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OPTIMIZATION ON STREPTOMYCES SPECIES ON GROWTH PROMOTION AND GRAIN YIELD IN BLACK GRAM FIELDS

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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, Plant Growth Promoting traits, Greenhouse 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. Theses *streptomyces* sp produce halo-zone range between 14 to 19 cm, for the pH grow range from 3 to 13, salinity 0% to 12%, temperature 20^{o}C to 50^{o}C, four carbon sources are used for carbon utilization such as glucose, fructose, sucrose, and starch, and heavy metals tolerances. Out of six *streptomyces* species KZSS-6 (*Streptomyces koyangensis*) was better results, and more efficient. So KZSS-6 strain was further evaluated on the greenhouse and black gram field conditions. The KZSS-6 strain was found to enhance plant root length, and yield. In greenhouse

condition the plant was grown in height 8 to 14cm, root length1 to 6cm, seeds 2 to 5 cm, total plant weight is 0.20 to 0.60mg, and total dry weight for plant 7 to 14mg. In the field experiment plant was grown in the root height and weight (10 to 13cm, 0.25 to 0.50mg), shoot length and weight (3.10 to 3.50cm), 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 the yield (11.5 to 29.50 tons).

Key words: - *Streptomyces koyangensis, antibacterial activity, optimization, greenhouse, field conditions*

1. Introduction

Agriculture one of the major contributing factors for global food supply. Present agriculture is facing several threats, like loss of soil fertility, poor soil quality, climatic changes, and in addition to large quantity pesticides. To overcome these challenges, eco-friendly approaches like biological pesticides can be utilized. To enhance the agricultural outputs sustainably, novelty and eco-friendly strategies must be employed in agriculture Gopalakrishnan *et al*. 2015; Majeed *et al*. 2018.

In the world, India is the largest producer of agricultural products. Since agriculture is a major contributing factor to the global food supply Preethi *et al*. 2020. Pulses (Black gram), are the major sources of proteins, micronutrients, and dietary fiber. 100g of black gram seeds contain 20.97 to 31.32g of proteins Yi-Shen Shuai and Fitz Gerald, 2018: Ali *et al*.2018; Rath and Das. 2021; Jegadeesan *et al*. 2021.

Streptomyces are filamentous Gram-positive bacteria, belonging to the order of *Actinomycetales* there are more than 570 groups available in nature. These *Actinomycetes* were found in soil, marine water, vermicompost, etc., Gopalakrishnan *et al*. 2014. When compared to other microorganisms, *Actinomycetes* are inhibited by a wide range of plants such as endophyte. Therefore, actinobacteria are efficient plant growth promoters, and have antagonistic activity on fungal pathogens and as bio control agent Vurukonda *et al*. 2018. Recently, *streptomyces* has been considered as a prospective bio control agent in agriculture, and also produces antibiotics and shows intense antagonistic activity through the production of various antifungal metabolites. Therefore, the *streptomyces* is more efficient against many other microorganisms present in rhizosphere of soil Schrey and Tarkka. 2008. *Streptomyces* sp., play major role in promoting of plant growth, plant protection, anti-microbial activity, and also produce secondary metabolites for commercial interest Gopalakrishnan *et al*. 2014.

Earlier, we reported a set of six *streptomyces* sp., were isolated from black gram soil and vermicompost samples. Those six strains (DVS-11, ESS-4, KZSS-6, NVS-10, TSS-2, and TVS-2) show antagonistic activity in *fusarium oxysporum* (FOC) in Black gram and show PGP traits.

These six strains were effective in control of heavy metals, sustaining of physiological properties and in production of antibiotics. Then, further green house and filed assay were conducted and results were obtained.

2. Materials and methods

2.1 Antibacterial activity by streak plate method

Antibacterial activity was performed by cross streak technique. Starch casein agar plates were prepared; isolates were streaked on the center of petri plates then incubated at 28^{O}C for 7days. Than the plates were streaked on test

organisms at angle of 90°, then the plates were incubated at 37°C for 12 hrs. The antibacterial activity was measured by inhibition of target bacteria and isolated *streptomyces* sp., (-) indicates the distance in millimeter (mm) inhibited by the isolates Oskay. 2009; Kumar *et al*. 2012; Sangkanu *et al*. 2017. The human bacterial pathogens that were use for the test *Bacillus subtiles* (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)* these bacterial cultures were procured from IMTECH, Chandigarh.

2.2 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 Gopalakrishnan *et al*. 2012: Gopalakrishnan *et al* 2014. After solidification, 3 days old *streptomyces* sp., culture was swabbed and was 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.

2.3 Metal's tolerances

The selected *streptomyces* sp., show tolerance against stress, by the addition of heavy metals to its growth environment Gelmi *et al*.1994; Sabry *et al*. 1997. PBS buffer is prepared and adjust the pH to 6.8.These are heavy metals are using the $Cuso_4$ (copper (II) sulfate, $Znso_4$ (Zink sulfate), $(ch_3\ coo)\ 2Pb.3h_2O$ (Lead acetate tri hydrate), $CH_3\ COONH_4$ (ammonium ethanoate), $K_2\ so_4$ (Potassium sulfate), and CH_3 COONA (sodium acetate). Heavy metals salts are prepared in different concentration in 10mm, 50mm, 100mm, 500mm, and 1000mm and were dissolved in PBS buffer. To prepare the starch casein agar plates, the centre of plate should have well with 12mm. On each plate six streptomyces sp., are streaked, and then incubated at 28 ± 2°C for 7 days and then length of growth was measured in cm.

