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Effect of *Streptomyces Sp* on Growth Promotion and Grain Yield in Black Gram Fields (*Vigna Mungo*)

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Review Article

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ABSTRACT

Six *streptomyces sp* ESS-4 (*Streptomyces fradiae*), DVS-11 (*Streptomyces olivoviridis*), KZSS-6 (*Streptomyces koyangensis*) NVS-10 (*Streptomyces flavofuscus*), TSS-3 (*Streptomyces griseorubens*), and TVS-2 (*Streptomyces lunaelactis*) were previously reported to antagonistic activity in fungal pathogens, PGP traits, green and field conditions in black gram. These isolates were characterized for their antibacterial activity for streak plate method, antibiotic resistances, and optimization of pH, salinity, temperature, carbon sources, and heavy metals. These *streptomyces sp* show inhibition to 11 antibiotics they produce the halo-zone produces 14 to 19 cm. This *streptomyces sp* grow in pH 3 to 13, salinity up to 0% to 12%, temperature up to 20-50, carbon sources for glucose, fructose, sucrose, and starch, and heavy metals tolerances. Out of six *sp*, KZSS-6 was better results. So KZSS-6 strain was further evaluated on the greenhouse and black gram field conditions. This *streptomyces* greenhouse condition they enhance the root length, yields enhanced when compared to the control. In greenhouse conditions black gram plant height 8 to 14 cm, root length 1 to 6 cm, the seeds 2 to 5cm, total plant weight is 0.20 to 0.60 mg, and total dry weight for plant 7 to 14 mg. In the field experiment, root height and weight (10 to 13 cm, 0.25 to 0.50 mg), shoot length and weight (3.10 to 3.50 cm), stem height and stem weight (22 to 32 cm, 4.0 to 4.25 gm), plant weight (5.50 to 7.50 gm), number of fruits weight (10, 100, and 1000) (0.4 to 0.50, 2.50 to 5.0 and 22 to 45 gm), and finally yield (11.5 to 29.50 tons).

INTRODUCTION

Agriculture is a major contributing factor to the global food supply. Now present circumstances agriculture was facing severe threats like loss of soil fertility, poor soil quality, climatic changes conditions, more use of pesticides. To overcome these challenges, eco-friendly approaches like bio-fertilizers. To enhance the agricultural outputs sustainably, novelty and eco-friendly strategies must be employed in agriculture, they reduced chemical pesticides. Plants, as non-locomotive organisms live. Pulses have contained are sources of proteins, micronutrients, and dietary fiber. Black gram plants, green gram plants have contained good proteins for 100 g of seeds the range of about 20.97 to 31.32 g and 25 to 30 g of protein, experts. India is one of the country largest pulses producers and consumers in the world. Preethi, R. *Vigna mungo* (L) hepper is commonly known as a black gram. One of the important Asiatic legumes crops these seeds contains a major protein and vitamins [1].

Streptomyces are filamentous gram (+) bacteria, belonging to the order of Actinomycetales, they are presented by more than 570. These actinomycetes were found in soil, marine water, vermicompost esperts, Streptomyces is to survive during the unfavorable environmental condition. Therefore, the streptomyces is more efficient against many other microorganisms present in rhizosphere soil. Recently *streptomyces* has been considered as a prospective bio control agent in agriculture, also to produces antibiotics and intense antagonistic activity through the production of various antifungal metabolites experts. When compared to other microorganisms, actinomycetes are inhibited by a wide range of plants as entophytes. Therefore, actinobacteria are efficient plant growth promoters, and potential to serve as an effective bio control agent experts. They are plays important role in plant growth-promoting, plant protection, and anti-microbial activity, also produce secondary metabolites for commercial interest, experts [2].

Earlier, we reported a set of six *streptomyces* strains were isolated from black gram soil and vermicompost samples. The strains are using this study namely DVS-11, ESS-4, KZSS-11, NVS-10, TSS-2, and TVS-2 which PGP traits and antagonistic activity of *fusarium oxysporum* in Black gram. Six *streptomyces* strains were effective control heavy metals, physiological properties and production of antibiotics. Then further green house and filed assay were conducted and results [3-8].

LITERATURE REVIEW

Antibacterial activity for streak plate method

Antimicrobial activity was performed by cross streak technique. SCA plates were prepared, isolates were streak on the center of Petri plates then incubated at 28ºC for 7days. Than the plates were streak on test organisms, at 90° then plates were incubation at 37ºC for 12 hrs. The anti-bacterial activity was measured by inhibition of target bacteria and isolated actinomycetes Oskay M, Kumar PS, Sangkanu S. The human bacterial pathogen was used for test, *Bacillus subtitles* (MTCC No. 10407), *Micrococcus luteus* (MTCC-106), *Staphylococcus aureus* (MTCC No. 6908), *Streptococcus mutans* (MTCC No. 890). Gram negative *bacteria Klebsiella pneumonia* (MTCC No. 9024), Proteus vulgaris (MTCC No. 744), *Pseudomonas aeruginosa* (MTCC No. 1034), *Saccharomyces cerevisiae* (MTCC-251). All the bacterial cultures were procured from IMTECH, Chandigarh [9].

