation

z = number of revertents initiated by the action of extract i.e. negative control

Table 2. Procedure followed for determining the antimutagenic potential of the extracts

	Without S9 mix	With S9 mix
Spontaneous reversion	Top agar (2 ml)+ Culture (100 µl)	Top agar (2 ml)+ Culture (100 µl)
Positive control	Top agar (2 ml)+ Culture (100 µl)+ NPD/SA (100 µl)	Top agar (2 ml)+ Culture (100 µl)+ 2-AF (100 µl)+ S9 mix (500 µl)
Negative control	Top agar (2 ml)+ Culture (100 µl)+ different concentrations of extracts (100 µl)	Top agar (2 ml)+ Culture (100 µl)+ different concentrations of extracts (100 µl))+ S9 mix (500 µl)
Co-incubation	Top agar (2 ml)+ Culture (100 µl)+ NPD/SA (100 µl)+ different concentrations of extracts (100 µl)	Top agar (2 ml)+ Culture (100 µl)+ 2-AF (100 µl)+ different concentrations of extracts (100 µl))+ S9 mix (500 µl)
Pre-incubation	Top agar (2 ml)+ Culture (100 µl)+ mixture (200 µl= 100 µl of NPD/SA+ different concentrations of extracts (100 µl) pre-incubated at 37°C for 30 minutes	Top agar (2 ml)+ Culture (100 µl)+ mixture (700 µl= 100 µl of 2-AF+ different concentrations of extracts (100 µl)+ S9 mix (500 µl) pre-incubated at 37°C for 30 minutes

NPD-4-nitro-o-phenylenediamine, SA-Sodium azide

Antimicrobial Activity

Antibacterial activity

The bacterial strains used for evaluating the antibacterial activity of the strains of *Streptomyces* spp. were procured from Microbial Type Culture Collection (MTCC) and Gene Bank, CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India (Table 3). Nutrient agar (NA) slants were used for maintaining bacterial cultures. They were regularly subcultured after every 30 days and kept at 4°C. The glycerol suspensions (20% v/v) were stored at -80°C. The antibacterial activity of extracts against various tested organisms (Gram positive and Gram negative bacteria) was analyzed by performing agar disc diffusion method.[35] The Muller-Hinton agar (MHA) was poured into the autoclaved petri plates under aseptic conditions and seeded with 100 µl of bacterial suspension (turbidity was compared to 0.5 MacFarland). The antibacterial activity was checked by impregnating sterile discs with 0.5 mg of extracts of both strains. The discs were kept on the surface of the medium and incubated at 4°C for half an hour for the

diffusion of the bioactive compounds. The plates were then kept for incubation at 37°C for 24-48 hours. The zone of inhibition was observed surrounding the discs which indicates the positive results. The measurement of zone of inhibition was taken in millimetres.

Table 3. List of test bacteria used

Bacteria	Reference number
Bacillus subtilis	MTCC 619
Salmonella typhi	MTCC 733
Escherichia coli	MTCC1885
Staphylococcus aureus	MTCC 96
Staphylococcus epidermidis	MTCC 435
Klebsiella pneumoniae	MTCC 109

Antifungal activity

The fungal phytopathogens used for testing the antifungal activity of the strains were procured from National Fungal Culture Collection of India, Pune (India) and Microbial Type Culture Collection (MTCC) and Gene Bank, CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India (Table 4). The fungal cultures were maintained on Potato Dextrose Agar (PDA) followed by regular sub culturing (every 30 days or so) and then stored at 4°C. They were preserved in glycerol suspension (20% v/v) as a stock at -20°C. Antifungal activity of extracts against different fungal cultures was determined by performing agar well diffusion method.[35] Potato dextrose agar (PDA) medium was poured into autoclaved petri plates under sterile conditions. The medium was seeded with 100 µl of fungal suspension. The seeded plate was punctured by a sterile cork borer and wells of 9 mm were made. The antifungal activity was tested by loading the wells with 200 µl of extracts (20 mg/ml). Plates were kept for incubation for 5 days at 28°C. Zone of inhibition around the wells confirmed positive result and the measurement was taken in millimetres.

Table 4. List of test fungi used

Fungal phytopathogen	Reference number
Alternaria citri	NFCCI Number: 2704
Botrytis cineria	NFCCI Number: 1305
Colletotrichum musae	NFCCI Number: 2256
Fusarium solani	NFCCI Number: 91
Rhizoctonia solani	NFCCI Number: 188
Alternaria brassicicola	MTCC 2102
Fusarium oxysporum	MTCC 284

Plant Growth Promotion Activity

Production of this plant growth promoting hormone (IAA) was analyzed by the method proposed by Bano and Musarrat (2003).[36] Yeast Malt (YM) broth (50 ml) having 0.2% L-tryptophan was inoculated with three 6 mm discs of cultures growing in SCNA (starch casein nitrate agar) and kept for incubation at 180 rpm at 28°C. Broth was centrifuged after 7 days of incubation for 15 minutes at 10,000 g at 4°C. After centrifugation, 2 ml of Salkowski reagent was added to 1ml of supernatant and again incubated for 25 minutes at room temperature. Development of pink colour indicated the production of indole-3-acetic acid and was measured at 530 nm. The amount production of indole-3-acetic acid (IAA) was analyzed in comparison with IAA standard.

To analyze the effects of strains on plant growth promotion activity, *in vivo* pot experiments were performed on *Triticum aestivum* (Wheat) of variety HD-3086 procured from Punjab Agriculture University, Ludhiana. Starch Casein Nitrate Agar (SCNA) medium was inoculated with cultures and kept for incubation for 7 days at 28°C until the occurrence of optimum sporulation. Five ml of autoclaved distilled water was added to the plate with 1% cellulose used as an adhesive. The mycelium and spores were loosened by gently scraping with the inoculation loop. The surface sterilized seeds were dipped for 24 hours in the cell suspension of *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 (10^8cfu/ml). The seeds were then air dried under sterilized conditions by placing them on autoclaved filter paper. When the seeds got dried, both the treated (bacterized) and non-treated seeds (control) were planted in pots using sterilized forcep. The different characteristics of soil *viz.* moisture content,[37] organic matter and organic carbon,[38] available nitrogen,[39] available phosphorus,[40] texture of soil (silt %, clay % and sand %),[41] available sodium and potassium content.[42, 43] were determined The growth parameters such as number of plants, fresh weight of plants, dry weight of plants, root length of plants, shoot length of plants, fresh weight of tillers, dry weight of tillers, number of tillers per plant and total number of seeds per plant were noted.

Statistical analysis

The experiments were performed in triplicates. The results were obtained as mean ± S.E. The regression equation was obtained by plotting graph between tested concentrations of extracts and inhibition of growth in Ames assay. The calculation of IC_{50} values was done using regression equation. The statistical analysis of the results was performed by one-way analysis of variance (ANOVA), two way ANOVA and Tukey's multiple comparison tests. The results were considered to be statistically significant at 5% level of significance.

RESULTS

The extracts obtained were *S. violascens* strain OS-6 chloroform extract, *S. violascens* strain OS-6 ethyl acetate extract, *Streptomyces* sp. strain TES-25 chloroform extract and *Streptomyces* sp. strain TES-25 ethyl acetate extract abbreviated as OCE, OEAE, TCE and TEAE respectively. The phytochemicals present in the extracts were evaluated by Ultra High Performance Liquid Chromatography (UHPLC) analysis. Among the extracts of *S. violascens* strain OS-6, epicatechin was present in maximum concentration of 701.757 ppm in OEAE extract. OCE extract contained highest concentration of kaempferol i.e. 164.054 ppm. TEAE extract showed maximum amount of epicatechin (593.060 ppm) while TCE extract exhibited high amount of kaempferol (46.101 ppm) among the extracts of *Streptomyces* sp. strain TES-25. All the extracts contained other polyphenolic compounds in lesser amounts. The different bioactivities in the extracts may be dependent on the presence of these polyphenolic compounds.

Antimutagenic activity

The antimutagenic capability of extracts in TA98 and TA100 strains of

Salmonella typhimurium without S9 mix or with S9 mix that acts as metabolic activator was determined by employing the protocol as described by Maron and Ames (1983) with minor modifications. It was observed that the extracts had more significant effect on reducing the mutagenicity initiated by 2-AF (indirect acting mutagen) in comparison to NPD and sodium azide (direct acting mutagens). The results of antimutagenic activity exhibited by different extracts of *S. violascens* strain OS-6 (OCE and OEAE) and *Streptomyces* sp. strain TES-25 (TCE and TEAE) are as follows:

S. violascens strain OS-6 chloroform extract (OCE)

The antimutagenic potential of OCE extract was determined as the percentage inhibition of the mutagenicity initiated by the direct and indirect acting mutagens (Table 5 and Fig. 1). The IC_{50} values of OCE extract are presented in Table 6. The reduction of his^+ revertents initiated by NPD in TA98 was 53.75% (IC_{50} value of 1299.84 µg/0.1 ml) in co-incubation mode and 55.07% (IC_{50} value = 1187.97 µg/0.1 ml) in pre-incubation mode at the maximum concentration. OCE extract exhibited antimutagenic activity of 60.98% (IC_{50} value of 953.37 µg/0.1ml) in TA98 against 2AF (indirect acting mutagen) in co-incubation mode of action while 66.53% (IC_{50} value = 607.89 µg/0.1 ml) in pre-incubation mode of treatment at the highest tested dose. In case of direct acting mutagens, maximum antimutagenic activity was observed in pre-incubation mode of treatment against TA100 with a reduction of sodium azide initiated mutagenicity by 48.80% (IC_{50} value = 2344.90 µg/0.1 ml) while the co-incubation mode of treatment showed 45.73% (IC_{50} value of 3010.92 µg/0.1 ml) reduction in mutagenicity at the highest dose (2500 µg/0.1ml). In TA100 strain, the observed % antimutagenicity was 62.85% (IC_{50} value of 888.91 µg/0.1 ml) in co-incubation mode of action and 69.23% (IC_{50} value of 543.48 µg/0.1 ml in pre-incubation mode of action at the highest concentration in case of indirect acting mutagen. The results showed significance at $p \leq 0.05$ using one-way ANOVA along with Tukey's multiple comparison tests.