To calculate the % of growth

$$\frac{\text{Complete inoculated length of streak – inhibited length}}{\text{Complete inoculated length}} \times 100$$

2.4 Effects of carbon source

The capacity of carbon sources utilized by isolated *streptomyces* was tested against four different carbon sources such as glucose, fructose, sucrose, and starch. All the four carbon sources are separately sterilized by membrane filtration and 1% carbon source was added to basalmineral salt agar media. The selected *streptomyces sp.,* were streaked on basal mineral salt agar media and incubated at 28 ± 2°C for five days Ordóñez-Robles *et al*. 2017. The plates were observed for the growth of *streptomyces* sp., and noted on the scale of 0-3 as follows; 0=no growth, 1= slight growth, 2=moderate growth, and 3= good growth.

2.5 Effect of Salinity

The *streptomyces* sp., were streaked on bennett`s agar (Himedia Laboratories, Mumbai, India), and amended with different concentrations of NaCl (0-12%, at 2% intervals) and incubation, at 28 ± 2°C for five days Gopalakrishnan *et al*. 2012. After incubation the plates were observed for growth of *streptomyces* sp. Based upon the growth the scale was given from 0-3 as follows; 0=no growth, 1= slight growth, 2=moderate growth, and 3= good growth.

2.6 Effect of pH

The selected *streptomyces* sp., were streaked on bennett`s agar (Himedia Laboratories, Mumbai, India), and adjusted with pH ranging from 5, 7, 9, and 11, and then incubated at 28 ± 2°C for five days Gopalakrishnan *et al*. 2012. After incubation, the plates were observed for the growth of *streptomyces* sp. Based upon growth the scale was given from 0-3 as follow; 0=no growth, 1= slight growth, 2=moderate growth, and 3= good growth.

2.7 Effect of temperature

The selected *streptomyces* sp., were streaked on bennett's agar (Himedia Laboratories, Mumbai, India) and then incubated at different temperatures of 20°C, 30°C,40°C, and 50°C for five days Gopalakrishnan *et al*. 2012. After incubation, the plates were observed for the growth of *streptomyces* sp.. Based upon growth the scale was given from 0-3 as follow; 0=no growth, 1= slight growth, 2=moderate growth, and 3= good growth.

2.8 Evaluation of *streptomyces* sp., PGP activity under greenhouse condition

Streptomyces sp., were found possess in greenhouse condition by using mass cultivating pot experiments. And then same six *streptomyces* sp., were assayed with sterile and non-sterile soils in the presences of FOC.

2.9 Mass cultivating pot experiments on *streptomyces* sp

Streptomyces sp were inoculated into starch casein agar medium and then incubated at 28 ± 2ºC for 3 to 5 days Mao *et al*. 2007; Thilagam and Hemalatha. 2019. 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 with additionally 10% maltose, 4% glucose**,** and 4% yeast extract, and then adjusted with pH 7.2. The media was prepared and inoculated with *streptomyces* sp. then 100 ml medium was transferred to 250ml flask; the flasks were incubated at 28 ± 2º C in rotary shaker with 170 rev for min. To prepare ISP-2 broth, add 4% yeast malt extract, transfer to the 48hr Log phage *streptomyces* sp. The inoculated flask was placed on rotary shaker incubator for 4-5 days. On the fifth day, 001 ml of FOC spores stock prepared as earlier was added to the culture medium.

2.10 Pot filling study

Each pot was filled with 1 kg soil (sterile and non-sterile soils are use in this study). Pot filling with sterile soil is done by aseptically, and inoculated with fungal spores into each of this 1 kg of sterile soil was mixed with 50 ml of FOC (concentration 10^{-6} spores per 1 gram of soil) spores' stock.

2.10 (a) Seed sowing

Black gram seeds were procured from commercial markets in GUNTUR. The seeds were selected base on the their appearance, surface sterilization was done by (with aqueous sodium hypochlorite NaOCl, 05% v/v, 2 min) and washed with sterile distilled water for 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.

2.10 (b) Growing of seedlings and plant growth analysis

In green house temperature was maintained 27^{o}C and humidity 55%. The treated pot was arranged in Completely Randomized Design (CRD) five replicates. The pots were maintaining 65% moisture level, and watered uniformly every day. Treatment posts were label, and maintained with certain distance. Antagonistic treatments allowed growing until 15 to 30 days.

2.10 (c) 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, estimation the 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.

$$Percentage\ of\ seed\ germination\ \% = \frac{\text{Number of greminated seeds}}{\text{Total number of seed sown}} \times 100$$

2.11 Evaluation of *streptomyces* for PGP potential on black gram under field conditions.

Field experiment was conducted were 2018–2019 (Rabi and Kharif season) 16.3763° N, 80.5277° E Nagarjuna University Andhra Pradesh, India. Experiment was conducted with 4 types of varieties, 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 t·ha^{-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 Preethi *et al*. 2021. The 10–14-day 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 with 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, hole 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, and 1000 seeds weight Gopalakrishnan *et al*. 2012.

$$\text{Percentage Yeild (\%) in tonnes} = \frac{\text{Square foot} \times \text{number of fruits} \times \text{seeds weight}}{100 \times \text{one hectare land}}$$

2.12 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).

3. Results

3.1 Anti-bacterial activity for bacterial pathogens

Six *streptomyces* species were screened against human bacterial pathogens. The percentage of activity was 44%, and then six isolates showed good antimicrobial activity against pathogens. The percentage of activity to each pathogen is listed below: Bacillus subtilies (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%) (Image 01; Table 01)

3.2 Effect on antibiotics

The six *streptomyces* sp were able to resistances to all 11 antibiotics. The zone of inhibition range is 14mm-20mm. 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 (image 2 and Table 02).

