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RESEARCH ARTICLE

BIO-EFFICACY OF ENDOPHYTIC ACTINOMYCETES FOR PLANT GROWTH PROMOTION AND MANAGEMENT OF FUNGAL WILT IN TOMATO (*SOLANUM LYCOPERSICUM*).

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Abstract

The objective of this study was the isolation and screening of endophytic actinomycetes isolates for their antagonistic potential and plant growth promoting activities. A total of 60 isolates were recovered from root, stem and leaf tissue of 30 tomato plants. Studies on plant growth promoting activities revealed that 44 showed abilities to produce indole acetic acid, 18 produced siderophores, 34 ammonia and 16 were observed to solubilize phosphate. In addition, production of hydrolytic enzymes such as chitinase, amylase and protease was demonstrated by these isolates. The efficacy of all endophytic actinomycetes was tested against *Rhizoctonia solani* pathogen in tomato, among 60 isolates obtained; seven endophytic actinomycetes were antagonistic to *Rhizoctonia solani in vitro*. Among them, potential isolate actinomycete was promising *in vivo*.

Introduction:-

Tomato (*Solanum lycopersicum)* is the most popular and agriculturally important commercial vegetable crop grown throughout the world. It is rich in vitamins A, B, C and has high lycopene content. Being versatile for culinary purposes, it is also one of the most commonly grown vegetable in the kitchen garden [1]. In India, it occupies an area of 801 thousand ha with a production of 22337 million tonnes [2]. Several diseases and disorders can influence tomato during the growing period. Loss of crop yield is a major concern in agriculture worldwide due to phytopathogens, especially fungi. Among the various diseases affecting the crop, *Rhizoctonia solani* is one of the most calamitous, soil-borne pathogen causes substantial loss to tomato crop [3].This pathogen can survive in soil within diseased plant material as mycelia or sclerotia during unfavorable environmental conditions for several years [4] causing root and crown rot. The chemical fungicides are used to control diseased crops. However, continuous use of fungicides has resulted in increased fungicide resistance, environmental pollution, detrimental effects on beneficial non-target organisms as well as severe effects on human life [5]. In recent years, natural or biological control has emphasised by providing an environment friendly, long lasting, inexpensive, safe alternate for the control of plant diseases [6]. The microorganisms are attractive source of natural compounds for pharmaceutical and agricultural industries. Therefore, the search has accelerated to screen microorganisms to find bioactive molecules (secondary metabolites and enzymes) antagonistic to plant pathogens [7]. Endophytes are microorganisms, proficiently and firmly colonize different plant tissues, from roots to all aerial parts, have been recognized, and has pivotal importance in agriculture [8] therefore, use of endophytic actinomycetes as biocontrol agent of soil borne diseases is of interest through their ability to colonize healthy plant tissues and produce antibiotics *in situ* [9]. Sreeja and Gopal [1] observed that *Streptomyces thermodiastaticus* an endophytic actinomycete isolated from tomato

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plants showed (44.63%) antimicrobial activity against *Ralstonia solanacearum* causes wilt in tomato. They are also known for their ability to promote plant growth by producing plant growth hormone such as indole acetic acid also help in nitrogen fixation, phosphate solubilisation and siderophore production [10]. In this context, the present study was taken up to know more about the bioefficacy of endophytic actinomycetes on plant growth promotion and management of fungal wilt in tomato.

Material and methods:-

Collection of samples and isolation of endophytic actinomycetes

A total of 60 endophytic actinomycetes isolates were isolated from healthy tomato plants. Local cultivars of both young (less than two weeks) and old plants (more than two weeks) were obtained from different locations and fungal culture of *Rhizocotnia solanii* was procured from the department of Microbiology, Punjab agricultural university, ludhiana, (India).

Determination of plant growth promoting activities

Phosphate Solubilization

Qualitative phosphate solubilization activity of endophytic actinomycetes isolates were analyzed on NBRI-BPB agar medium. Actinomycetes cultures were inoculated on plates containing NBRI-BPB medium and incubated at 28^0C for 7 days and the colonies forming yellow halo zone were considered as phosphate solubilizers and were selected allowed to grow for 7 days in broth was carried out using Erlenmeyer flasks (250ml) containing 50 ml of Pikovskaya medium grown for 7 days for quantitative estimation of P- solubilization using a standard method done by Olsen and Sommers [11].