S. violascens strain OS-6 ethyl acetate extract (OEAE)

OEAE extract possessed antimutagenic response of 61.14% with IC_{50} value of 620.17 µg/0.1 ml in co-incubation mode of experiment and 65.86% (IC_{50} value = 419.89 µg/0.1 ml) in pre-incubation mode of experiment in TA98 strain against direct acting mutagen (NPD) at the highest examined dose (Table 7 and Fig. 2). Table 8 depicts the IC_{50} values of OEAE extract. In case of indirect acting mutagen, the antimutagenic response recorded was 72.61% (IC_{50} value of 275.89 µg/0.1 ml) in co-incubation method of treatment and 84.11% (IC_{50} value = 32.79 µg/0.1 ml) in pre-incubation mode of treatment in TA98 strain at the highest tested concentration. In case of TA100 strain, it reduced the mutagenicity initiated by sodium azide (the direct acting mutagen) by 54.68% (IC_{50} value of 1339.43 µg/0.1 ml) in co-incubation mode of treatment and 60.61% (IC_{50} value of 1012.32 µg/0.1 ml) in pre-incubation mode of treatment at the highest tested dose. The extract was very effective in decreasing the his^+ revertants in TA100 strain against indirect acting mutagen (2-AF) by 75.099% (IC_{50} value of 239.85 µg/0.1 ml) in co-incubation method of treatment and 85.65% (IC_{50} value of 4.66 µg/0.1 ml) in pre-incubation mode of treatment at the highest dose. It was observed that the antimutagenic response was strongly enhanced in case of indirect acting mutagen as compared to direct acting mutagens. The results showed statistical significance at 5% level of significance by one-way ANOVA along with Tukey's multiple comparison tests.

***Streptomyces* sp. strain TES-25 chloroform extract (TCE)**

Antimutagenic effect of TCE extract against the direct and indirect acting mutagens in TA98 and TA100 strains of *S. typhimurium* is presented in Table 9 and Fig. 3. The IC_{50} values of TCE extract are presented in Table 10. The results expressed that the decrease in number of his^+ revertants was less in case of absence of metabolic activation. In TA98 tester strain, a moderate antimutagenic response was observed with inhibitory effect of 53.49% (IC_{50} value = 1556.197 µg/0.1 ml) in co-incubation mode of treatment and 54.95% (IC_{50} value of 1366.49 µg/0.1 ml) in pre-incubation mode of treatment against NPD. The extract considerably decreased the number of revertant colonies by 59.49% (IC_{50} value = 1199.91 µg/0.1 ml) in co-incubation mode of treatment and 64.49% (IC_{50} value of 589.93 µg/0.1 ml) in pre-incubation mode of treatment against 2-AF (S9 mediated mutagen) at a dose of 2500 µg/0.1 ml in TA98 tester strain. In TA100 tester strain inhibitory effect of 43.90% (IC_{50} value of 4491.76 µg/0.1 ml) in co-incubation method of treatment and 45.52% (IC_{50} value of 3604.72 µg/0.1 ml) in pre-incubation mode of analysis was recorded against sodium azide at the highest tested dose while against 2-AF was 59.28% (IC_{50} value of 1118.79 µg/0.1 ml) in co-incubation mode of treatment and 67.85% (IC_{50} value = 441.42 µg/0.1 ml) in pre-incubation method of treatment at the highest dose. The results revealed that the inhibitory effect was dose dependent. One-way ANOVA as well as Tukey's multiple comparison tests indicated that the results were significant at $p \leq 0.05$ level of significance.

***Streptomyces* sp. strain TES-25 ethyl acetate extract (TEAE)**

The antimutagenic response of TEAE extract is presented in Table 11 and Fig. 4.

Table 12 presents the IC_{50} values of TEAE extract. In case of NPD (direct acting mutagen), the extract showed an inhibitory effect of 60.76% (IC_{50} value = 699.24 µg/0.1 ml) in pre-incubation mode of treatment followed by 58.44% (IC_{50} value of 732.16 µg/0.1 ml) in co-incubation method of experimentation in TA98 tester strain at the highest tested concentration. The rat liver homogenate led to a considerable increase in the antimutagenic response in both the tester strains. The reduction in the his^+ revertent colonies was 66.19% (IC_{50} value of 445.86 µg/0.1 ml) in co-incubation mode of treatment and 82.42% (IC_{50} value = 61.56 µg/0.1 ml) in pre-incubation mode of experiment against 2-AF having microsomal mammalian activation system in TA98 tester strain at the highest checked dose. The extract exerted antimutagenic activity of 49.16% (IC_{50} value of 2143.08 µg/0.1 ml) in co-incubation mode of treatment and 54.25% (IC_{50} value of 1422.26 µg/0.1 ml) in pre-incubation mode of treatment against sodium azide without metabolic activation in TA100 strain at the highest concentration tested. The inhibitory effect was more in TA100 strain with percentage inhibition of 67.45% (IC_{50} value of 407.48 µg/0.1 ml) in co-incubation mode of treatment and 84.11% (IC_{50} value = 39.25 µg/0.1 ml) in pre-incubation mode of treatment at the highest tested concentration of 2500 µg/0.1 ml. The inhibitory effect was increased in a dose dependent manner. The results were found to be significant one-way ANOVA along with Tukey's multiple comparison tests at $p \leq 0.05$ level of significance.

Table 5: Antimutagenic activity of chloroform extract of *S. violascens* strain OS-6 (OCE) against direct acting mutagens (4-nitro-o phenylenediamine and sodium azide) and S9-dependent mutagen (2-aminofluorene) in TA98 and TA100 strains of *Salmonella typhimurium*

Treatment	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh (%)± SE	Mean ± SE	Inh(%)± SE	Mean ± SE	Inh (%)± SE	Mean ± SE	Inh (%)± SE
Spontaneous		38.67±8.57	-	44.33±6.98	-	173.67±31.88	-	207±9.29	-
Positive									
NPD	20	1646.33±50.54	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1650 ±86.60	-	-	-
2-AF	20	-	-	4729.33±407.64	-	-	-	1971.67±53.98	-
Negative									
	100	35.67±2.33	-	44.67±6.64	-	205.67±12.45	-	216.67±11.46	-
	250	39.33±5.61	-	42.00±5.51	-	199.33±13.59	-	218.33± 15.93	-
	500	40.33±4.67	-	39.67±6.44	-	207.67±16.15	-	211.33±17.03	-
	1000	41.00±4.73	-	39.67±4.63	-	229±9.87	-	219 ±15.53	-
	2500	40.67±5.04	-	39.33±4.33	-	210±13.86	-	202 ±16.29	-
					Co-incubation				
	100	1176±29.51	29.03±3.16	3770±64.83	19.56 ±5.20	1454±57.26	12.88±7.28	1524.67± 41.32	25.41±2.797
	250	1017.33±24.92	38.93±3.55	3213.67±46.22	31.36 ±5.63	1278±66.57	24.79±7.83	1384± 29.14	33.50±1.19
	500	968.33±32.03	42.11±2.697	2608.33±22.92	44.42 ±4.57	1166±29.53	33.13±4.64	1300± 33.72	38.08±3.19
	1000	850.00±18.82	49.57±0.61	2302.67±47.94	51.12 ±3.71	1073.67±32.67	40.33±2.26	1007.33± 35.87	54.92±3.32
	2500	782.00±38.55	53.75±2.38	1840.67±29.55	60.98 ±3.54	986.33±6.17	45.73±3.24	858±38.07	62.85±2.80
HSD			12.4544		21.4018		25.5897		12.8646
F-Ratio (4,10)			12.9207*		12.5638*		5.5707*		31.3575*
					Pre-incubation				
	100	1156±70.38	30.28±4.86	3233.67±39.73	30.99±5.40	1370±10.68	18.82±4.36	1420.33±33.74	31.20±3.77
	250	1015.33±36.77	39.05±3.90	3046.33±50.09	35.17 ±4.18	1282.33±54.58	25.13±2.50	1323.67±47.96	36.77±4.49
	500	960.67±13.32	42.60±1.81	2550.67±38.58	45.82 ±3.65	1123.33±42.73	36.41±0.98	1125.67± 22.81	47.97±2.81
	1000	848.67±45.499	49.73±1.58	1912 ±35.73	59.53 ±3.25	1024 ±39.69	43.73±3.72	926.67± 37.35	59.595±2.44
	2500	761.67±30.60	55.07±1.90	1586.33±13.09	66.53 ±2.81	946 ±34.53	48.799±1.39	747.67± 55.92	69.23±1.58
HSD			14.4412		18.4124		13.4804		14.8199
F-Ratio (4,10)			9.5373*		14.8978*		18.6511*		24.3395*

	TA98 (without S9)	TA98 (with S9)	TA100 (without S9)	TA100 (with S9)
Treatment	F-ratio $_{(1, 20)}$ = 0.1326	F-ratio $_{(1, 20)}$ = 5.0741*	F-ratio $_{(1, 20)}$ = 1.3298	F-ratio $_{(1, 20)}$ = 10.1071*
Dose	F-ratio $_{(4, 20)}$ = 21.9405*	F-ratio $_{(4, 20)}$ = 26.6968*	F-ratio $_{(4, 20)}$ = 16.722*	F-ratio $_{(4, 20)}$ = 54.3663*
Treatment×Dose	F-ratio $_{(4, 20)}$ = 0.0202	F-ratio $_{(4, 20)}$ = 0.4163	F-ratio $_{(4, 20)}$ = 0.1022	F-ratio $_{(4, 20)}$ = 0.3442
HSD	14.5284	21.5086	22.0352	14.9511

Inh- Inhibition; * represents the significance at $p \leq 0.05$
Values are shown as Mean ± S.E.