3.3 Heavy Metals Tolerances

Heavy metals tolerances $Cuso_4$, $Znso_4$, $C4H_{12}O_7Pb$, and CH_3COONH_4 are shown KZSS-6, ESS-4, NVS-10, DVS-11 and TSS-3 tolerance levels. $Cuso_4$ metal tolerances rang from 4 -32 out of six *Streptomyces* sp., KZSS-6 are found to more activity, $Znso_4$ metal tolerances range is from 11-32, ESS-4 was found to be more activity then other isolates, $C4H_{12}O_7Pb$ metal tolerances range of five isolates is from 3-26, more activity is found in TSS-3 then the other isolates, CH_3COONH_4 metal tolerances range is from 2-14, more activity is found in KZSS-6. Out of six isolates only in TSS-3 shows none of the four metal tolerances ($Cuso_4$, $Znso_4$, $C4H_{12}O_7Pb$, and CH_3COONH_4) (Table 03 (i, ii, iii and iv).

3.4 Carbon sources

Six *streptomyces* sp., were characterized for their carbon utilization traits, like fructose (excepted ESS-4, NVS-10, and DVS-11) and glucose (except ESS-4 and TSS-3) as a monosaccharide sucrose as disaccharides and starch as a polysaccharide, of the six *streptomyces* sp., ESS-4 utilized higher amount of fructose, glucose and starch and DVS-11utilized maximum amount of sucrose table 04.

3.5 Effects of Temperature pH, and Salinity:

Six *streptomyces* were able to grow in temperature range from 20^{o}C to 40^{o}C, expected 50^{o}C (KZSS-6 tolerates up to 30^{o}C), six *streptomyces* sp., were grown in pH range between 5 to 13, no growth was observed 5, and 13. Out of six

isolates TVS-2 shows at the pH range 9. Six *streptomyces* sp., were grown in various NaCl concentrations up to 0 to 12%, KZSS-6 shows maximum growth of up to 12%, reaming five *streptomyces* are grown from 0 to 10% table 5, 6, and 7.

3.6 Greenhouse and M. S Medium Growth Factors

Four varieties of seeds were grown in green house and M. S medium conditions. Plant height ranges from 7.50 to 14.30 cm, root length range from 1.80 to 6.50 cm, height seeds range from 2.80 to 5.7 cm, range from leaf length 1.6 to 4.33 cm, total plant weight range from 0.19 to 0.48 gm and finally dry plant weight (mg) range from 7.60 to 14.50 image-4 and table of 8 (i) and (ii).

3.7 Evaluations field condition

Under field conditions, the KZSS-6 significantly enhanced height of the plant, leaf, shoot, and root, length plant, leaf, shoot, root, grain weight, and grain yield percentage. Grain yield was enhanced up to 9-12%, and stover yield 15 to 30%. Shoot length (mm^{-2})10 to 25%, plant height (plant cm^{-1}) 15 to 30%, and root length (plant cm^{-1}) were also enhancing 12 to 24% respectively table of 9 (i) and (ii).

4. Discussion

Sengupta *et al*. 2015 reported antimicrobial activity of 54 *streptomyces sp* were isolates from soil, 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*. Satheeja and Jebakumar 2011 investigated five *streptomyces* which exhibited antibacterial effects against clinical isolates of methicillin-susceptible S. aureus and S. typhi. wadetwar and Patil. 2013 studied 78 isolates were isolated from soil, and then 23 isolates showed antimicrobial activity against *B. subtilis, B. cereus, S. aureus and E. coli. Few isolates showed activity against P. vulgaris*. Nandini J *et al*. 2012 reported that *Streptomyces thermolilacinus* and *Streptomyces werreansis* showed significant antagonistic activities against some important gram negative and positive pathogenic bacteria.

Salinity stress, soil pH, and temperature were causes for major threat plant growth and crop yield. Askar A *et al*. 2011 reported that *Streptomyces spororaveus RDS28* growth condition was at 31^{o}C and pH at 7.5, and the medium contains carbon source like glucose. Singh and Rai. 2012 reported that *Streptomyces rimosus* sp produce antibiotics, growth at different pH levels 7.0 to 7.4, different temperatures range from 20-30^{0}C, various carbon sources like glucose and fructose. Hayder NH, Mahmood MS 2016 studied *Streptomyces rochei M78* isolates were observed using starch casein broth (SCB) as the best production medium, at initial pH 7.0. Hayder NH, Mahmood MS 2016 studied that starch and casein +yeast extract + peptone appeared to be the best Carbon (C) and Nitrogen (N) sources respectively. The C:N ratio 4:1 after 72 h of incubation for optimal production of antibacterial metabolites.

Alekhya and Gopalakrishnan. 2017 to reported six isolates were found to have tolerance to wide range of physiological conditions including pH 11, salinity at 10% and temperature at 40^{o}C and growth in different environmental conditions. Bhosale *et al*. 2015 to studies in *streptomyces* werraensis the optimization studies for pH as 8.0, salinity (5%), starch and ammonium sulphate as suitable carbon and nitrogen sources respectively.

Bundale *et al*. 2015 investigated on the *streptomyces spectabilis*, *Streptomyces purpurascens*, *Streptomyces coeruleorubidus* and *Strepto-myces lavendofoliae*. *Streptomyces spectabilis* were observed to have starch and casein as the carbon and nitrogen sources, at pH 7, and temperature at 30˚C. *Streptomyces spectabilis*, utilizes cellobiose and peptone as the carbon and nitrogen sources, on the 5th day at pH 5 and temperature at 30^{o}C. *Streptomyces coeruleorubidus*, maximal production resulted on the sixth day at pH 6 and temperature 35^{o}C. Khattab *et al*. 2016 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 30^{o}C.