Antibiotic assay (or) antibiotic resistances

The potential isolates were tested for their antibiotic susceptibility and resistance activity against 11 different antibiotics such as Amoxicillin, Ampicillin, Cefpodoxime, Chloramphenicol, Ciprofloxacin, Neomycin, Novobiocin, Penicillin, Rifampicin, Tetracycline, Vancomycin. Muller Hinton Agar media was prepared, autoclaved at 121°C for 15 minutes at 15 lbs pressure, cooled and poured into Petri plates. After solidification, 3 days old Actinomycetes culture was swabbed and followed by placing the antibiotic disc aseptically. The plates were incubated at 28 ± 2°C for four to five days. The results were noted as the zone of inhibition in mm [10].

Metal's tolerances

The selected actinomycetes against the stress, by the addition of heavy metals to its growth environment. PBS buffer prepared and adjust the pH to 6.8. Heavy metals are using the $CuSO_4$ (Copper (II) Sulfate, $ZnSO_4$ (Zink Sulfate), (CH_3COO) $2Pb.3H_2O$ (Lead acetate tri hydrate), CH_3COONH_4 (Ammonium Ethanoate), K_2So_4 (Potassium sulfate), and CH_3COONa (Sodium acetate), Heavy metals salts are prepared in different concentration in 10 mm, 50 mm, 100 mm, 500 mm, and 1000 mm were dissolved PBS buffer. To prepare the starch casein agar, centre of plate well in 12 mm. On each plate six actinomycetes stains are streaks, incubated at 28 ± 2°C for 7 days [11].

To calculated the % of growth=Complete inoculated length of streak–inhibited length X 100
Complete inoculated length

Effects of carbon source

The capacity of carbon sources utilization by isolated Actinomycetes was tested against four different carbon sources such as glucose, fructose, sucrose, and starch. All the four carbon sources separately sterilized by membrane filtration and 1% carbon source were added to basalmineral salt agar media. The selected Actinomycetes were streaked on basal mineral salt agar media and incubated at 28 ± 2°C for five days. The plates were observed for the growth of Actinomycetes and noted as the scale of 0-3 as follows; 0=no growth, 1=slight growth, 2=moderate growth, and 3=good growth [12-15].

Effect of Salinity

The Actinomycetes strains were streaked on Bennett`s agar (Himedia Laboratories, Mumbai, India), amended with different concentrations of NaCl (0-12%, at 2% intervals) and incubation, at 28 ± 2°C for five days. After incubation the plates were observed for growth of Actinomycetes. Based upon the growth the scale was given as 0-3 as follows; 0=no growth, 1=slight growth, 2=moderate growth, and 3=good growth [16].

Effect of pH

The selected Actinomycetes were streaked on Bennett`s agar (Himedia Laboratories, Mumbai, India), adjusted with pH ranging from 5,7,9, and 11, incubated at 28 ± 2°C for five days. After incubation, the plates were observed for the growth of Actinomycetes. Based upon growth the scale was given 0-3 as follow; 0=no growth, 1=slight growth, 2=moderate growth, and 3=good growth [17].

Effect of temperature

The selected Actinomycetes were streaked on Bennett's agar (Himedia Laboratories, Mumbai, India) and incubated at different temperatures of 20°C, 30°C, 40°C, and 50°C for five days. After incubation, the plates were observed for the growth of Actinomycetes. Based upon growth the scale was given 0-3 as follow; 0=no growth, 1= slight growth, 2=moderate growth, and 3=good growth [18].

Evaluation of actinobacterial PGP activity under greenhouse condition

Six strains were possessed in greenhouse condition by using mass cultivating pot experiments. Six strains were assayed with sterile and non-sterile soils in the presences of FOC.

Mass culturing of *streptomyces spps* fermentation broth

Six strains were inoculated into starch casein agar medium and incubated at 28 ± 2°C for 3 to 5. Agar blocks were prepared, 1 sq cm of agar block from the periphery of actively growing colony was cut and inoculated in 100 ml of ISP-2 broth and additionally 10% maltose, 4% glucose and 4% yeast extract, adjusted with pH 7.2. The media was prepared and inoculated the selected actinomycetes. The medium was transfer to in each flask 100 ml and to take 250 ml flask, the flasks were incubated 28 ± 2°C in rotary shaker with stirring at 170 rev min. To prepare ISP-2 broth additionally added 4% yeast malt extract, transfer to the 48 hr log phage actinobacterial broth culture added. The inoculated flask was placed on incubator rotary shaker for 4-5 days. On the fifth day, 001 ml of FOC spores stock prepared as earlier was added to the culture medium [19].

Pot filling the study

Each pot was filling with 1 kg soil (sterile and non-sterile soils using this study). Pot filling with sterile soil in aseptically, and inoculated with fungal spores for which each 1 kg of sterile soil was mixed with 50 ml of FOC spores' stock (concentration 10-6 spores per 1 gram of soil) [20-25].

Seed sowing: Black gram seeds were procured from commercial markets in GUNTUR. The seeds were selected by appearance, surface sterilization (with aqueous sodium hypochlorite—NaOCl, 05% v/v, 2 min) and washed with sterile distilled water three times. One (or) two seeds were sown per pot thus giving a total of 10 seeds (five replication pots) for each treatment in study [26-30].

Growing of seedlings and plant growth analysis: In green house temperature was maintain 27°C and humidity 55%. In treatment pot were arranged in a Completely Randomized Design (CRD) five replicates. The pot was maintaining 65% moisture level, and watered uniformly every day. Treatment post was label, and maintained with succent distances. Antagonistic treatments allowed growing until 15 to 30 days.