Table 6. IC_{50} (µg/0.1 ml) values of chloroform extract of *S. violascens* strain OS-6 (OCE) in Ames assay

	TA98				TA100			
	Without S9		With S9		Without S9		With S9	
	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation
Regression equation	y = 7.679ln(x)-5.045	y = 7.702ln(x)-4.519	y = 13.08ln(x)-39.81	y = 12.06ln(x)-27.36	y = 10.36ln(x)-33.03	y = 9.955ln(x)-27.29	y = 12.22ln(x)-33.04	y = 12.54ln(x)-28.98
R^2 value	0.976	0.990	0.987	0.956	0.977	0.970	0.956	0.976
IC_{50} value	1299.84	1187.97	953.37	607.89	3010.92	2344.90	888.91	543.48

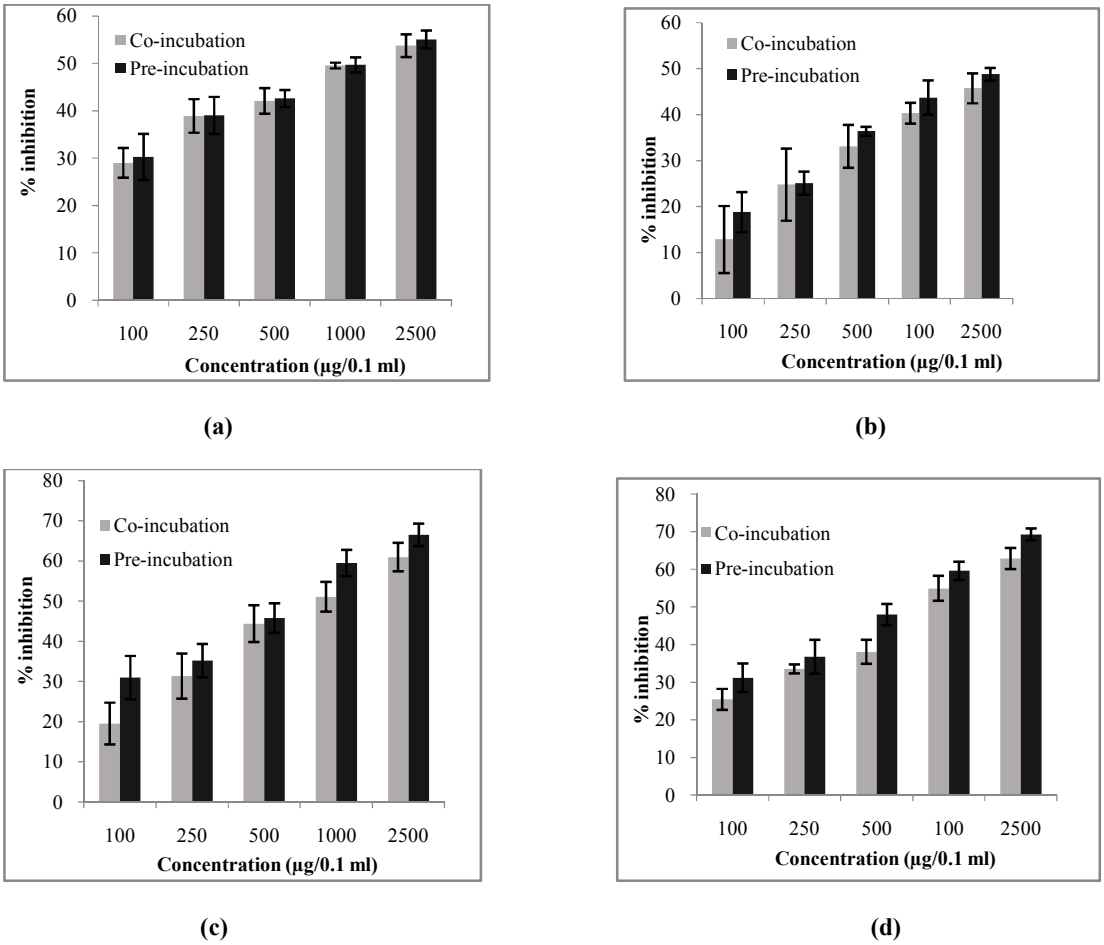


Fig. 1. Antimutagenic activity of chloroform extract of *S. violascens* strain OS-6 (OCE) on (a) *S. typhimurium* strain TA98 without S9 against 4-nitro-o-phenylenediamine (NPD) (b) *S. typhimurium* strain TA100 without S9 against sodium azide (c) *S. typhimurium* strain TA98 with S9 against 2-aminofluorene (2-AF) (d) *S. typhimurium* strain TA100 with S9 against 2-aminofluorene (2-AF).

Table 7. Antimutagenic activity of ethyl acetate extract of *S. violascens* strain OS-6 (OEAE) against direct acting mutagens (4-nitro-o phenylenediamine and sodium azide) and S9-dependent mutagen (2-aminofluorene) in TA98 and TA100 strains of *Salmonella typhimurium*

Treatment	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh (%)± SE	Mean ± SE	Inh(%)± SE	Mean ± SE	Inh (%)± SE	Mean ± SE	Inh (%)± SE
Spontaneous		53±4.36	-	42.33±7.06	-	179.33±16.25	-	179.33±35.78	-
Positive									
NPD	20	1637±44.80	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1626.33±80.27	-	-	-
2-AF	20	-	-	6202.67±314.00	-	-	-	2216.67±104.48	-
Negative									
	100	41.67±3.18	-	43.33±6.69	-	220.33±6.39	-	215.33±14.43	-
	250	44.33±5.78	-	39.67±6.01	-	210.67±9.40	-	209.33±17.13	-
	500	44.67±4.63	-	37±6.66	-	215.67±9.21	-	220.67±22.45	-
	1000	38.33±3.48	-	38±5.51	-	211±11.72	-	219.67±17.90	-
	2500	45±4.36	-	38.67±6.12	-	214±13.796	-	224±14.73	-
					Co-incubation				
	100	1076.33±33.28	35.12±1.75	3757.67±34.197	39.39±3.03	1272±27.18	24.92 ±2.53	1419±38.14	39.31±4.87
	250	981.67±28.72	41.15±0.05	3422±55.68	44.91±2.01	1134.33±52.59	34.72 ±1.04	1175.67±32.95	51.72±2.04
	500	875.33±38.39	47.67±3.30	2555.33±30.60	58.98±1.74	1049±30.62	40.73 ±2.41	1032.33±39.07	58.99±3.12
	1000	745±55.72	55.72±3.57	2029±18.48	67.495±2.08	930.33±26.86	48.72 ±3.91	915.33±37.83	65.16±1.37
	2500	662±20.13	61.14±2.33	1714±33.60	72.61±2.06	846.33±45.83	54.68 ±5.77	715.67±34.85	75.099±2.23
HSD			11.7879		10.3615		16.3494		13.8722
F-Ratio (4,10)			17.313*		40.9676*		11.0143*		20.5971*
					Pre-incubation				
	100	1012.67±20.21	38.97 ±2.99	2681.33±53.69	57.01±1.74	1259.67±31.71	25.77±2.94	873.67±20.28	67.01±1.02
	250	920.33±30.95	44.87±3.22	1987±47.09	68.15±2.42	1123.33±40.75	35.00 ±4.96	749.67±37.02	73.14±1.81
	500	850.67±35.37	49.23±3.52	1546.33±27.76	75.37±1.61	1033.33±38.65	41.48 ±5.91	636.67±20.96	78.96±1.85
	1000	696.33±20.795	58.77±1.62	1307±41.97	79.31±1.27	924.67±64.41	49.34 ±5.43	588.67±7.17	81.53±0.31
	2500	591±10.02	65.68 ±0.46	1013.67±21.33	84.11±0.76	763.67±37.77	60.61±4.96	505.33±50.15	85.65±2.51
HSD			12.2244		7.6886		22.9952		7.8061
F-Ratio (4,10)			16.6402*		40.9672*		7.2775*		18.9429*

	TA98 (without S9)	TA98 (with S9)	TA100 (without S9)	TA100 (with S9)
Treatment	F-ratio $_{(1, 20)}$ = 4.1935	F-ratio $_{(1, 20)}$ = 168.5993*	F-ratio $_{(1, 20)}$ = 0.3866	F-ratio $_{(1, 20)}$ = 157.3022*
Dose	F-ratio $_{(4, 20)}$ = 33.8325*	F-ratio $_{(4, 20)}$ = 78.9179*	F-ratio $_{(4, 20)}$ = 16.9107*	F-ratio $_{(4, 20)}$ = 36.9732*
Treatment×Dose	F-ratio $_{(4, 20)}$ = 0.0961	F-ratio $_{(4, 20)}$ = 3.0171*	F-ratio $_{(4, 20)}$ = 0.1537	F-ratio $_{(4, 20)}$ = 3.4254*
HSD	12.9378	9.8298	21.4956	12.1269

Inh- Inhibition; * represents the significance at $p \leq 0.05$
Values are shown as Mean ± S.E.

Table 8. IC_{50} (µg/0.1 ml) values of ethyl acetate extract of *S. violascens* strain OS-6 (OEAE) in Ames assay

	TA98				TA100			
	Without S9		With S9		Without S9		With S9	
	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation
Regression equation	y = 8.462ln(x)-4.431	y = 8.567ln(x)-1.739	y = 11.25ln(x)-13.27	y = 8.363ln(x)+20.81	y = 9.380ln(x)-17.54	y = 10.74ln(x)-24.35	y = 10.89ln(x)-9.652	y = 5.831ln(x)+41.01
R^2 value	0.988	0.980	0.956	0.958	0.992	0.996	0.994	0.978
IC_{50} value	620.17	419.89	275.89	32.79	1339.43	1012.32	239.85	4.66