Yasin *et al*. 2018 reported that in rhizobacteria and two bacterial growing black gram plants in greenhouse conditions were found to the improve growth, exhibit maximum growth potential and growth-promoting attributes were evaluated. *Pseudomonas fluorescens* SA8 with kinetin (10µM) inoculated in black gram plants exhibited improved water relation, under salt stress. Liu *et al*. 2020 reported the artificial inoculation in wheat plant in green house, exhibit growth in both adult stage and seedling stages. Therefore, the inoculation in the field condition shows selective resistance to wheat sharp eyespot.

5. Conclusion

Comparing the above mentioned results with this study, we can conclude six *streptomyces* sp were found to have antibacterial activities eight human pathogenic bacteria, it shows (30 to 40%) activity. Antibiotic resistances assay was performed to 11 know antibiotics; the zone of inhibition six *streptomyces* sp was from (10 to 30 mm). Carbon utilization was tested; the six streptomyces sp utilized four carbons, and high utilization of starch was found. These six *streptomyces* sp showed tolerance to a wide range of physiological conditions including pH 11, temperatures at 40 ^{o}C and salinity at 12% in the above information, and the choose KZSS-6 (*Streptomyces koyangensis*) is more active than other isolates. Our findings confirm the streptomyces species have grown the Vigna mungo, the bacteria are exhibited multiple antagonistic plant pathogens, plant growth-promoting activities, salinity tolerances, seedling establishment, root, shoot and yield growth, enhancing the soil growth and productivity. In greenhouse and field conditions to understand the natural interaction with other soil native microflora and host fungal pathogens.

Table 01 Antibacterial activity in streak plate technique (mm)

S.No	Isolate cultural	*Bacillus subtitles*	*Micrococcus luteus*	*Staphylococcus aureus*	*Streptococcus mutans*	*Klebsiella pneumonia*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiae*
1	ESS-4	24	24	21	05	5	12	16	16
2	DVS-11	16	17	13	16	18	18	09	21
3	KZSS-6	27	16	24	22	16	23	2	21
4	NVS-10	08	15	08	02	17	12	13	12
5	TVS-2	17	16	17	12	13	12	12	14
6	TSS-3	23	0	20	06	23	24	13	15

(-) indicates the distance in millimeter (mm) inhibited by the isolates.

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%).

Table 02 Antibiotic assays for most promising isolates in zone of mm

The results were noted as the zone of inhibition in mm.

Am= Amoxyclav, AMP= Ampicillin, CF= Cefpodoxime, CP= Chloraphnicol, CIP= Ciproflaxacin, NE= Neomycin, NB= Novobiocin, P= Penicillin, RF= Rifampicin, TR=

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	0.0	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	1.60	1.49	1.49	1.31	1.58
SED	1.66	0.70	1.03	0.82	0.77	1.22	1.13	1.60	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

Tetracycline, VC= Vencomycin

SE =standard error; SED= Standard errors of differences of means LSD = (5% Level) least significant difference; CV = Coefficient of variance.

Table no – 03 Heavy metals tolerances (in mm)

3. i Cuso4 (Copper sulphate)

Length of growth was measured in cm. SE =standard error; SED= Standard errors of differences of means, CV = coefficient of variance.

S. no	$Cuso_4$ 10mm	$Cuso_4$ 50mm	$Cuso_4$ 100mm	$Cuso_4$ 500mm	$Cuso_4$1000mm
TVS-2	00	00	00	00	00
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	00	00	00	00	00
Mean	14.57	12.00	13.42	14.90	17.57
SE ±	4.59	3.976	3.75	5.63	4.82
CV %	83.47	87.66	73.95	83.27	72.2

3. ii $Znso_4$ (Zinc sulfate)

S. no	$Znso_4$ 10mm	$Znso_4$ 50mm	$Znso_4$ 100mm	$Znso_4$ 500mm	$Znso_4$ 1000mm
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	16.00	7.85	9.42	16.42
SE ±	3.92	4.28	2.89	3.59	5.06
CV %	92.24	65.55	90.10	93.30	75.45

Length of growth was measured in cm. SE =standard error; SED= Standard errors of differences of means, CV = coefficient of variance.

3. iii $C_4H_{12}O_7Pb$ (Lead acetate trihydrate)

S. no	$C_4H_{12}O_7Pb$ 10mm	$C_4H_{12}O_7Pb$ 50 mm	$C_4H_{12}O_7Pb$ 100 mm	$C_4H_{12}O_7Pb$ 500 mm	$C_4H_{12}O_7Pb$ 1000mm
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	0.0	16.28	14.42	4.71	13.57
SE ±	0.0	4.53	3.92	1.68	3.97
CV %	0.0	67.65	66.56	87.61	68.54

Length of growth was measured in cm. SE =standard error; SED= Standard errors of differences of means, CV = Coefficient of variance.

3. iv CH3COONH4 (Ammonium acetate)

S. no	CH_3COONH_4 10MM	CH_3COONH_4 50MM	CH_3COONH_4 100MM	CH_3COONH_4 500MM	CH_3COONH_4 1000MM
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	00	00	3.142	00
SE ±	1.911	00	00	3.93	00
CV %	142.48	00	00	115.88	00

Length of growth was measured in cm. SE =standard error; SED= Standard errors of differences of means, CV = coefficient of variance.

Table 04 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***	0.60**
LSD (5%)	1.186	0.7764	1.765	1.316
CV %	127.3	35.3	63.1	43.2

Responses of the six streptomyces to carbon sources 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)

***Statistically significant at 0.001.

Table no- 05 Effects of salinity

Responses of the six streptomyces to salinity were record as follows, 0= no growth;

1 = slight growth; 2 = moderate growth; 3 = good growth.