Measurement of plant growth parameters: The black gram plants were grown in 15 to 30 days, measure the root length, plant height, the plants were uprooted, fresh biomass, and disease index. In black gram root length was measure from the base of the shoot to the tip of the primary root and shoot length was measured from the base of the shoot to tip of primary leaf. Seed germination was calculated in percent by using the formula.

$$\text{Percent of seed germination} = (\text{number of germinated seeds})/(\text{total number of seed sown}) \times 100$$

Evaluation of Streptomyces for PGP potential on black gram under field conditions

Field experiment was conducted in 2018-2019 (rabi and kharif season) 16.3763°N, 80.5277°E Nagarjuna university Andhra Pradesh, India. Experiment was conducted with 4 types of veritas, like Poush, PU-31, TBG-104, Q-31 soda (90 to 120 days) these seeds were collected form Acharya N G Ranga Agricultural University, Guntur which normally yields 1.2–1.5 tha^{-1}. The experiment was laid out in a randomized complete block design with 3 replicates and subplot sizes of 10 m × 7.5 m. The *Streptomyces spp* were grown on starch casein broth at 28°C for 5 days. The control contained no Streptomyces strains. The 10-14 days old single seedlings (black gram) were uprooted from the green house, black gram plant roots were dipped in the respective *Streptomyces spp*. broth (containing 10-8 CFU•mL^{-1}) for 50 min and transplanted at site soil, the plants were planted in row-to-row with spacing of 25 cm and a plant-to-plant spacing of 25 cm. Broth culture (1000 mL 10^{-8}) was applied one in 15 days until flowering stage along with irrigation. The black gram crop was harvested manually on 2018 and 2019 and observed for plant height, whole plant weight, stem height, stem width, stem weight, internodes number of fruits, leaf width, leaf weight, shoot length, shoot height, root height, root weight, number of root nodules, root nodules weight, one fruit weight, 10 seeds weight, 100 seeds weight, 1000 seeds weight.

$$\text{percentage Yeild (\%) tonnes} = (\text{square foot} \times \text{number of fruits} \times \text{seeds weight})/(100 \times \text{one hectare land})$$

Statistical analysis

The antagonistic activity was conducted for the completely randomized design with three replicates in three fungal pathogens *viz.*, physiological properties like Temperature, pH salinity, carbon source utilization, and antibiotic production. The data were subjected to Analysis of Variance (ANOVA) and the mean values were compared at a 5% Least Significant Difference (5% LSD) and Coefficient of Variation (% CV) [31].

RESULTS

Anti-bacterial activity for bacterial pathogens

Six isolates were screened against human bacterial pathogens. The percentage of activity was only 44 (41.50%) isolates showed good antimicrobial activity against pathogens. The percentage of activity to each pathogen is listed below: Bacillus subtitles (18%) *Micrococcus luteus* (14%), *Staphylococcus aureus* (18%), Streptococcus mutans (20%). Gram negative bacteria *Klebsiella pneumonia* (28%), Proteus vulgaris (16%), *Pseudomonas aeruginosa* (18%), *Saccharomyces cerevisiae* (16%), (Table 1 and Figure 1).

Effect on antibiotics

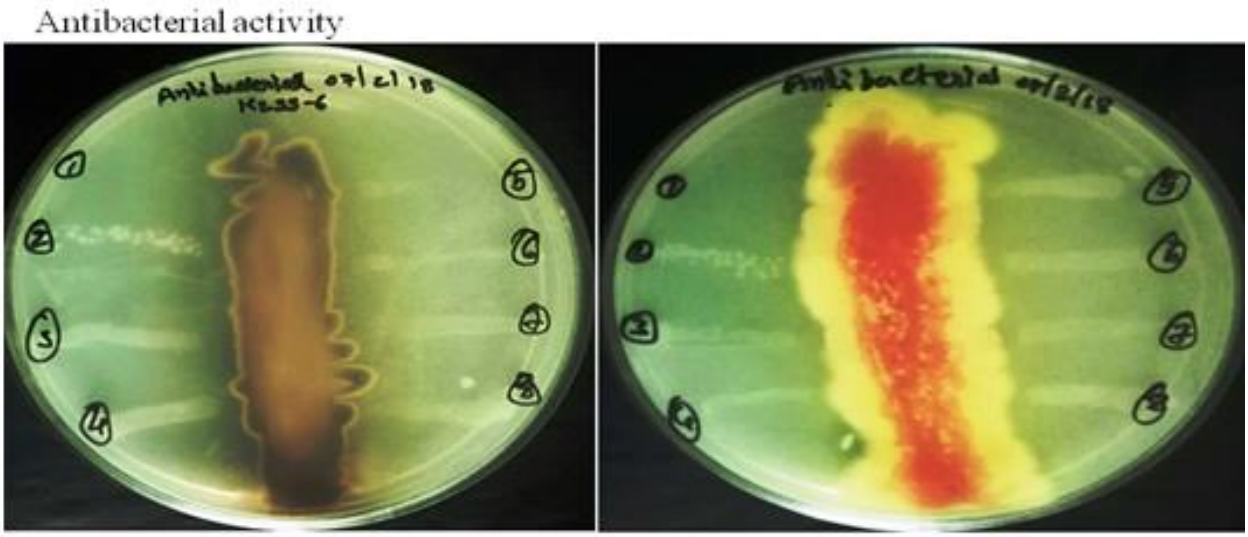
The six antagonistic isolates were able to resist all 11 antibiotics. The zone of inhibition range is 14 mm-20 mm. DVS-11 showed maximum resistance to Amoxiclav, Ampicillin, Ciprofloxacin, and Vancomycin, ESS-4 showed maximum resistance to Cefpodoxime and Tetracycline, TVS-2 showed maximum resistance to Chloramphenicol and Rifampicin, NVS-10 showed maximum resistance to Neomycin and Penicillin and KZSS-6 showed maximum resistance to Novobiocin. The results are represented in the Figure 2 and Table 2.