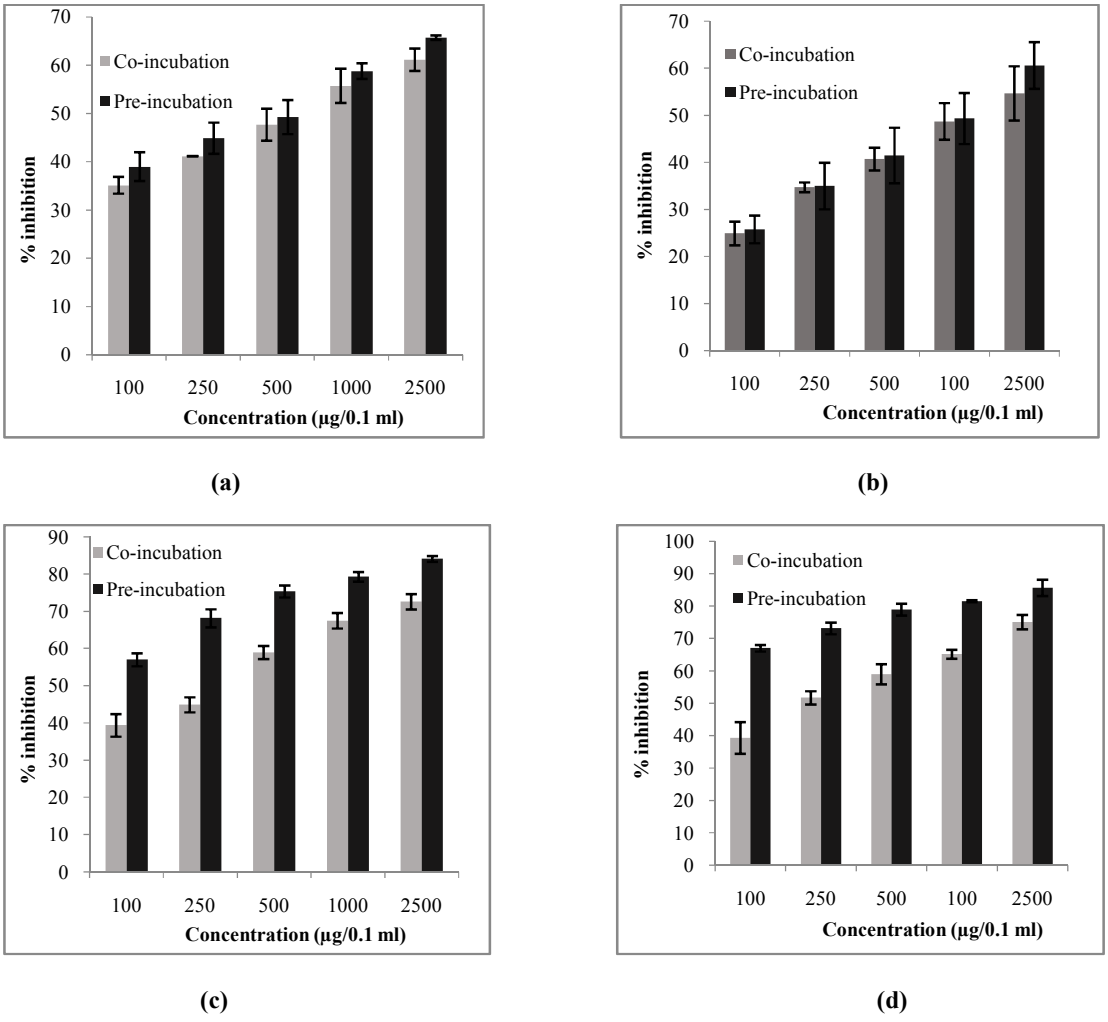


Fig. 2. Antimutagenic activity of ethyl acetate extract of *S. violascens* strain OS-6 (OEAE) on (a) *S. typhimurium* strain TA98 without S9 against 4-nitro-o-phenylenediamine (NPD) (b) *S. typhimurium* strain TA100 without S9 against sodium azide (c) *S. typhimurium* strain TA98 with S9 against 2-aminofluorene (2-AF) (d) *S. typhimurium* strain TA100 with S9 against 2-aminofluorene (2-AF).

Table 9. Antimutagenic activity of chloroform extract of *Streptomyces* sp. strain TES-25 (TCE) against direct acting mutagens (4-nitro-o-phenylenediamine and sodium azide) and S9-dependent mutagen (2-aminofluorene) in TA98 and TA100 strains of *Salmonella typhimurium*

Treatment	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh (%)± SE	Mean ± SE	Inh(%)± SE	Mean ± SE	Inh (%)± SE	Mean ± SE	Inh (%)± SE
Spontaneous		44.67±8.25	-	46.67 ±5.04	-	163.67±31.698	-	162.33±17.15	-
Positive									
NPD	20	1675±67.84	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1650.33±85.04	-	-	-
2-AF	20	-	-	5754 ±162.41	-	-	-	2348.67±36.77	-
Negative									
	100	44±4.36	-	35.67±4.63	-	205.67±12.45	-	217.33±16.48	-
	250	40.33±4.096	-	44.33±3.76	-	199.33±13.59	-	204.67±15.21	-
	500	44±3.61	-	40.67±6.12	-	207.67±16.15	-	204.33±21.07	-
	1000	34.33±1.76	-	44.67±6.06	-	229±9.87	-	216.67±13.96	-
	2500	43.33±2.91	-	42±2.89	-	210±13.86	-	228±15.72	-
					Co-incubation				
	100	1260±68.595	24.97±6.26	4736.33±106.81	17.76±0.96	1377.67±73.69	18.93±1.37	1850.33±28.82	23.34±1.47
	250	1151±100.13	32.31±3.42	4415.33±52.06	23.29±2.84	1225±40.08	28.83±4.298	1693±42.55	30.59±1.12
	500	1021±94.85	40.24±3.93	3596.33±43.91	37.65±2.05	1168.67±34.79	33.03±4.38	1526.33±43.91	38.32±1.89
	1000	903.67±28.49	46.98±0.495	2872±22.48	50.40±1.45	1081.67±25.30	39.77±2.09	1259.33±77.81	51.15±3.58
	2500	798.67±11.096	53.49 ±2.65	2353.67±28.01	59.49±0.68	1012±27.68	43.90±3.94	1092±45.90	59.28±1.17
HSD			17.8405		8.2503		16.0194		9.5789
F-Ratio (4,10)			8.7466*		98.5752*		8.0031*		50.8813*
					Pre-incubation				
	100	1269.33±76.999	25.02±1.85	4036±50.74	29.89±2.71	1343.33±28.29	20.65±5.23	1768.67±77.39	27.18±3.05
	250	1129±4.16	33.15±2.90	3406.33±51.2	41.01±2.14	1169.33±23.78	32.77±3.07	1380±48.88	45.11±3.47
	500	985.67±34.98	41.96±4.19	2908.33±48.12	49.72±1.61	1106.67±43.04	37.58±2.596	1154±45.71	55.65±3.33
	1000	894.67±22.88	47.48±1.17	2546.33±32.84	56.14±0.75	1072.33±36.67	40.44±2.73	1026.67±39.43	61.94±2.42
	2500	772.33±44.47	54.95±4.61	2066.33±28.24	64.49±1.35	991.67±20.74	45.52±2.07	909.67±34.75	67.85±1.07
HSD			14.9945		8.5562		15.4612		13.0527
F-Ratio (4,10)			13.3076*		52.9907*		8.0706*		32.546*

	TA98 (without S9)	TA98 (with S9)	TA100 (without S9)	TA100 (with S9)
Treatment	F-ratio $_{(1, 20)}$ = 0.1668	F-ratio $_{(1, 20)}$ = 84.8696*	F-ratio $_{(1, 20)}$ = 1.3631	F-ratio $_{(1, 20)}$ = 49.9883*
Dose	F-ratio $_{(4, 20)}$ = 21.2508*	F-ratio $_{(4, 20)}$ = 145.6945*	F-ratio $_{(4, 20)}$ = 15.9517*	F-ratio $_{(4, 20)}$ = 75.6641*
Treatment×Dose	F-ratio $_{(4, 20)}$ = 0.0186	F-ratio $_{(4, 20)}$ = 4.2125*	F-ratio $_{(4, 20)}$ = 0.1196	F-ratio $_{(4, 20)}$ = 2.2640
HSD	17.7549	9.0554	16.9616	12.3346

Inh- Inhibition; * represents the significance at $p \leq 0.05$; Values are shown as Mean ± S.E.

Table 10. IC_{50} (µg/0.1 ml) values of chloroform extract of *Streptomyces* sp. strain TES-25 (TCE) in Ames assay

	TA98				TA100			
	Without S9		With S9		Without S9		With S9	
	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation
Regression equation	y = 9.129ln(x)-17.14	y = 9.462ln(x)-18.29	y = 13.99ln(x)-49.25	y = 10.77ln(x)-18.71	y = 7.778ln(x)-15.44	y = 7.381ln(x)-10.48	y = 11.74ln(x)-32.42	y = 12.55ln(x)-26.49
R^2 value	0.994	0.993	0.969	0.993	0.978	0.937	0.98	0.944
IC_{50} value	1556.197	1366.49	1199.91	589.93	4491.76	3604.72	1118.79	441.42

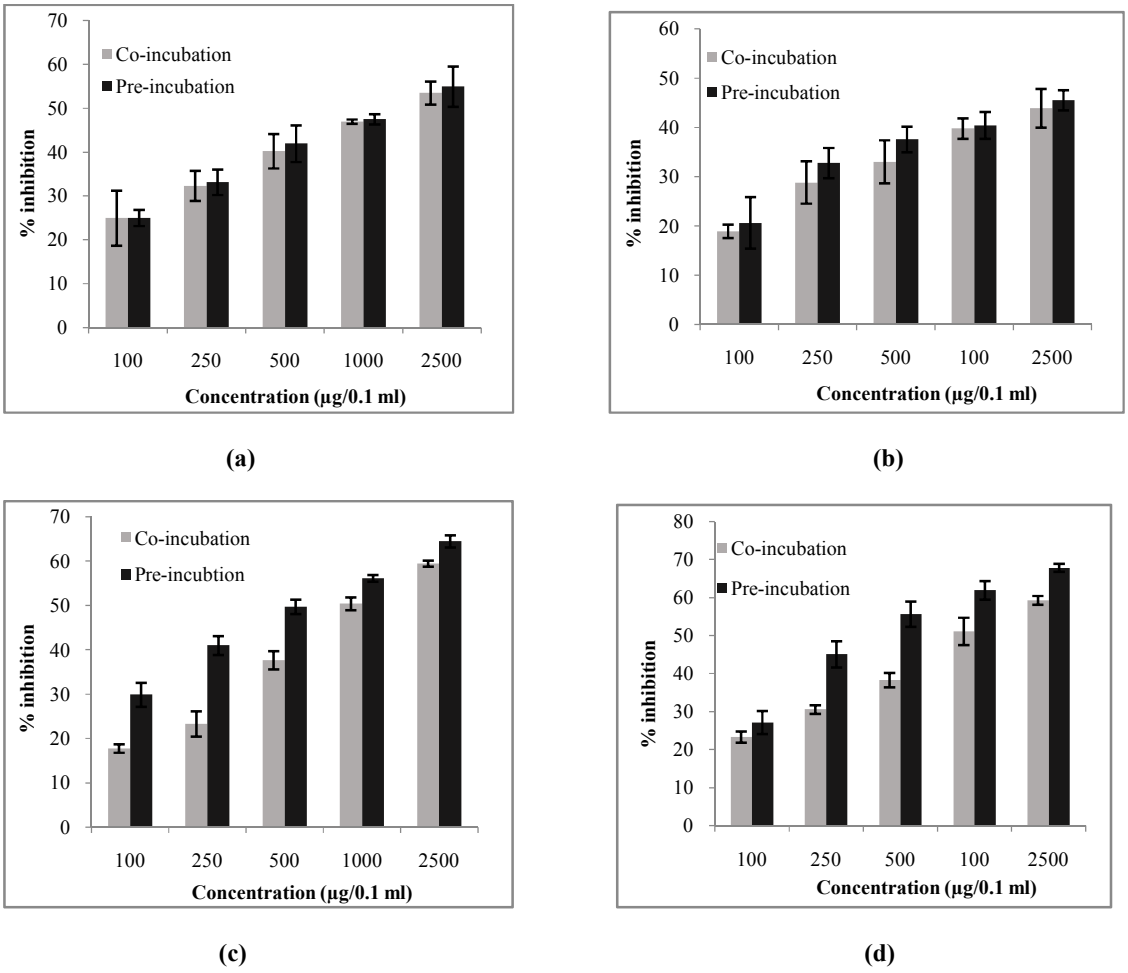


Fig. 3. Antimutagenic activity of chloroform extract of *Streptomyces* sp. strain TES-25 (TCE) on (a) *S. typhimurium* strain TA98 without S9 against 4-nitro-o-phenylenediamine (NPD) (b) *S. typhimurium* strain TA100 without S9 against sodium azide (c) *S. typhimurium* strain TA98 with S9 against 2-aminofluorene (2-AF) (d) *S. typhimurium* strain TA100 with S9 against 2-aminofluorene (2-AF).