Name of the isolates	0% Nacl	2 %	4 %	6%	8 %	10%	12 %
TVS-2	2.33	2	2.33	1.00	3.00	1.0	0.67
KZSS-6	2.67	3	2.00	1.00	2.00	2.67	2.0
ESS-4	2.33	2.33	1.667	1.00	2.00	1.67	1.33
NVS-10	2.67	2.33	1.00	1.00	0.667	0.67	00
DVS-11	3.0	3	3.00	3.00	3.00	2.67	0.33
TSS-3	2.67	2	1.00	1.00	1.00	0.67	1.33
Control	0.0	0.0	0.00	0.00	0.00	00	00
Mean	2.24	2.095	1.571	1.14	1.667	1.33	0.81
SE ±	0.50**	0.23**	0.27**	0.00	0.17**	0.42**	0.37**
LSD (5%)	1.109	0.5012	0.5930	0.00	0.3882	0.924	0.824
CV %	27.9	13.4	21.2	0.00	13.1	39.0	57.2

SE =standard error;

= least significant difference, CV = coefficient of variance.

**statistically significant at 0.01 (P values)

***Statistically significant at 0.001.

Table no-06 effect of pH and Temperature

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.

Name of the Isolates	20 ºC	30 ºC	40 ºC	50 ºC	pH-5	pH-7	pH-9	pH-11	pH-13
TVS-2	3	3	2	0	0	3	2	2	0
KZSS-6	3	3	3	0	0	3	2	2	0
ESS-4	1	2	2	0	0	3	0	0	0
NVS-10	1	2	1	0	0	3	1	3	0
DVS-11	3	3	2	0	0	3	3	2	0
TSS-3	2	2	2	0	0	3	3	1	0
control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
Mean	1.86	2.19	1.71	0	0	2.5	1.57	1	0
SE ±	0	0.17	0	0	0	0	0.41	0.27	0
LSD 5%	0	0.3882	0	0	0	0	0.89	0.59	0
CV %	0	10	0	0	0	0	32.1	17.9	0

SE =standard error; LSD = least significant difference; CV = coefficient of variance.

**statistically significant at 0.01 (P values)

***Statistically significant at 0.001.

Table no-07 (i) Evaluation of *streptomyces* sp., PGP activity under greenhouse condition

Name the variety seeds	Plant Hight (cm)	Root length (cm)	Hight of the seed (cm)	Leaf length (cm)	Total plant weight (mg)	Total dry weight for plant (mg)
Poush	14.66	4.76	4.70	4.33	0.48	14.66
PU-31	13.63	3.73	5.7	3.73	0.24	13.63
TBN-104	12.70	6.80	4.80	3.23	0.19	12.70
Q-31 soda	13.80	3.76	5.43	2.53	0.21	13.80
Mean	13.70	4.76	5.15	3.45	0.28	13.70
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

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

Table no 7 (ii) 14 days after greenhouse conditions

Name the variety seeds	Plant Hight (cm)	Root length (cm)	Hight of the seed (cm)	Leaf length (cm)	Total plant weight (mg)	Total dry weight for plant (mg)
Poush	11.63	3.80	3.13	2.73	0.54	11.63
PU-31	10.86	2.36	4.73	2.10	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

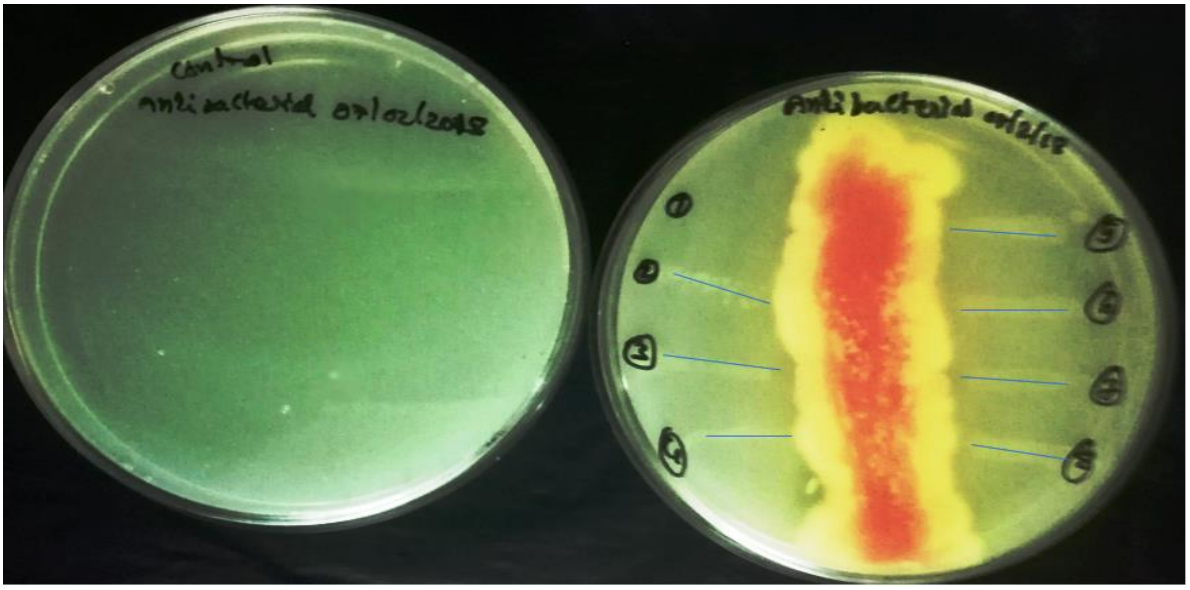
Table 08 Harvesting period for black gram crop (Root, shoot, stem and leaf)