Table 1. Antibacterial activity in streak plate technique

S. No	Isolate cultura	*Bacillus subtitles*	*Micrococcu luteus*	*Staphyloc-occus aureus*	*Streptococc us mutans*	Klebsiella pneumonia	Klebsiella pneumonia	Pseudomonas aeruginosa	Saccharom-yces cerevisiae
1	ESS-4	++	[Indexing at]	++	+	-	++	[Indexing at]	++
2	DVS-11	++	[Indexing at]	++	[Indexing at]	[Indexing at]	++	+	[Indexing at]
3	KZSS-6	[Indexing at]	++	[Indexing at]	-	++	[Indexing at] +	-	++
4	NVS-10	+	[Indexing at]	+	[Indexing at]	++	+	[Indexing at]	[Indexing at]
5	TVS-2	[Indexing at]	+	++	[Indexing at]	++	[Indexing at]	++	-
6	TSS-3	++	-	[Indexing at]	+	++	++	++	[Indexing at]

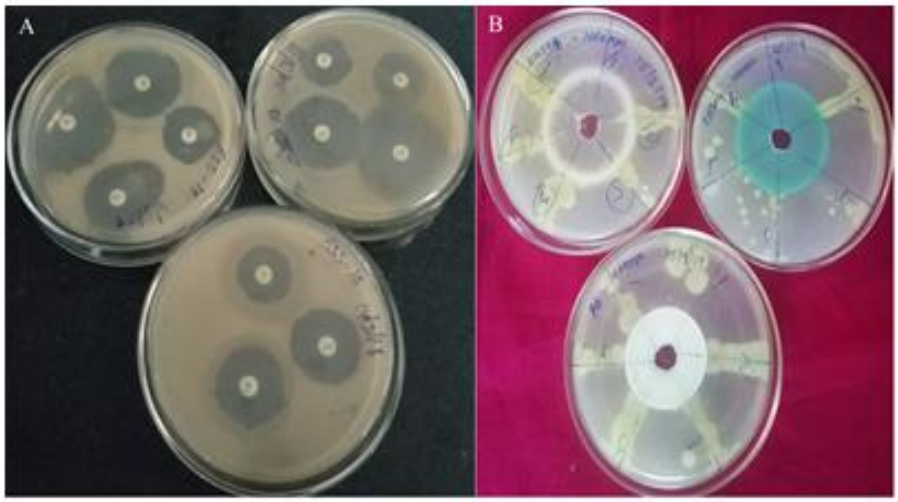
The bacterial pathogens are showed the percentage of antibacterial activity *Bacillus subtitles* (18%) *Micrococcus luteus* (14%), *Staphylococcus aureus* (18%), *Streptococcus mutans* (20%). Gram negative bacteria *Klebsiella pneumonia* (28%), *Proteus vulgaris* (16%), *Pseudomonas aeruginosa* (18%), *Saccharomyces cerevisiae* (16%).

Figure 1. Antibacteria activity for bacterail straians.



Antibacterial activity represent *Micrococcus luteus, Bacillus subtitles, Staphylococcus aureus, Streptococcus mutans,* Gram negative bacteria *Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Saccharomyces cerevisiae.*

Figure 2. Antibiotic activity and metals tolerance



A is represent to the antibiotic activity, by using 13 types of antibiotic,
B is corresponds to the metals protection by using four types metals.

Table 2. Antibiotic assays for most promising isolates

Isolates	AM	AMP	CF	CP	CIP	NE	NB	P	RF	TR	VC
TVS-2	18	16.33	15	20	16.33	18	19	15.67	20	16.33	16.67
KZSS-6	15.67	17.33	15.33	14.67	17	17.33	20.33	15.33	14	17.33	17.33
ESS-4	18	16.33	17.67	15.33	17	18.33	13.67	15.33	15	19.67	15.33
NVS-10	15.33	17	16.33	18.33	16.33	18.63	17.33	18.33	19	16	18.67
DVS-11	18.33	17	17.33	15.67	17.67	16.33	18.33	17.67	16.67	16	18.67
TSS-3	14.33	16.33	18.67	19	15.33	15.67	18	19.67	18	16	19.33
Control	0	0	0	0	0	0	0	0	0	0	0
Mean	14.24	14.33	14.33	14.71	14.24	14.9	15.24	14.57	14.67	14.48	15.14
SE ±	2.35	1	1.46	1.17	1.09	1.73	1.6	1.49	1.49	1.31	1.58
SED	1.66	0.7	1.03	0.82	0.77	1.22	1.13	1.6	1.05	0.93	1.12
LSD5%	5.13	2.179	3.193	2.551	2.393	3.777	3.494	3.248	3.26	2.875	3.461
CV %	20.3	8.5	12.5	9.7	9.4	14.2	12.9	12.5	12.5	11.2	12.8

Am=Amoxyclav; AMP= AMPICILLIN; CF=Cefpodoxime; CP=Chloraphnicol; CIP=Ciproflaxacin; NE=Neomycin; NB=Novobiocin; P=Penicillin; RF=Rifampicin; TR=Tetracycline; VC=Vencomycin; SE=Standard Error; SED=Standard Errors of Differences of Means LSD=(5% Level) East significant difference; CV=Coefficient of Variance.