Table 11. Antimutagenic activity of ethyl acetate extract of *Streptomyces* sp. strain TES-25 (TEAE) against direct acting mutagens (4-nitro-o phenylenediamine and sodium azide) and S9-dependent mutagen (2-aminofluorene) in TA98 and TA100 strains of *Salmonella typhimurium*

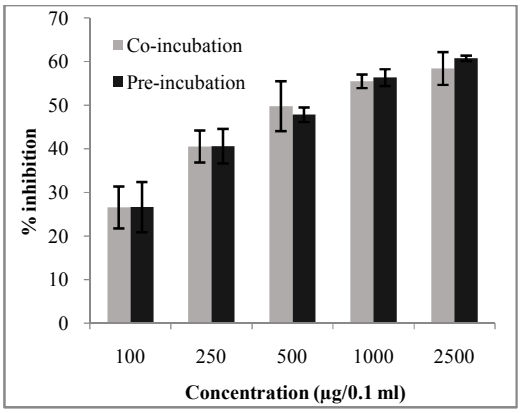
Treatment	Dose (µg/0.1 ml)	TA98				TA100			
		Mean ± SE	Without S9 Inh (%)± SE	With S9 Mean ± SE	Inh(%)± SE	Mean ± SE	Without S9 Inh (%)± SE	With S9 Mean ± SE	Inh (%)± SE
Spontaneous		51.33±2.85	-	46.67±8.65	-	171.33±30.07	-	217±15.13	-
Positive									
NPD	20	1675±67.84	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1579±30.12	-	-	-
2-AF	20	-	-	6820.33±56.53	-	-	-	2855±36.295	-
Negative									
	100	53.67±7.31	-	36.33±4.81	-	208.33±9.56	-	205.67±14.62	-
	250	44.67±3.18	-	43.33±6.23	-	210±14.98	-	245±20.13	-
	500	34.33±3.53	-	38±5.51	-	215.67±15.43	-	204.67±12.02	-
	1000	33.33±5.46	-	36±5.57	-	223±13.23	-	208.33±19.24	-
	2500	32.33±5.61	-	36±5.29	-	207.67±10.48	-	213.67±17.42	-
					Co-incubation				
	100	1239±46.88	26.58±4.81	4685.33±44.03	31.45±1.21	1351.67±41.34	16.43±3.92	2004.33±22.06	32.10±0.33
	250	1012±57.42	40.55±3.65	3627.67±55.41	47.11±0.64	1273.67±35.23	22.28±2.19	1592.67±51.90	48.39±1.95
	500	853±75.74	49.79±5.72	3336.67±26.598	51.36±0.30	1043.33±49.13	39.37±2.90	1464±20.84	52.48±0.196
	1000	764.33±47.39	55.495±1.55	2757.67±192.12	59.90±2.63	993±14.36	43.12±2.18	1261.67±12.44	60.21±0.21
	2500	711±42.57	58.44±3.76	2329±43.097	66.19±0.95	904.67±34.51	49.16±3.01	1073.67±10.48	67.45±0.27
HSD			19.2570		6.5165		13.536		4.1942
F-Ratio (4,10)			9.7078*		89.9994*		23.2405*		219.7772*
					Pre-incubation				
	100	1235.70±41.95	26.67±5.75	3093.67±43.64	54.93±0.84	1229.33±26.46	25.33±3.44	1287.33±34.48	59.13±1.35
	250	1007.30±24.36	40.65±3.969	2660.67±47.68	61.37±0.67	1057±6.08	38.11±0.64	1192.67±50.92	63.64±2.52
	500	888.33±19.15	47.84±1.67	2215.67±25.69	67.88±0.56	1004.33±12.81	42.15±1.09	1066±36.72	67.47±2.03
	1000	747.33±4.48	56.36±1.92	1740.67±24.54	74.87±0.50	956.67±38.71	45.93±3.21	878±23.76	74.73±1.25
	2500	677.67±38.97	60.76±0.65	1227.67±35.18	82.42±0.72	833.33±46.26	54.25±4.22	634.33±26.96	84.11±1.39
HSD			15.5132		3.1071		13.4127		8.2558
F-Ratio (4,10)			16.3994*		262.9785*		13.6707*		30.6478*

	TA98 (without S9)	TA98 (with S9)	TA100 (without S9)	TA100 (with S9)
Treatment	F-ratio $_{(1, 20)}$ = 0.0142	F-ratio $_{(1, 20)}$ = 606.01*	F-ratio $_{(1, 20)}$ = 14.9365*	F-ratio $_{(1, 20)}$ = 394.5976*
Dose	F-ratio $_{(4, 20)}$ = 24.5981*	F-ratio $_{(4, 20)}$ = 238.4488*	F-ratio $_{(4, 20)}$ = 35.2053*	F-ratio $_{(4, 20)}$ = 131.8673*
Treatment×Dose	F-ratio $_{(4, 20)}$ = 0.0845	F-ratio $_{(4, 20)}$ = 5.6314*	F-ratio $_{(4, 20)}$ = 1.7934	F-ratio $_{(4, 20)}$ = 7.0281*
HSD	18.8393	5.500	14.5176	7.0549

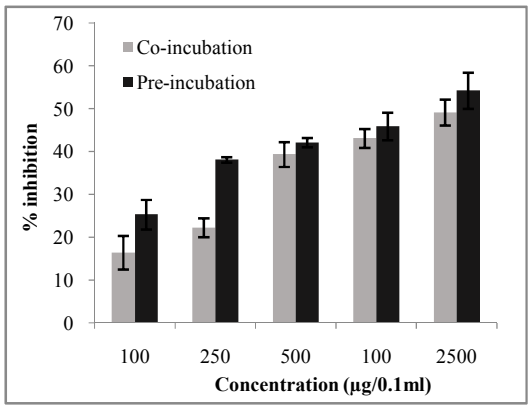
Inh- Inhibition
* represents the significance at $p \leq 0.05$
Values are shown as Mean ± S.E.

Table 12. IC_{50} (µg/0.1 ml) values of ethyl acetate extract of *Streptomyces* sp. strain TES-25 (TEAE) in Ames assay

	TA98				TA100			
	Without S9		With S9		Without S9		With S9	
	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation	Co-incubation	Pre-incubation
Regression Equation	y = 10.03ln(x)-16.2	y = 10.70ln(x)-20.09	y = 10.54ln(x)-14.33	y = 8.729ln(x)+14.04	y = 10.92ln(x)-33.84	y = 8.461ln(x)-11.42	y = 10.59ln(x)-13.73	y = 7.799ln(x)+21.34
R^2 value	0.928	0.964	0.966	0.996	0.931	0.966	0.964	0.966
IC_{50} value	732.16	699.24	445.86	61.56	2143.08	1422.26	407.48	39.25



(a)



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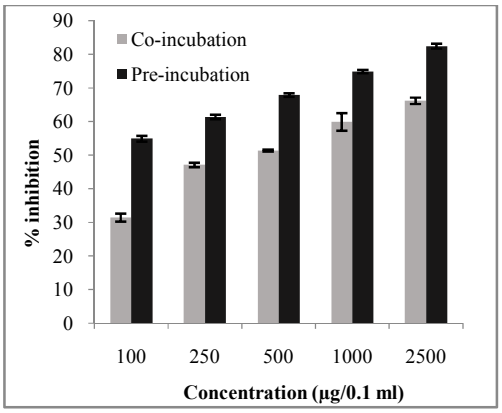

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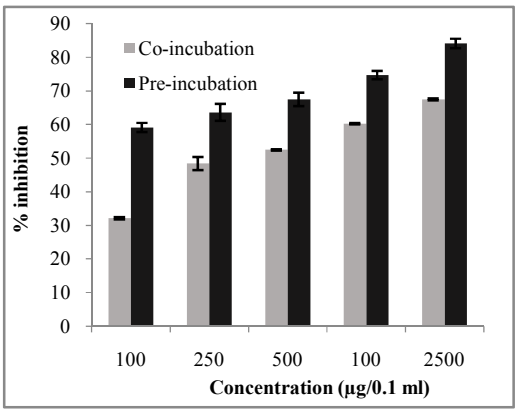


(d)

Fig. 4. Antimutagenic activity of ethyl acetate extract of *Streptomyces* sp. strain TES-25 (TEAE) on (a) *S. typhimurium* strain TA98 without S9 against 4-nitro-o-phenylenediamine (NPD) (b) *S. typhimurium* strain TA100 without S9 against sodium azide (c) *S. typhimurium* strain TA98 with S9 against 2-aminofluorene (2-AF) (d) *S. typhimurium* strain TA100 with S9 against 2-aminofluorene (2-AF).