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

plant height (cm plant $^{-1}$)	Whole plant wight (g m $^{-2}$)	Number of fruits (no)	Number of root nodules (no)	Root nodules weight (g m $^{-2}$)	One fruit weight (g m $^{-2}$)	10 Grains weight (g m $^{-2}$)	100 Grains weight (g m $^{-2}$)	1000 Grains weight (g m $^{-2}$)	Yield % in control (tones)	Yield % in filed (tones)
35.33	7.62	17	20	0.21	0.57	0.52	2.52	22.34	21.77	29.52
32.66	5.64	12.66	38.33	0.26	0.41	0.5	4.37	47.29	12.31	15.11
26.66	6.44	14.33	29	0.17	0.37	0.45	4.47	44.41	12.43	17.49
33.33	6.36	17.33	40.33	0.2	0.36	0.44	4.94	49.36	13.94	17.80
32	6.52	15.33	31.91	0.21	0.43	0.47	4.07	40.85	15.1125	19.98
1.44	0.31	0.86	3.62	0.01	0.03	0.01	0.41	4.84	1.74	2.5
47.5971	47.64	47.64	50.13	49.04	49.53	48.35	49.33	50.52	50.27	51.01

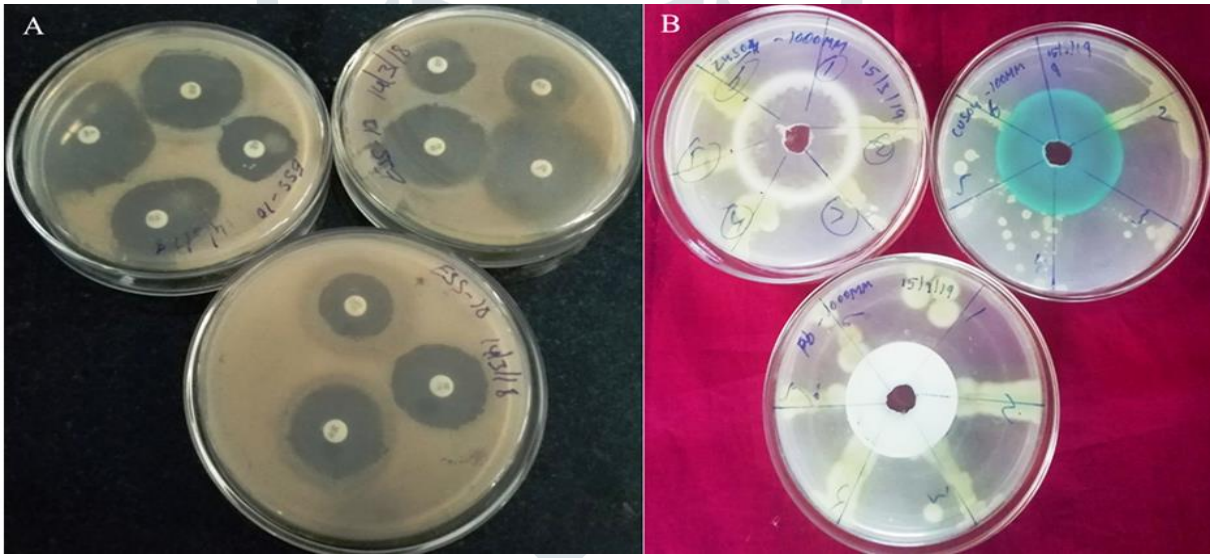
Responses of the KZSS-6 to field trials were record, with four types of black gram seeds as follows. SE =standard error; %CV = coefficient of variance.

Figure 01 Antibacteria activity for bacterail straians



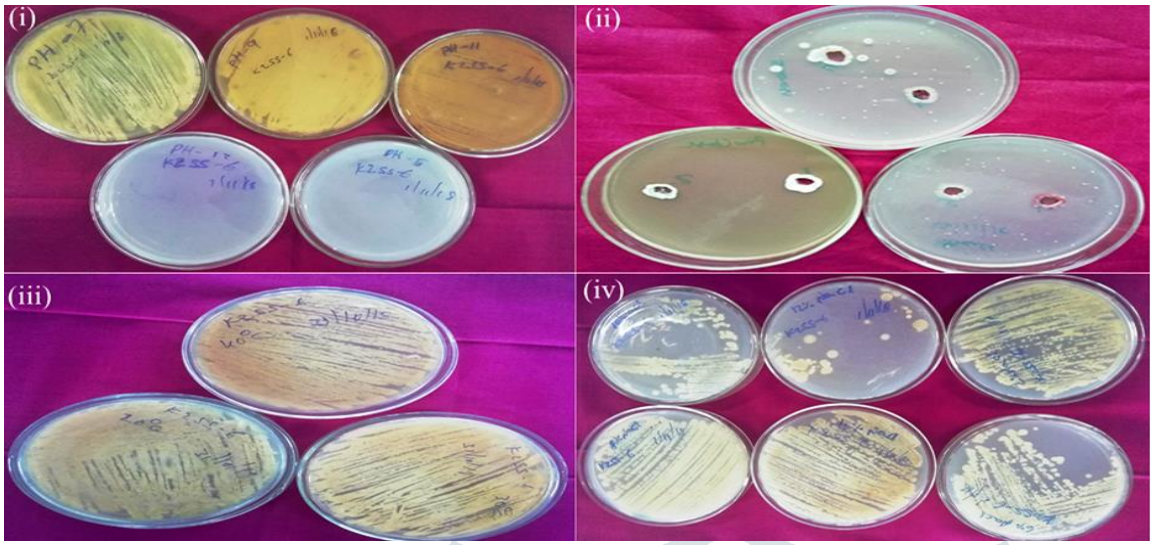
This figure represent in the antibacterial activity on *Micrococcus luteus, Bacillus subtiles, Staphylococcus aureus, Streptococccus mutans,* Gram Negative bacteria *Klebsiells pneumonia, Proteus vulagris, Pseudomonas aeruginosa, Saccharamyces cerevisiae.*