Heavy metals tolerances

Heavy metals tolerances $CuSO_4$, $ZnSO_4$, $C_4H_{12}O_7Pb$, and CH_3COONH_4 are shown KZSS-6, ESS-4, NVS-10, DVS-11 and TSS-3 tolerance levels. $CuSO_4$ metal tolerances rang is of five isolates 4-32 out of five KZSS-6 are more activity, $ZnSO_4$ metal tolerances rang is 11-32 more activity in ESS-4, $C_4H_{12}O_7Pb$ metal tolerances rang is of five isolates 3-26 more activity in TSS-3, CH_3COONH_4 metal tolerances rang is 2-14 more activity in KZSS-6. Out of six isolates only TSS-3 where's none they grew at four metals ($CuSO_4$, $ZnSO_4$, $C_4H_{12}O_7Pb$, and CH_3COONH_4). The results are represented in the Table 3 and 4, Figure 3.

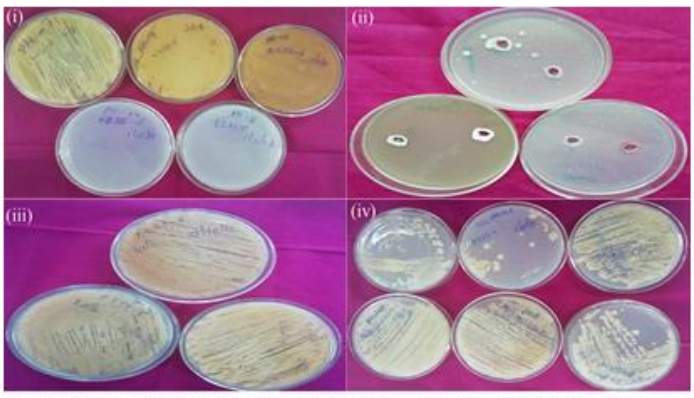
Table 3. Heavy metals tolerances $CuSO_4$

S.no	$CuSO_4$ 10 mm	$CuSO_4$ 50 mm	$CuSO_4$ 100 mm	$CuSO_4$ 500 mm	$CuSO_4$1000 mm
TVS-2	0	0	0	0	0
KZSS-6	32	26	13	26	32
ESS-4	20	16	20	26	26
NVS-10	15	20	15	0	18
DVS-11	10	18	22	30	21
TSS-3	25	4	24	29	26
Control	0	0	0	0	0
Mean	14.57	12	13.42	14.9	17.57
SE ±	4.59	3.976	3.75	5.63	4.82
CV %	83.47	87.66	73.95	83.27	72.2
SE=Standard Error; SED=Standard Errors of Differences of means; CV=coefficient of variance.					

Table 4. Heavy metals tolerances $ZnSO_4$

s.no	$ZnSO_4$ 10 mm	$ZnSO_4$ 50 mm	$ZnSO_4$ 100 mm	$ZnSO_4$ 500 mm	$ZnSO_4$ 1000 mm
TVS-2	0	0	0	0	0
KZSS-6	22	28	13	15	29
ESS-4	13	20	13	11	32
NVS-10	15	19	18	17	18
DVS-11	23	21	11	23	26
TSS-3	0	24	0	0	10
Mean	10.42	16	7.85	9.42	16.42
SE ±	3.92	4.28	2.89	3.59	5.06
CV %	92.24	65.55	90.1	93.3	75.45
SE=standard error; SED=Standard errors of differences of means; CV=coefficient of variance.					

Figure 3. Carbon sources, NaCl, pH and temperature.