Indole acetic acid (IAA) production

The production of IAA by endophytic actinomycetes isolates were estimated according to Gordon and Weber [12] with some modifications. Endophytic actinomycetes were inoculated in 100 ml Erlenmeyer flasks containing yeast malt extract broth for 10 days in a rotary shaker at 200 rpm, 28ºC for 7 days. The 10 ml of grown culture was taken in eppendorff tubes and centrifuged at 10,000 rpm for 20 minutes and 1 ml supernatant was mixed with 2 ml Salkowski's reagent and incubated for 30 min at room temperature. Development of a pink color indicated IAA production.

Ammonia production

The endophytic actinomycetes isolates were tested for the production of ammonia using the method described by Cappucino and Sherman [13]. In this method 20 µl of seed culture was propagated in 10 ml of peptone water and incubated at 28°C with shaking at 120 rpm for 10 d. Subsequently, 0.5 ml of Nesseler's reagent was added to the culture and the development of brown to yellow color indicated a positive test for ammonia production. The absorbance was measured at 530 nm using a Thermo scientific (Multiskan GO) spectrophotometer, compared with the standard curve of $(NH_4)_2SO_4$ and expressed in mg/ml.

Siderophore production

Siderophore production of the endophytic actinomycetes was determined by the method of Schwyn and Neilands [14]. A loop full of culture was inoculated on Chrome azurol S (CAS) agar medium and incubated at 28± 2°C for 5 days. The colony with a halo zone of yellow-orange color was considered positive for siderophore production. The positive isolates were cultured in yeast malt extract broth and incubated at 28ºC for 10 days with shaking at 120 rpm for 10 days. Catechol-type siderophores were estimated by Arnow's method [15] and hydroxamate siderophores were determined using the Csaky test [16].

Chitinase activity

The test for chitinase production was performed by the procedure described by (Taechowisan and Lumyong [17], Tang-um and Niamsup [18]. Colloidal chitin was prepared from the chitin by the modified method of Hsu and Lockwood [19]. The clear halo zone measured on colloidal chitin agar medium showed positive chitinase activity. For the quantitative estimation of chitinase activity 0.6% and 1% colloidal chitin concentration was used. Colloidal chitin broth was used as a production medium with pH 7 and incubated at 28ºC in the incubator shaker at 150- 160 rev min-1 for 7 days. Spores were inoculated to a concentration of 10^5 ml^{-1}. Chitinase activity in the supernatant was determined by the procedure of Taechowisan and Lumyong [17], Tang-um and Niamsup [18]. The amount of N-acetyl glucosamine (GlcNAc) released in the supernatant was spectrophotometrically measured by the method of

Somogyi-Nelson [20] on the 520-nm absorbance. One unit (U) of chitinase activity was defined as the amount of enzyme required to produce 1 mol of reducing sugar per min. under the conditions of the experiment.

In vitro antagonistic bioassay

The endophytic actinomycetes isolates were evaluated for their antagonistic activity against phytopathogenic fungus Rhizoctonia solani by dual-culture in vitro assay. Colony growth inhibition (%) was calculated by using the formula: C – T/C x 100, where C is the colony growth of pathogen in control and T is the colony growth of pathogen in dual culture.

Green house experiment

The most potent endophytic actinomycete isolate *Streptomyces* sp. was grown on starch caisein nutrient agar (SCNA) broth at 28°C for seven days with continuous shaking at 150 rpm. The cells were centrifuged at 10,000 rpm for 15 min and the pellets diluted with distilled water to yield a final concentration of 10^6 CFU/ml. The endophytic actinomycete suspension was used to treat targeted plants under green house conditions. Soil was taken from field and sterilized by autoclaving at 121^0C for 1 hr for 3 consecutive days. Tomato seeds (Variety-*Punjab sartaj*) were grown in pots, using completely randomized block design (CRD) with 4 treatments and 3 replications each. Five seeds were sown per pot containing sterile soil. The treatments were (T_0) Control with sterile soil only, (T_1) Seeds treated with fungal spore suspension(*Rhizocotnia solanii*), (T_2) Seeds treated with filtrate of potential actinomycete isolate (TR60) + fungal spore suspension and (T_3) Seeds treated with filtrate of with potential actinomycete isolate. Observations on per cent wilt incidence, days to flowering, number of fruits per plant, per fruit weight and yield per plant were recorded and statistically analysed using ANOVA.