Figure02 Antibiotic activity and metals tolerance



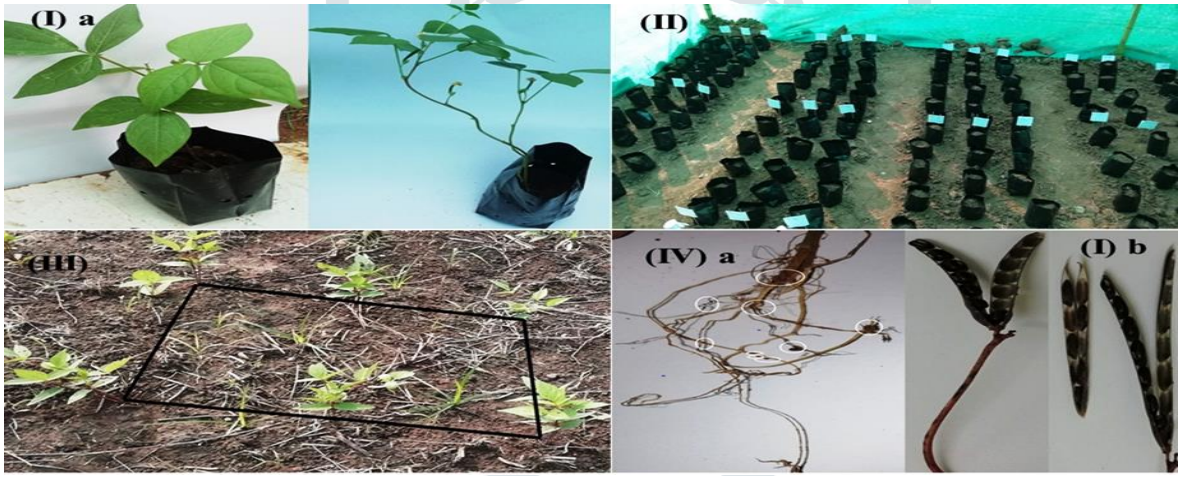
This figure A represent in the antibiotic activity, by using 13 types of antibiotics. Figure B corresponds to the protection of the metal by using four types of metals.

Figure 03 Carbon sources, Nacl, pH and temperature



This figure represents physiological properties of six streptomyces sp., (i) The growth plates in pH ranging from (5,7,9, and 11) (ii) The carbon sources utilization of six streptomyces sp,(glucose, fructose, sucrose, and starch) (iii) The growing the various temperature, (20ºC, 30ºC, 40ºC, and 50ºC) (iv) The six streptomyces sp are growth in Nacl concentration, (0, 2, 4, 6, 8, 10, and 12%).

Image 04 Green-house and Field conditions



These figure represent (I) a the pot mixture packed (pot with 1 kg soil) under greenhouse conditions. (II) Plant growth in the pot, culture with soil (1kg of sterile soil was mixed with 50 ml of FOC spores. (III) Represent the field trials in black gram soils, with a completely randomized design (CRD). (IV)a corresponds to the no of root nodules, and (b) represents the black gram harvesting seeds. Figure 04 Green house and field trials conditions