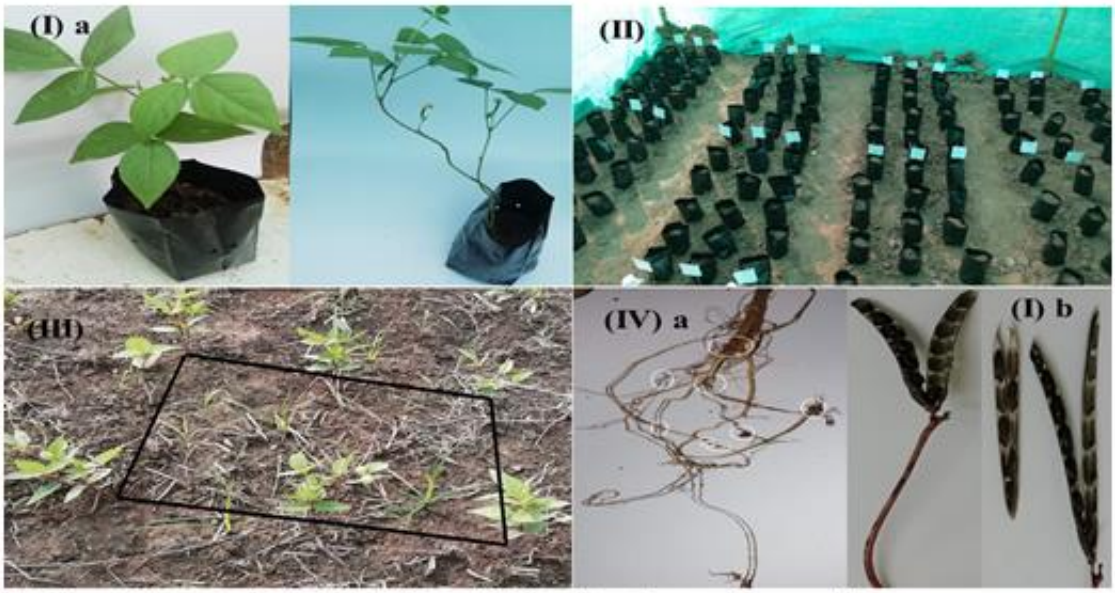


This images represents **physiological properties** (i) The pH, with different pH ranging from (5, 7, 9, and 11). (ii) The carbon sources, four different carbon sources such as (glucose, fructose, sucrose, and starch). (iii) The temperature, different temperatures of (20°C, 30°C,40°C, and 50°C). (iv) The NaCl concertation, different concentrations of NaCl (0, 2, 4, 6, 8, 10, and 12%).

Table 5. Heavy metals tolerances $C_4H_{12}O_7Pb$

s.no	$C_4H_{12}O_7Pb$ 10 mm	$C_4H_{12}O_7Pb$ 50 mm	$C_4H_{12}O_7Pb$ 100 mm	$C_4H_{12}O_7Pb$ 500 mm	$C_4H_{12}O_7Pb$ 1000 mm
TVS-2	0	0	0	0	0
KZSS-6	0	22	20	12	16
ESS-4	0	22	19	9	13
NVS-10	0	21	21	5	19
DVS-11	0	17	26	4	25
TSS-3	0	31	15	3	22
Mean	0	16.28	14.42	4.71	13.57
SE ±	0	4.53	3.92	1.68	3.97
CV %	0	67.65	66.56	87.61	68.54
SE=standard error; SED=Standard errors of differences of means; CV=coefficient of variance.					

Figure 4. Green house and field trials conditions.



(I) The pot mixture packed (pot was filling with 1 kg soil) under greenhouse condition.
(II) Plant grown in pot, culture with soil (1 kg of sterile soil was mixed with 50 ml of FOC spores.
(III) Represent to the filed trials in black gram soils, with a completely randomized design (CRD).
(IV) (A) Corresponds to the no of root nodules. (B) represent to the black gram harvesting seeds.

Effects of temperature pH, and Salinity

All the six promising isolates were able to grow in temperature up to 20°C to 40°C, expect 50°C (KZSS-6 tolerates up to 30°C), six isolates were grown in pH range between 5 to 13, expect (5, 13) out of six isolates TVS-2 up to range 9. Six isolates were grown in various NaCl concentration up to 0 to 12%, six isolates are performed (KZSS-6 up to 12%) The results are represented in the Tables 6-8.

Table 6. Heavy Metals Tolerances CH_3COONH_4

s.no	CH_3COONH_4 10 mm	CH_3COONH_4 50 mm	CH_3COONH_4 100 mm	CH_3COONH_4 500 mm	CH_3COONH_4 1000 mm
TVS-2	0	0	0	0	0
KZSS-6	14	0	0	7	0
ESS-4	0	0	0	0	0
NVS-10	2	0	0	7	0
DVS-11	9	0	0	0	0
TSS-3	5	0	0	8	0
Mean	3.28	0	0	3.142	0
SE ±	1.911	0	0	3.93	0
CV %	142.48	0	0	115.88	0
SE=standard error; SED=Standard errors of differences of means, CV=coefficient of variance.					

Table 7. Carbon sources.

Name of isolates	Fructose	Glucose	Sucrose	Starch
DVS-11	2.33	3	2	3.33
ESS-4	0.67	2	0.67	1.33
KZSS-6	0	0	1.33	2
NVS-10	0	0.667	1.67	1
TVS-2	0	3	3.33	2.33
TSS-3	0.67	0	2	2
Control	0	0	0	0
Mean	0.52	0.238	1.57	1.71
SE ±	0.54**	0.35**	0.81 [Google scholar]	0.60**
LSD (5%)	1.186	0.7764	1.765	1.316
CV %	127.3	35.3	63.1	43.2
SE=standard error; LSD=least significant difference; CV=coefficient of variance. **statistically significant at 0.01 (P values), [Google scholar]Statistically significant at 0.001.				