Antimicrobial Activity

Antibacterial activity

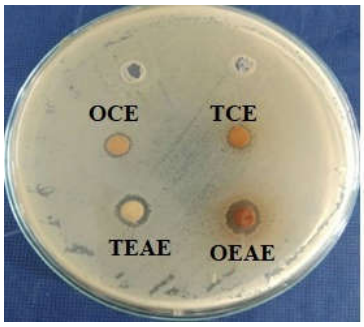
The antibacterial activity of the extracts of *S. violascens* strain OS-6 (OCE and OEAE) and *Streptomyces* sp. strain TES-25 (TCE and TEAE) was tested against *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae* by performing disc diffusion method (Table 13 and Fig. 5) The extracts of both the strains did not show activity against the tested gram negative bacteria (*Salmonella typhi* and *Escherichia coli*) except *Klebsiella pneumoniae* while they inhibited the growth of gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*). The maximum antibacterial activity was shown by OEAE extract against *Staphylococcus aureus* with zone of inhibition of 16 mm followed by OCE extract creating a zone of inhibition of 12 mm against *Staphylococcus aureus* among the extracts of *S. violascens* strain OS-6. In case of extracts of *Streptomyces* sp. strain TES-25, the highest antibacterial activity was observed in TEAE extract with zone of inhibition of 12 mm as compared to TCE extract having zone of inhibition of 11 mm against *Staphylococcus aureus*.

Table 13. Antibacterial activity of extracts of *Streptomyces* spp.

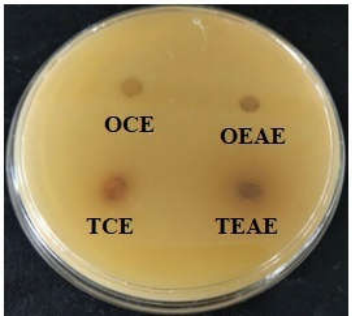
Test organism	Zone of inhibition (in mm) of extracts			
	S. violascens strain OS-6		*Streptomyces* sp. strain TES-25	
	OCE	OEAE	TCE	TEAE
Bacillus subtilis	8 (S)	11 (S)	8 (S)	10(S)
Salmonella typhi	0 (R)	0 (R)	0 (R)	0 (R)
Escherichia coli	0 (R)	0 (R)	0 (R)	0 (R)
Staphylococcus aureus	12 (S)	16 (S)	11 (S)	12 (S)
Staphylococcus epidermidis	9 (S)	11 (S)	8 (S)	10 (S)
Klebsiella pneumoniae	8 (S)	12 (S)	7(S)	8 (S)

The alphabet 'R' represents resistant while 'S' represents sensitive

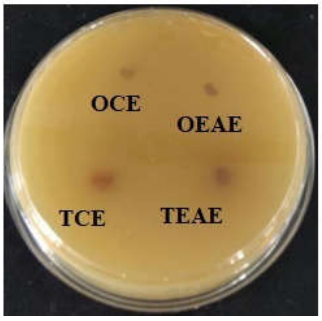
OCE- chloroform extract of *S. violascens* strain OS-6, OEAE- ethyl acetate extract of *S. violascens* strain OS-6, TCE- chloroform extract of *Streptomyces* sp. strain TES-25, TEAE- ethyl acetate extract of *Streptomyces* sp. strainTES-25



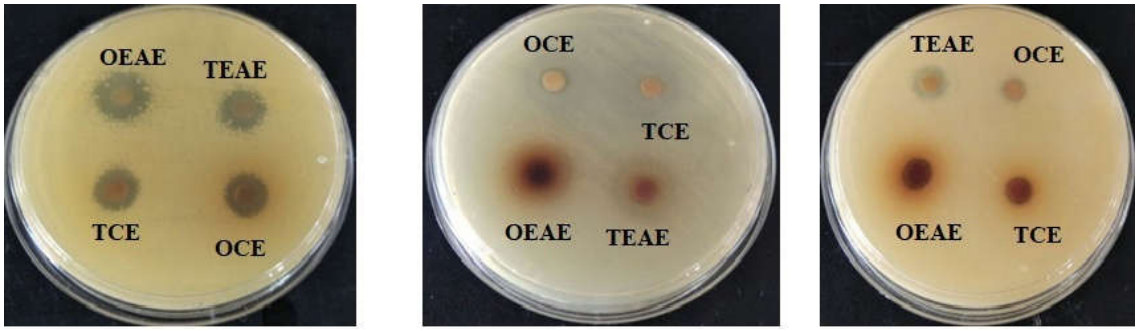
Bacillus subtilis



Salmonella typhi



Escherichia coli



Staphylococcus aureus ***Staphylococcus epidermidis*** ***Klebsiella pneumoniae***

Fig. 5. Antibacterial activity of chloroform extract of *S. violascens* strain OS-6 (OCE), ethyl acetate extract of *S. violascens* strain OS-6 (OEAE), chloroform extract of *Streptomyces* sp. strain TES-25 (TCE) and ethyl acetate extract of *Streptomyces* sp. strain TES-25 (TEAE) in disc diffusion method.

Antifungal activity

The antifungal activity of the extracts (OCE, OEAE, TCE and TEAE) of both the strains was checked against *Alternaria citri*, *Botrytis cineria*, *Colletotrichum musae*, *Fusarium solani*, *Rhizoctonia solani*, *Alternaria brassicicola* and *Fusarium oxysporum* using well diffusion method. The extracts showed antifungal activity against all the tested fungi except *Botrytis cineria*. The maximum antifungal activity was exhibited by OEAE extract against *Colletotrichum musae* creating zone of inhibition of 18 mm in comparison to OCE extract with zone of inhibition of 14 mm against *Colletotrichum musae*. Antifungal activity was also observed in OCE extract against *Alternaria brassicicola* with zone of inhibition of 14 mm. The highest antifungal activity was observed in TEAE extract creating a zone of inhibition of 16 mm against *Colletotrichum musae* and *Alternaria brassicicola*. TCE extract showed less activity as compared to TEAE extract with zone of inhibition of 15 mm against *Colletotrichum musae*. Table 14 and Fig. 6 represent the results of the antifungal potential of the extracts. It was observed that the extracts showed more antifungal activity as compared to antibacterial activity.

Table 14. Antifungal activity of extracts of *Streptomyces* spp.

Test organism	Zone of inhibition (in mm) of extracts			
	S. violascens strain OS-6		*Streptomyces* sp. strain TES-25	
	OCE	OEAE	TCE	TEAE
Alternaria citri	12 (S)	16 (S)	12 (S)	14 (S)
Botrytis cineria	0 (R)	0 (R)	0 (R)	0 (R)
Colletotrichum musae	14 (S)	18 (S)	15 (S)	16 (S)
Fusarium solani	11 (S)	13 (S)	11 (S)	12 (S)
Rhizoctonia solani	11.5 (S)	13 (S)	11 (S)	12 (S)
Alternaria brassicicola	14 (S)	16.5 (S)	11 (S)	16 (S)
Fusarium oxysporum	10 (S)	12 (S)	0 (R)	11 (S)

The alphabet 'R' represents resistant while 'S' represents sensitive

OCE- chloroform extract of *S. violascens* strain OS-6, OEAE- ethyl acetate extract of *S. violascens* strain OS-6, TCE- chloroform extract of *Streptomyces* sp. strain TES-25, TEAE- ethyl acetate extract of *Streptomyces* sp. strain TES-25

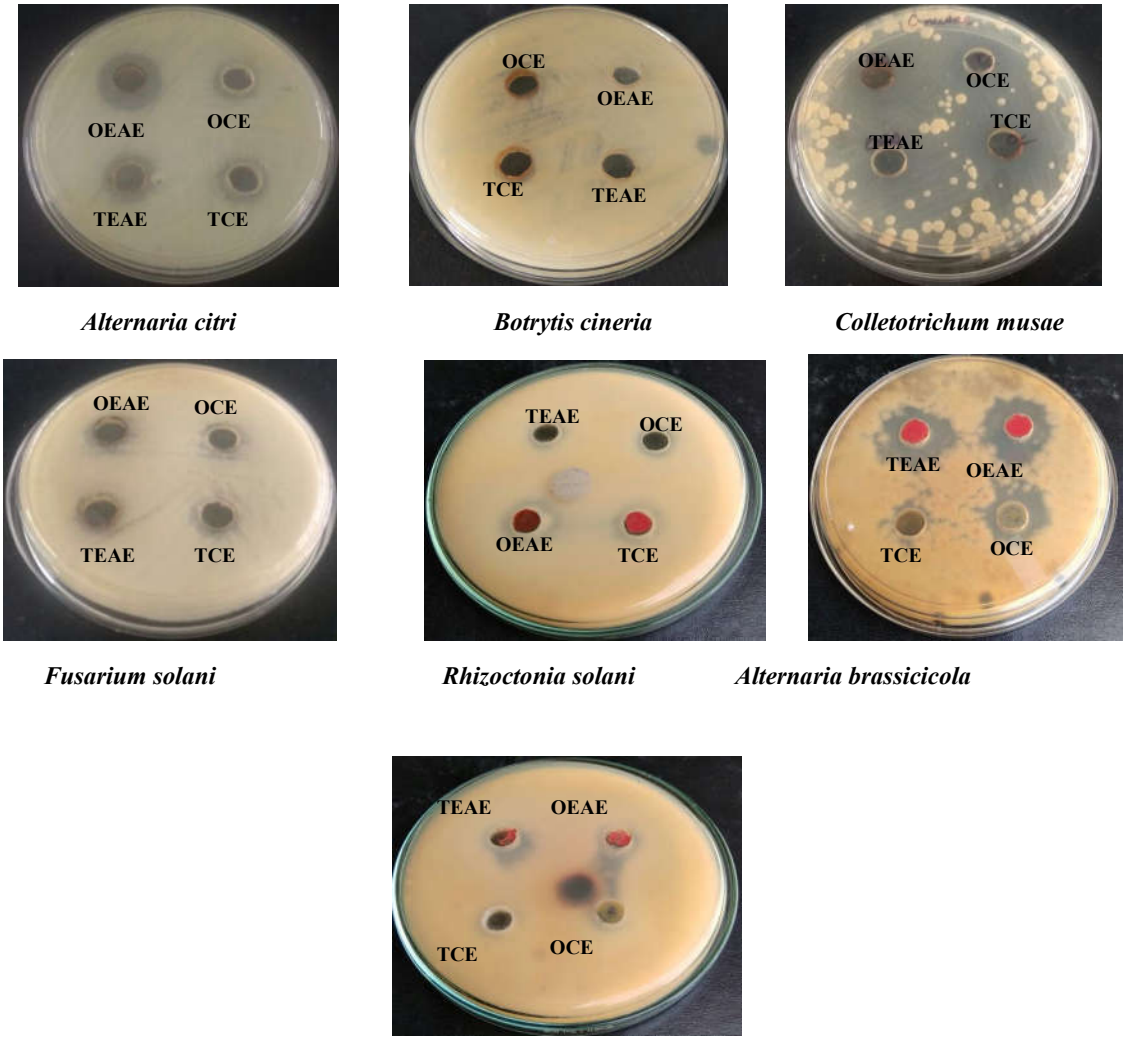


Fig. 6. Antifungal activity of chloroform extract of *S. violascens* strain OS-6 (OCE), ethyl acetate extract of *S. violascens* strain OS-6 (OEAE), chloroform extract of *Streptomyces* sp. strain TES-25 (TCE) and ethyl acetate extract of *Streptomyces* sp. strain TES-25 (TEAE) in well diffusion method.