Molecular characterization and phylogenetic analysis of antagonistic endophytic actinomycetes

Total DNA of endophytic actinomycetes isolate from cells, processed for genomic DNA extraction. The fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA, a single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1366bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nr data base of NCBI gen bank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

Results and Discussion:-

Isolation and identification of endophytic actinomycetes

A total of 60 endophytic actinomycetes were isolated from root, stem and leaf tissues of 30 healthy tomato plants, and identified based on their morphological and biochemical characteristics. Out of 60 isolates, 39 belonged *Streptomyces* sp. followed by 7 *Nocardia* sp., 5 *Micromonospora* sp., 6 *Microbisspora* sp. and 3 *Actinopolyspora sp*. Results were found in agreement with previous findings of endophytic actinomycetes isolation from, banana, chilli and *Saccharum officinarum* roots, leaf and stem tissues [21, 22, 23].

Plant growth promoting traits of endophytic actinomycetes.

Phosphate solubilization and IAA production Among the 60 endophytic actinomycetes isolates, 16 (26.67%) were able to solubilize inorganic phosphate and were identified as potential phosphate solubilizing isolates based on a clear halo zone around the colony on NBRI-BPB agar medium. Quantitative estimation of phosphate solubilization by the endophytic actinomycetes ranged from 4.2 ±0,02 - 32.42±0.2 mg/ml (Table 1). Forty-four endophytic actinomycetes (73.33%) were positive for IAA production; quantitative range of IAA production was found from 2.38±0.21 to 124.37±0.26 µg/ml (Table 1) with the highest by *Streptomyces* sp. TR60.

Ammonia and siderophore production by endophytic actinomycetes.

Thirty-four isolates were positive for the production of ammonia at levels ranging from 1.84±0.001 to 29.37±0.06 mg/ml. Isolate *Streptomyces* sp. TS14 produced the maximum amount of ammonia (29.37±0.06 mg/ ml) followed by *Streptomyces* sp TR60 (27.05±0.19 mg/ml). Siderophore production was detected in 18 (30%) isolates on CAS agar media, forming clear orange halo zone around the colonies. The seven isolates produced catachol type siderophore (at levels ranging from 2.8±0.2 to 92.50±0.2 µg/ml), whereas, 16 isolates produced hydroxamatetype

siderophore (range from 3.9±0.2 to 134.50±0.09 µg/ml). Isolate *Streptomyces* sp. TR60 produced greatest amount siderophore (Table 1).

Chitinase production.

On the basis of maximum antifungal activity as well as hydrolytic enzymes production, the endophytic actinomycete isolate *Streptomyces* sp. TR60 was selected for qualitative production of chitinase enzyme by plate agar assay. A clear zone surrounding the actinomycetes colony was observed, indicating that *Streptomyces* sp. TR60 produced chitinase. Maximum chitinase activity was observed on 4th day 0.061 U/ml at 0.6% colloidal chitin concentration. With 1% colloidal chitin substrate concentration, the maximum activity was observed 0.059 U/ml on 4th day (Fig 1) as compared to standard (Fig1.2). Similar observations were reported by [26, 27] during production of chitinase from *S. marcescens* and *S. lividans*, where by enzyme production was observed at exponential stage i.e. 84 h.