Table 8. Effects of salinity

Name of the isolates	0% NaCl	0.02	0.04	0.06	0.08	0.1	0.12
TVS-2	2.33	2	2.33	1	3	1	0.67
KZSS-6	2.67	3	2	1	2	2.67	2
ESS-4	2.33	2.33	1.667	1	2	1.67	1.33
NVS-10	2.67	2.33	1	1	0.667	0.67	0
DVS-11	3	3	3	3	3	2.67	0.33
TSS-3	2.67	2	1	1	1	0.67	1.33
Control	0	0	0	0	0	0	0
Mean	2.24	2.095	1.571	1.14	1.667	1.33	0.81
SE ±	0.50**	0.23**	0.27**	0	0.17**	0.42**	0.37**
LSD (5%)	1.109	0.5012	0.593	0	0.3882	0.924	0.824
CV %	27.9	13.4	21.2	0	13.1	39	57.2

Responses of the six Actinomycetes to salinity were record as follows,
0=no growth; 1=slight growth; 2=moderate growth; 3=good growth.
SE =standard error; LSD=least significant difference, CV=Coefficient of Variance.
**statistically significant at 0.01 (P values)
[Google scholar]Statistically significant at 0.001.

Greenhouse and MS medium growth factors

Four verities of seeds were growing green house and medium conditions. Plant height (cm) 7.50 to 14.30 ranges, root length (cm) 1.80 to 6.50 range, height seeds (cm) 2.80 to 5.7 range, leaf length 1.6 to 4.33 range, total plant weight (gm) 0.19 to 0.48 range and finally dry plant weight (mg) 7.60 to 14.50 range. Results are represented in the table of 9 and 10.

Table 9. Effect of pH and temperature.

Name of the Isolates	20°C	30°C	40°C	50°C	pH-5	pH-7	pH-9	pH-11	pH-13
TVS-2	3	3	2	0	0	3	2.67	2.667	0
KZSS-6	3	3	3	0	0	3	2.67	2.667	0
ESS-4	1	2.33	2	0	0	3	0.33	0.333	0
NVS-10	1	2	1	0	0	3	1.67	3	0
DVS-11	3	3	2	0	0	3	3	2.667	0
TSS-3	2	2	2	0	0	3	67	1.667	0
control	0	0	0	0	0	0	0	0	0
Mean	1.86	2.19	1.71	0	0	2.57	1.57	1.857	0
SE ±	0	0.17**	0	0	0	0	0.41**	0.27**	0
LSD 5%	0	0.3882	0	0	0	0	0.897	0.593	0
CV %	0	10	0	0	0	0	32.1	17.9	0

Responses of the six Actinomycetes to pH and temperature were record as follows, 0= no growth; 1=slight growth; 2=moderate growth; 3=good growth.
SE=standard error; LSD=least significant difference; CV=coefficient of variance.
**statistically significant at 0.01 (P values)
[Google scholar]Statistically significant at 0.001.

Table 10. Normal greenhouse condition

Name the variety seeds	Plant Height (cm)	Root length (cm)	Height of the seed (cm)	Leaf length (cm)	Total plant weight (mg)	Total dry weight for plant (mg)
Poush	11.63	3.8	3.13	2.73	0.54	11.63
PU-31	10.86	2.36	4.73	2.1	0.27	10.86
TBN-104	7.16	1.33	2.26	1.6	0.31	7.16
Q-31 soda	8.26	2.83	4.23	1.86	0.26	8.26
Mean	9.48	2.58	3.59	2.075	0.35	9.48
SE	1.82	0.88	0.95	0.41	0.11	1.82
% CV	43.02	45.95	43.75	42.26	45.77	43.02

SE=standard error; SED=Standard errors of differences of means, CV= coefficient of variance.

Evaluations filed condition

Under field condition, the KZSS-6 significantly enhanced plant height (cm plant^{-1}) leaf, shoot, and root high, leaf, shoot and root length, grains weight, 1000 grains weight, and grain yield percentage. Grain was enhanced by 9-12%, and stover yield 15 to 30%. Shoot length (mm^{-2})10 to 25%, plant height (cm plant^{-1}) 15 to 30%, and root length (cm plant^{-1}) were also enhancing, 12 to 24% respectively. The results are represented in the Tables 11-13.

Table 11. 14 days after greenhouse conditions.

Name the variety seeds	Plant Height (cm)	Root length (cm)	Height of the seed (cm)	Leaf length (cm)	Total plant weight (mg)	Total dry weight for plant (mg)
Poush	14.66	4.76	4.7	4.33	0.48	14.66
PU-31	13.63	3.73	5.7	3.73	0.24	13.63
TBN-104	12.7	6.8	4.8	3.23	0.19	12.7
Q-31 soda	13.8	3.76	5.43	2.53	0.21	13.8
Mean	13.7	4.76	5.15	3.45	0.28	13.7
SE	0.31	0.56	0.18	0.29	0.05	0.31
% CV	47.97	50.67	47.74	48.53	4476	47.97

Table 12. Harvesting period for black gram crop

S.No	Root lenght (cm plant $^{-1}$)	root weight (g m $^{-2}$)	shoot height (cm plant $^{-1}$)	shoot length (cm plant $^{-1}$)	stem height (cm plant $^{-1}$)	stem weight (g m $^{-2}$)	stem width (cm plant $^{-1}$)	leaf width (cm plant $^{-1}$)	leaf weight (g m $^{-2}$)	Internodes (no's)
POUSH	12.66	0.49	3.76	5.33	32	4.24	0.33	5.84	0.45	9
PU-31	11.66	0.31	3.4	4.93	28.66	4.25	0.3	5.76	0.35	8
Q-31	13	0.21	3.96	5.13	22.66	4.08	0.4	5.87	0.35	7.33
TBG-104	10.66	0.27	3.26	4.73	27.33	4.1	0.4	6	0.26	5.66
MEAN	12	0.32	3.6	5.03	27.66	4.17	0.35	5.87	0.35	7.5
SE ±	0.4	0.04	0.12	0.1	1.49	0.03	0.01	0.03	0.03	0.54
% CV	47.73	54	47.76	48.06	47.628	48.6	49.14	48.71	48.45	47.99