Plant Growth Promotion Activity

Both the cultures i.e. *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 were found to produce indole-3-acetic acid. The culture *S. violascens* strain OS-6 produced 48.4 µg/ml while *Streptomyces* sp. strain TES-25 produced 35.2 µg/ml of IAA. Fig. 7 represents standard curve of IAA. The soil used for growing plants possessed moisture content of 0.032 g in 100 g of soil with 4.76% of organic matter, 2.76% of organic carbon having alkaline pH of 7.6. The available phosphorus present was 131.15 ± 2.76 mg/kg while available nitrogen was 254.33 ± 16.16 mg/kg. The soil was classified as sandy (silt = 10.26%, clay = 0.81%, sand = 88.93%). The available sodium and available potassium were 294.0 ± 7.4 mg/kg and 124.5 ± 8.35 mg/kg respectively. The application of *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 as seed dressing exhibited positive response on different plant growth parameters on *Triticum aestivum* (wheat) plants such as root length, shoot length, number of plants, fresh weight per plant, dry weight per plant, fresh weight per tiller, dry weight per tiller, number of tillers per plant and number of seeds per plant (Table 15 and Table 16). The control and treated plants showed healthy germination. The percentage of seed germination in control plants was 77 % while plants treated with both strains the percentage was 92%. The root length, shoot length, number of plants, fresh weight per plant, dry weight per plant,

fresh weight per tiller, dry weight per tiller, number of tillers per plant and number of seeds per plant was enhanced by 10.60%, 17.15%, 19.57%, 41%, 57.80%, 55.74%, 54.39%, 11.48%, 27% with *S. violascens* strain OS-6 respectively while the percentage of enhancement observed in *Streptomyces* sp. strain TES-25 was 0.27%, 10.70%, 19.57%, 39.46%, 33.94%, 35.25%, 22.46%, 5.74% and 14.27% in the above mentioned characteristics respectively (Table 17). Fig. 8 and Fig. 9 show the plant growth promoting potential of strains as compared to control.

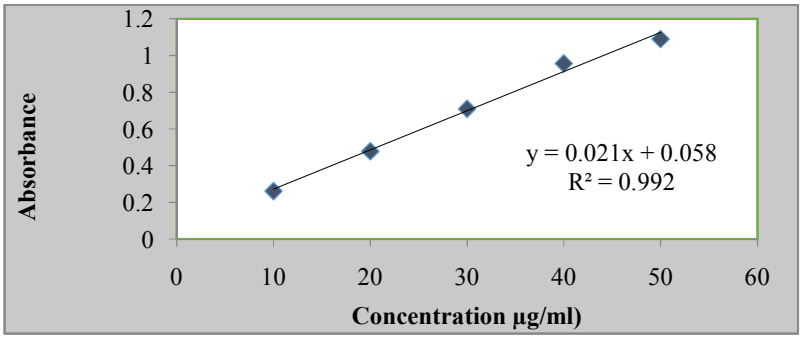


Fig. 7: Standard curve of Indole-3-acetic acid (IAA).

Table 15. Plant growth promotion activity of strains of *Streptomyces* spp. in *Triticum aestivum* (wheat)

Treatment	No. of plants	Fresh weight of plants (g)	Dry weight of plants (g)	Fresh weight of tillers (g)	Dry weight of tillers (g)	Total number of tillers	Total number of seeds
Control	46	120	50.01	56.01	26.27	56	748
S. violascens strain OS-6	55	202.54	94.68	104.58	48.54	75	1136
Streptomyces sp. strain TES-25	55	200.05	80.23	90.78	38.39	71	1022

Table 16. Effect of strains of *Streptomyces* spp. on growth parameters of *Triticum aestivum* (wheat) plants grown in natural conditions

Treatment	Root length (cm) ± SE	Shoot length (cm) ± SE	Fresh weigh per plant (g) ± SE	Dry weight per plant (g) ± SE	Fresh weight per tiller (g) ± SE	Dry weight per tiller (g) ± SE	No. of tillers per plant ± SE	No. of seeds per plant ± SE
Control	7.45 ± 0.40	46.82 ± 1.37	2.61 ± 0.18	1.09 ± 0.07	1.22 ± 0.11	0.57 ± 0.04	1.22 ± 0.098	16.26 ± 1.43
S. violascens strain OS-6	8.24 ± 0.26	54.85 ± 1.22	3.68 ± 0.28	1.72 ± 0.099	1.90 ± 0.15	0.88 ± 0.07	1.36 ± 0.09	20.65 ± 1.61
Streptomyces sp. strain TES-25	7.47 ± 0.396	51.83 ± 1.28	3.64 ± 0.24	1.46 ± 0.07	1.65 ± 0.12	0.698 ± 0.07	1.29 ± 0.09	18.58 ± 1.76
F-ratio (2, 153)	1.6742	9.4201*	5.8385*	14.4803*	6.7558*	6.5092*	0.5878	1.7538
HSD	1.1855	4.3181	0.8143	0.2743	0.4348	0.2033	0.3139	5.4538

*represents the significance at $p \leq 0.05$

Values are shown as Mean ± S.E

Table 17. Percentage increase of growth characteristics of *Triticum aestivum* (wheat) plants after treatment with strains of *Streptomyces* spp.

	Treatment	
Growth parameter	*S. violascens* strain OS-6	*Streptomyces* sp. strain TES-25
Root length	10.60	0.27
Shoot length	17.15	10.70
No. of plants	19.57	19.57
Fresh weight per plant	41.00	39.46
Dry weight per plant	57.80	33.94
Fresh weight per tiller	55.74	35.25
Dry weight per tiller	54.39	22.46
No. of tillers per plant	11.48	5.74
No. of seeds per plant	27.0	14.27

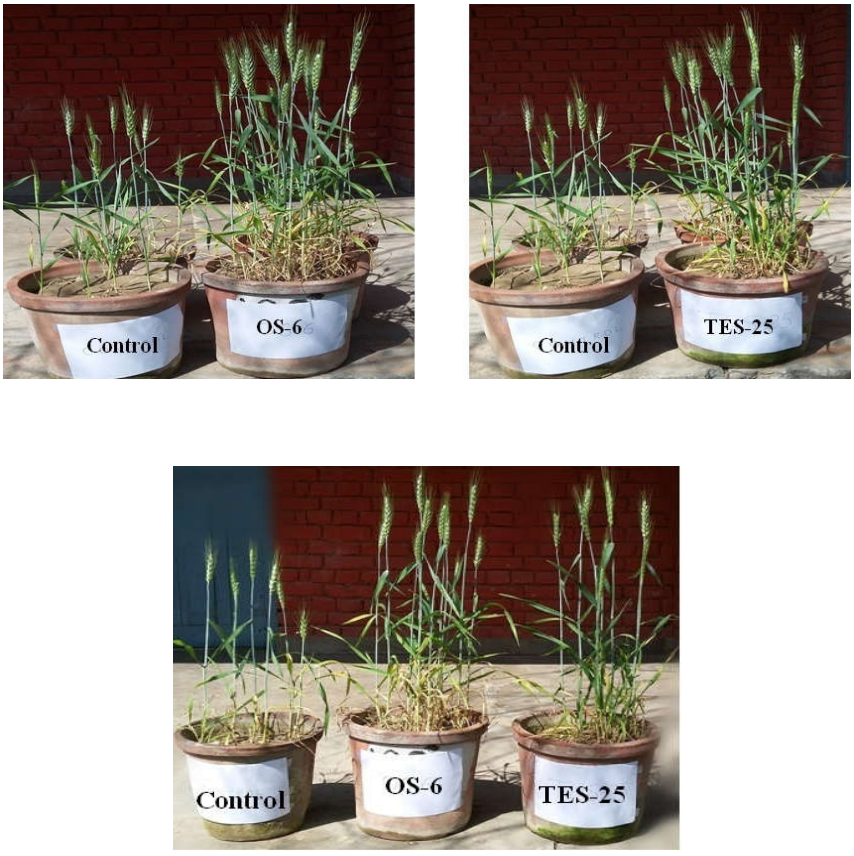


Fig. 8. Photographs showing plant growth promotion activity of strains of *Streptomyces* spp. over control in *Triticum aestivum* (wheat).

Fig. 9. Photographs of *Triticum aestivum* (wheat) control plant and plants treated with *Streptomyces* spp.

DISCUSSION

The early stages in carcinogenesis are marked by onset of mutations. The structure of DNA is maintained by inhibition of reactive oxygen species produced by extrinsic and intrinsic agents by modulation of phase I and phase II enzymes and hence suppressing the occurrence of mutations. Ames Assay was carried out to evaluate the antimutagenic potential of *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25. The Ames test determines that the substance under analysis has mutagenic property and hence can be carcinogenic as cancer is associated with mutations. It is defined as a reverse mutation test involving mutated *S. typhimurium* strains and is used to evaluate the mutagenic activity of chemicals. The antimutagenic activity of extracts was analyzed using NPD and sodium azide (direct acting mutagens) and 2-aminofluorene (indirect acting mutagen) in Ames Assay. The frameshift and base pair substitution mutations describe the antimutagenic mechanism of actions of extracts. The frameshift mutations were tested using *Salmonella typhimurium* TA98 strain while base pair substitution mutations were tested using *S. typhimurium* TA100 strain. The extracts at concentrations of 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml and 2500 µg/ml did not show any toxicity or mutagenic activity to both tester strains in the presence or absence of S9 mix but showed their antimutagenic activity. The non-mutagenic activity of the extracts may be due to their inability to interact with DNA and did not inhibit the synthesis of DNA and thus brought about the initiation of SOS repair mechanism.[44]All the extracts possessed antimutagenic activity in a dose dependent fashion. Dose dependent activity was also reported by Aqil et al. (2008)[34] in Ames assay. The results were significant at 5% level of significance in one way ANOVA. The extracts exhibited more antimutagenic effect in pre-incubation mode of experimentation as compared to co-incubation mode. The extracts showed percent inhibition of antimutagenicity ranged from 43.90% to 85.65%. The regression values ranged from 0.92 to 0.99. The extracts exhibited moderate inhibitory effect in case of direct acting mutagens without S9 mix. The least IC_{50} value among extracts of *S. violascens* strain OS-6 was possessed by OEAE extract in pre-incubation mode of action in TA98 (32.79 µg/ml) and TA100 (4.66 µg/ml) strains in the presence of metabolic activation. Among the extracts of *Streptomyces* sp. strain TES-25, the highest antimutagenic response was observed in TEAE extract with IC_{50} values of 61.56 µg/ml in TA98 strain with S9 mix and 39.25 µg/ml in TA100 strain with metabolic activation in pre-incubation method of treatment. The reduction in revertent colonies was observed against direct and indirect acting mutagens in both strains. The results demonstrated that the antimutagenic activity may be due to the presence of epicatechin as determined in the UHPLC analysis. This polyphenolic compound along with other polyphenolic compounds might be responsible for providing the antimutagenic activity to the extracts. The addition