References

1. Ali, R., Saeed, S. M. G., Ali, S. A., Sayed, S. A., Ahmed, R., & Mobin, L. (2018). Effect of black gram flour as egg replacer on microstructure of biscuit dough and its impact on edible qualities. *Journal of Food Measurement and Characterization*, *12*(3), 1641-1647.
2. Al-Askar, A. A., & Khair, A. (2011). In vitro antifungal activity of Streptomyces spororaveus RDS 28 against some phytopathogenic fungi. *African Journal of Agricultural Research*, *6*(12), 2835-2842.
3. Bhosale, H. J., Kadam, T. A., Fulwad, S. G., Karale, M. A., & Kanse, O. S. (2015). Optimization of antifungal compound production by a moderately halophilic streptomyces werraensis hb-11. Int J Pharm Sci Res, 6(3), 1190-9.
4. Bundale, S., Begde, D., Nashikkar, N., Kadam, T., & Upadhyay, A. (2015). Optimization of culture conditions for production of bioactive metabolites by Streptomyces spp. isolated from soil. *Advances in Microbiology*, *5*(06), 441.
5. Gelmi, M., Apostoli, P., Cabibbo, E., Porru, S., Alessio, L., & Turano, A. (1994). Resistance to cadmium salts and metal absorption by different microbial species. *Current Microbiology*, *29*(6), 335-341.
6. Gopalakrishnan, S., Humayun, P., Vadlamudi, S., Vijayabharathi, R., Bhimineni, R. K., & Rupela, O. (2012). Plant growth-promoting traits of Streptomyces with biocontrol potential isolated from herbal vermicompost. *Biocontrol Science and Technology*, *22*(10), 1199-1210.
7. Gopalakrishnan, S., Vadlamudi, S., Bandikinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., ... & Varshney, R. K. (2014). Evaluation of Streptomyces strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. *Microbiological Research*, *169*(1), 40-48.
8. Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L., & Krishnamurthy, L. (2015). Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech*, *5*(4), 355-377.
9. Hayder, N. H., & Mahmood, M. S. (2016). Production, Purification and characterization of bioactive compounds from locally Streptomycesrochei M78. *Iraqi Journal of Science*, *57*(2B), 1165-1183.
10. Jegadeesan, S., Raizada, A., Dhanasekar, P., & Suprasanna, P. (2021). Draft genome sequence of the pulse crop blackgram [Vigna mungo (L.) Hepper] reveals potential R-genes. *Scientific reports*, *11*(1), 1-10.
11. Khattab, A. I., Babiker, E. H., & Saeed, H. A. (2016). Streptomyces: isolation, optimization of culture conditions and extraction of secondary metabolites. *International Current Pharmaceutical Journal*, *5*(3), 27-32.
12. Kloepper, J. W. (1994). Plant growth-promoting rhizobacteria (other systems). *Azospirillum/plant associations*, *187*, 137-166.
13. Kumar, P. S., Raj, J. P. P., Duraipandiyan, V., & Ignacimuthu, S. (2012). Antibacterial activity of some actinomycetes from Tamil Nadu, India. *Asian Pacific journal of tropical biomedicine*, *2*(12), 936-943.
14. Liu, J., Anderson, N. P., & Mundt, C. C. (2020). Methods for Screening Wheat Genotypes for Resistance to Sharp Eyespot in the Field and Greenhouse. *Plant Disease*, *104*(12), 3192-3196.
15. Majeed, A., Muhammad, Z., & Ahmad, H. (2018). Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. *Plant cell reports*, *37*(12), 1599-1609.
16. Jodhawat, N., Gehlot, P., Songara, D., & Kaur, S. (2012). MOLECULAR CHARACTERIZATION OF SECONDARY METABOLITE PRODUCING STREPTOMYCES SPECIES. *Journal of Drug Delivery and Therapeutics*, *2*(5).
17. Oskay, M. (2009). Antifungal and antibacterial compounds from Streptomyces strains. *African Journal of Biotechnology*, *8*(13).
18. Preethi, R., Deotale, S. M., Moses, J. A., & Anandharamakrishnan, C. (2020). Conductive hydro drying of beetroot (Beta vulgaris L) pulp: Insights for natural food colorant applications. *Journal of Food Process Engineering*, *43*(12), e13557.
19. Preethi, R., Moses, J. A., & Anandharamakrishnan, C. (2021). Effect of conductive hydro-drying on physiochemical and functional properties of two pulse protein extracts: Green gram (Vigna radiata) and black gram (Vigna mungo). *Food Chemistry*, *343*, 128551.
20. Rath, A., & Das, A. B. (2021). Chromium stress induced oxidative burst in Vigna mungo (L.) Hepper: physio-molecular and antioxidative enzymes regulation in cellular homeostasis. *Physiology and Molecular Biology of Plants*, *27*(2), 265-279.

21. Sengupta, Sohan, Arnab Pramanik, Abhrajyoti Ghosh, and Maitree Bhattacharyya. "Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem." *BMC microbiology* 15, no. 1 (2015): 1-16.
22. Sabry, S. A., Ghozlan, H. A., & Abou-Zeid, D. M. (1997). Metal tolerance and antibiotic resistance patterns of a bacterial population isolated from sea water. *Journal of applied Microbiology*, *82*(2), 245-252.
23. Sangkanu, S., Rukachaisirikul, V., Suriyachadkun, C., & Phongpaichit, S. (2017). Evaluation of antibacterial potential of mangrove sediment-derived actinomycetes. *Microbial pathogenesis*, *112*, 303-312.
24. Schrey, S. D., & Tarkka, M. T. (2008). Friends and foes: streptomycetes as modulators of plant disease and symbiosis. *Antonie Van Leeuwenhoek*, *94*(1), 11-19.
25. Shimizu, M., Yazawa, S., & Ushijima, Y. (2009). A promising strain of endophytic Streptomyces sp. for biological control of cucumber anthracnose. *Journal of General Plant Pathology*, *75*(1), 27-36.
26. Singh, N. E. H. A., & Rai, V. I. B. H. U. T. I. (2012). Optimization of cultural parameters for antifungal and antibacterial metabolite from microbial isolate; Streptomyces rimosus MTCC 10792 from soil of Chhattisgarh. *Int J Pharm Pharm Sci*, *4*(4), 94-101.
27. Thilagam, R., & Hemalatha, N. (2019). Plant growth promotion and chilli anthracnose disease suppression ability of rhizosphere soil actinobacteria. *Journal of applied microbiology*, *126*(6), 1835-1849.
28. Varma, A., Verma, S., Sudha, Sahay, N., Bütehorn, B., & Franken, P. (1999). Piriformospora indica, a cultivable plant-growth-promoting root endophyte. *Applied and environmental Microbiology*, *65*(6), 2741-2744.
29. Vurukonda, S. S. K. P., Giovanardi, D., & Stefani, E. (2018). Plant growth promoting and biocontrol activity of Streptomyces spp. as endophytes. *International journal of molecular sciences*, *19*(4), 952.
30. Wadetwar, R. N., & Patil, A. T. (2013). Isolation and characterization of bioactive actinomycetes from soil in and around Nagpur. *International journal of pharmaceutical sciences and research*, *4*(4), 1428.
31. Yasin, N. A., Khan, W. U., Ahmad, S. R., Ali, A., Ahmad, A., & Akram, W. (2018). Imperative roles of halotolerant plant growth-promoting rhizobacteria and kinetin in improving salt tolerance and growth of black gram (Phaseolus mungo). *Environmental Science and Pollution Research*, *25*(5), 4491-4505.
32. Yi-Shen, Z., Shuai, S., & FitzGerald, R. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food & nutrition research*, *62*.