Table 13. Harvesting period for black gram crop

S.No	plant height (cm plant $^{-1}$)	hole plant wight (gm^{-2})	number of fruits (no)	number of root nodules (no)	root nodules weight (gm^{-2})	one fruit weight (gm^{-2})	10 grains weight (gm^{-2})	100 grains weight (gm^{-2})	1000 grains weight (gm^{-2})	Yield % in control (tones)	Yield % in filed (tones)
POUSH	35.33	7.62	17	20	0.21	0.57	0.52	2.52	22.34	21.77	29.52
PU-31	32.66	5.64	12.66	38.33	0.26	0.41	0.5	4.37	47.29	12.31	15.11
Q-31	26.66	6.44	14.33	29	0.17	0.37	0.45	4.47	44.41	12.43	17.49
TBG-104	33.33	6.36	17.33	40.33	0.2	0.36	0.44	4.94	49.36	13.94	17.8
MEAN	32	6.52	15.33	31.91	0.21	0.43	0.47	4.07	40.85	15.1125	19.98
SE ±	1.44	0.31	0.86	3.62	0.01	0.03	0.01	0.41	4.84	1.74	2.5
% CV	47.5971	47.64	47.64	50.13	49.04	49.53	48.35	49.33	50.52	50.27	51.01
Responses of the KZSS-6 to field trials were record, with four types of black gram seeds as follows. SE=standard error; %CV=coefficient of variance.											

DISCUSSION

Antimicrobial activity of 54 actinomycetes isolates and 3 isolates showed antibacterial activity against *E.coli*, *S. aureus, Bacillus subtilis, P. aeruginosa, Eterobacter aeruginosa, Salmonella typhi, Salmonella typhimurium* and *Vibrio c olerae Sengupta,* et al. 2015. Five *Streptomyces* which exhibited antibacterial effects against clinical isolates of methicillin-susceptible S. aureus and S. typhi Satheeja and Jebakumar 2011. 78 isolates 23 isolates showed antimicrobial activity against *B subtilis, B cereus, S aureus and E coli.*

Salinity stress, soil pH, and temperature was causes a major threat for plant growth and crop yield. *Streptomyces spororaveus RDS28* growth condition was 31°C and pH of 7.5, then medium contains carbon source like glucose. *Streptomyces thermolilacinus* and *Streptomyces werreansis* showed significant antagonistic activities against some important gram negative and positive pathogenic bacteria. *Streptomyces rimosus subsp* antibiotic production including, growth at different pH values, different temperatures, different carbon sources.

Streptomyces rochei M78 isolate were observed using Starch Casein Broth (SCB) as the best production medium, at initial pH 7.0 Starch and casein+yeast extract+peptone appeared to be the best carbon and nitrogen sources respectively and C:N ratio of 4:1 after 72 hr of incubation for optimal production of antibacterial metabolites Hayder NH, Mahmood MS 2016. Six isolates were found to have tolerance to wide range of physiological conditions including pH 11, salinity at 10% and temperature at 40°C and grown in different environmental conditions. *Streptomyces werraensis* the optimization studies for pH as 8.0, salinity (5%), starch and ammonium sulphate as suitable carbon and nitrogen sources respectively.

Streptomyces spectabilis, Streptomyces purpurascens, Streptomyces coeruleorubidus and *Strepto-myces lavendofoliae. Streptomyces spectabilis* were observed to be starch and casein as the carbon and nitrogen sources, pH 7, temperature 30ºC, *streptomyces spectabilis*, cellobiose and peptone as the carbon and nitrogen sources, on the 5 th day at pH 5 at 30ºC. *Streptomyces coeruleorubidus*, maximal production resulted on the sixth day at pH 6 and temperature of 35ºC, 2015. The results revealed nine strains of Streptomyces of them two (PS1 and PS28) isolates exhibited high activity against pathogenic bacteria. The optimum growth conditions were pH 7.5, temperature at 30ºC.

Rhizobacteria, the two bacterial growing black gram plants in greenhouse conditions to improve growth, to exhibited maximum growth potential and growth-promoting attributes were evaluated. SA8 with kinetin (10 µM) inoculated in black gram plants exhibited improved water relation, under salt stress. The artificial inoculation in wheat plant green house, to growth in both adult stage and seedling stages. Therefore, the inoculation in the field condition selecting resistance to wheat sharp eyespot.

CONCLUSION

In the present work six *Streptomyces sp* was antimicrobial activity against the microbial pathogens in eight pathogenic bacteria its shows (30 to 40%) activity. Antibiotics assay to performed, the zone of inhibition of the six *Streptomyces* sp was (10 to 30 mm). Carbon utilization to performed, the six *streptomyces sp* were utilized four carbons, high utilization was starch. These six isolates showed tolerance to a wide range of physiological conditions including pH 11, 40°C temperatures and 12% salinity in above information, and then choose the KZSS-6 (*Streptomyces koyangensis*) is more active than other isolates. In greenhouse and field conditions to understand the natural interaction with other soil native microflora, and host fungal pathogens.

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