of S9 mix initiated the increase in the antimutagenic response in all the extracts particularly in OEAE extract of *S. violascens* strain OS-6 and TEAE extract of *Streptomyces* sp. strain TES-25 against both strains of *S. typhimurium*. The ethyl acetate extract contains some therapeutically significant molecules. The ethyl acetate extract exhibited the maximum antimutagenic activity on account of presence of different polyphenolic compounds mainly epicatechin against 2-aminofluorene initiated mutagenicity. The previous studies showed that epicatechin possess antimutagenic potential against a large number of mutagens. [45, 46, 47, 48] The compounds and different extracts from plants and microorganisms were analyzed for their mutagenic and cytotoxic potential performing similar studies. [49, 50, 51, 52]

The multidentate ligand nature of polyphenols enable them to bind the protein molecules present on the periphery on more than single site. [53] The polyphenolic compounds usually epicatechin (modulators) present in the ethyl acetate extracts change the organization and function carried out by cytochrome P-450 enzyme by interfering with the metabolic initiation of 2-AF and hence defending against the chemically initiated process of mutagenesis. The 2-AF is converted to N-hydroxy-2-aminoflourene by cytochrome P-450 enzyme on direct interaction with DNA and mutagen.[54] The liver glycosides induced the competitive suppression of P-450 isozymes which may be responsible for the enhancement of inhibitory activity along with the S9 mix.[55]After metabolic activation, such type of protective activity in the form of antioxidant activity is linked to the function possessed by isoforms of cytochrome P-450s having reductase or oxygenase in the system of detoxification of destroying compounds involved in the generation of free radicals, oxygen radicals and reactive oxygen species.[56, 57] The promutagens are oxidized by metabolically active cytochrome P-450 during competitive suppression by glycosides that might be responsible for providing the antimutagenic activity to the extracts along with S9 mix thus bringing about the conversion of promutagens.[58] The alteration in the function and organization of cytochrome P-450 enzyme results in defending the mutagenesis induced by chemicals that bring about differential pathways and metabolism of mutagens.[53] The current study revealed the desmutagenic effect in which mutagen is directly inactivated before the mutation in genes that is shown by all extracts against the direct and indirect acting mutagens. The polyphenolic compounds also regulate the estradiol-dependent cancer.[59]Polyphenolic compounds exhibiting the antioxidant potential also possess anticancer and antimutagenic activities on account of their capability of scavenging the free radicals and initiating the enzymes having antioxidant activity.[60] The significant role of ethyl acetate extracts on account of their antimutagenic activity has been related to their antioxidant potential. The secondary compounds possess the property of scavenging the free radicals, decreasing the mutagenicity, ameliorating the alkylated damage of DNA and ceasing the mutations in cells.[61]Hence, natural antimutagens are capable of controlling the changes in cells by inhibiting the mutations initiated by genotoxic substances that bring about different degenerative diseases such as cancer.[62] The appropriate method to decrease the incidence of cancer is to incorporate the chemopreventive agents possessing multiple functions. The daily consumption of 1 g of polyphenolic compounds in fruits or vegetables reduced the rate of mutagenesis and carcinogenesis.[63] The phyto-originated medicines have been proved to be very effective in providing protection against the mutations and cancer without any side effects.[64, 65]

The antibiotic resistant pathogens have lead to the enhanced necessity of the unexplored antimicrobial compounds. Actinobacteria produce safe, novel and effective antimicrobial products to inhibit the growth of the drug-resistant pathogens.[66, 67] The pharmaceutical research laboratories screen thousands of actinobacteria per year especially *Streptomyces* strains for obtaining undiscovered bioactive compounds that can be used to isolate new antibiotics as novel antimicrobial compounds.[68, 69, 70, 71, 72] In the present study, the extracts were analyzed for their antimicrobial activity against bacterial strains and fungal phytopathogens. The antibacterial activity of the extracts was tested against both gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram negative bacteria (*Salmonella typhi*, *Escherichia coli* and *Klebsiella pneuminiae*). The antifungal activity of the extracts was examined against *Alternaria citri*, *Botrytis cineria*, *Colletotrichum musae*, *Fusarium solani*, *Rhizoctonia solani*, *Alternaria brassicicola* and *Fusarium oxysporum*. The extracts exhibited

antibacterial potential against tested gram positive bacteria but did not show activity against tested gram negative bacteria except *Klebsiella pneuminiae* to which they showed moderate activity. The maximum antibacterial activity was observed in case of OEAE extract against *Staphylococcus aureus* among the extracts of *S. violascens* strain OS-6. TEAE extract produced highest antibacterial response against *Staphylococcus aureus* among the extracts of *Streptomyces* sp. strain TES-25. The maximum antifungal activity was shown by OEAE extract against *Colletotrichum musae* in the extracts of *S. violascens* strain OS-6. Among the extracts of *Streptomyces* sp. strain TES-25, the highest antifungal activity was observed in TEAE against *Colletotrichum musae* and *Alternaria brassicicola*. All the extracts showed antifungal activity against the tested fungal phytopathogens. The ethyl acetate extracts of both the strains showed more antimicrobial activity as compared to the chloroform extracts. The previous studies also revealed that ethyl acetate was the best solvent for the extraction of antimicrobial compounds.[69] Pugazhvendan et al. (2010)[73] and Baskaran et al. (2016)[74] also reported that the maximum antimicrobial activity was exhibited by ethyl acetate extracts of different *Streptomyces* spp.

The present work showed that the extracts were more effective in case of gram positive bacteria as compared to gram negative bacteria. Similar results were also reported by Ilic et al. (2007),[75]Arasu et al. (2008),[69] Al-Hulu et al. (2011),[76] Sosovele et al. (2012),[77] Ramazani et al. (2013),[78] Kekuda et al. (2013)[79] where the different strains of *Streptomyces* spp. showed more antibacterial effect against gram positive bacteria in comparison to gram negative bacteria. The reason behind it is the difference in the morphology of cells walls of bacteria. An outer lipopolysaccharide membrane is present in the cell walls of gram negative bacteria and therefore it shows impermeability to lipophilic solutes and the porins hinder the entry of hydrophilic solutes.[80] The cell wall of gram positive bacteria has only peptidoglycan layer which is permeable to most of the antibacterial compounds. Various studies conducted by Al-Zahrani (2007),[81] Arasu et al. (2008),[69] Choi et al. (2012),[82] Hunadanamra et al. (2013),[83] Nguyen and Kim (2015),[84] Dezfully and Ramanayaka (2015),[85] Baskaran et al. (2016)[74] showed the antibacterial and antifungal activity of the different strains of *Streptomyces* spp. which are in accordance with the present work.

The strains of *Streptomyces* spp. were analyzed for their plant growth promoting potential. The strains showed the production of indole-3-acetic acid. *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 produced 48.4 µg/ml and 35.2 µg/ml of indole-3-acetic acid respectively. The results for the production of IAA are in accordance with the previous reports.[86, 87, 88, 89] The root exudates provide tryptophan to the microorganism of rhizosphere region which leads to the enhanced production of auxins by them. The plant hormones produced by streptomycetes help in providing stimulus for the enhanced growth of plants.[90, 91]These act as chemical messengers. Auxins are plant hormones having an indole ring that enhance growth of plant by providing stimulus for germination of seeds, growth of seedlings, elongation of cells and initiating the growth of roots.[92]

Indole-3-acetic acid (IAA) is included in the auxin family and is produced by different species of *Streptomyces viz. S. lydicus, S. olivaceoviridis*, *S. hygroscopicus, S. rimosus*, *S. griseoviridis* and *S. rochei* residing in the rhizospeheric soil and enhance growth of plants by increasing germination of seeds, elongation of roots and dry weight of roots.[19, 24, 93] The different species produce different amount of IAA.[92, 94] The production of IAA is affected by conditions of culture, stage of growth and substrate availability. The plant growth promoting potential of the strains was studied *in vivo* bioassay in *Triticum aestivum* (wheat) seeds. The treatment of seeds with *S. violascens* strain OS-6 and *Streptomyces* sp. strain TES-25 resulted in the significant increase of root length, shoot length, number of plants, fresh weight of plants, dry weight of plants, fresh weight of tillers, dry weight of tillers, number of tillers per plant and number of seeds per plant. There are many reports that prove that *Streptomyces* spp. possess plant growth promotion activity.[18, 19, 20, 21, 23] Treatment of seeds with *S. griseus* resulted in increase of different growth parameters in oat, wheat and carrot as compared to control.[95] *Streptomyces* strains have also enhanced plant growth as seed or soil inoculants in rice, sorghum and chickpea.[23, 29, 96, 97] Goudjal et al. (2013)[92] and Khamna et al. (2010)[93] reported the improvement in germination of seeds and elongation of roots on

treatment of seeds of different crops with *Streptomyces* spp. The three endophytic *Streptomyces* spp. when inoculated on tomato seeds resulted in the enhanced germination of seeds, elongation of roots and shoots on account of production of IAA and siderophore.[88] The two rhizosphere-competent strains of *Streptomyces* (*S. atrovirens* and *S. filipinensis*) lead to the increase in growth of tomato plants in comparison to plants that were treated with non rhizosphere-competent strains.[19] The continuous secretion of compounds like lecithins, glutens, flavonoids and polysaccharides bring recognition between plants and microorganisms[98] and influence the root colonization of microorganisms.[99]

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