In vitro antagonistic activity of endophytic actinomycete isolates against phytopathogenic fungus

Out of sixty isolates, 7 isolates (11.67%) were displaying antagonistic activity against *Rhizoctonia solani*. The antagonistic activity of endophytic actinomycete isolates against phytopathogenic fungus was observed to fall in a range of 4.60±0.03% to 39.85±0.36%. It was observed that root endophytes were better antagonistic agents as compared to stem and leaf isolates. Isolate TR60 showed the maximum percent inhibition against *Rhizoctonia solani* (39.85±0.36%) when compared with control. Passari *et al* [28] recovered forty-two endophytic actinomycetes from medicinal plants were evaluated for their antagonistic potential and plant growth-promoting abilities. Twenty-two isolates which showed the inhibitory activity against at least one pathogen were subsequently tested for their plant-growth promoting activities and were compared genotypically using DNA based fingerprinting.

Scanning electron microscopy (SEM)

Scanning electron microscopy was employed to evaluate the effects of *Streptomyces* sp TR60 on the fungal cell walls of *Rhizoctonia solani*. The co-culture containing *R. solani* and endophytic actinomycete isolate TR60 as well as *R. solani* culture alone as a control was selected for experiment. Results obtained showed that control appeared sectored regular vegetative cells along with large roughly spherical spores (Fig 2A) whereas fungal colony co-cultured with TR60 showed aberrant vegetative cell structure of the hyphae. Further, the fungal hyphae appeared like flattened ribbons having several pits at the poles (Fig 2B) as well as presence of bulbous structures at the edges of the inhibited fungal colonies on the PDA plates was evident (Fig3B). Our results are in conformity with several studies carried out by other investigators. Rawlinson *et al* [29] observed that the bacterial cells examined using SEM, were totally deformed and exhibited severe destruction. The surfaces of the bacterial cells were damaged and had become rough and swollen, but unlysed.

In vivo plant growth activity of tomato seedlings under pot culture conditions.

The most potent isolate *Streptomyces* sp. TR60 identified by 16S rRNA gene sequencing as strains of *Streptomyces fulvissimus* (Table 3) was used for *in vivo* greenhouse experiment on tomato. Inoculation of with *Streptomyces* TR60, showed a significant ($p<0.05$) increase in yield parameters such as days to flowering, root and shoot length treated with the endophytic actinomycetes were superior in performance when compared to the control (Table 2). In the present study the plants treated with *Streptomyces fulvissimus* (TR60) isolate recorded the minimum number of days (31.67) for flowering, followed by seeds treated with filtrate of potential actinomycete isolate and fungal spore suspension (36.66) as compared to absolute control.

Wilt incidence seeds treated with only fungus was recorded of 92.30% that reduced to 53.62% seeds treated with *Streptomyces fulvissimus* + fungus (table 2). Hence, it is concluded from the study that the endophytic actinomycetes, *Streptomyces fulvissimus* from tomato showed superior performance among the all isolates in plant growth promotion as well as in the management of fungal wilt in tomato. This promising endophytic actinomycete has a potential to be developed as a biocontrol agent for the management of fungal wilt in tomato.

Table 1:-Relative production of IAA, P-solubilization, Ammonia and siderophores by endophytic actinomycete from *Solanum lycopersicum* (tomato)

Isolates	IAA (µg/ml)	Phosphate solubilization (mg/100ml)	Ammonia (mg/ml)	Siderophore	
				Hydroxamate (µg/ml)	Catechol (µg/ml)
TR1	8.69±0.12	-	5.49±0.01	-	-
TR2	6.4±0.06	6.5 ±0.2	3.66±0.06	-	-
TL3	51.89±0.33	11.6 ±0.03	11.66±0.005	92.1±0.2	20.2±0.5
TS4	29.07±0.07	17.1 ±0.16	6.42±0.003	-	-
TR5	22.12±0.32	16.5 ±0.02	-	-	16.2±0.1
TR6	5.62±0.22	-	-	-	-
TL7	18.07±0.32	-	19.43±0.006	3.9±0.2	-
TL8	13.43±0.027	-	7.99±0.013	-	-
TL10	2.38±0.21	4.4 ±0.03	26.30±0.041	-	-
TL11	7.6±0.04	14.9 ±0.04	20.47±0.003	-	-
TR12	9.16±0.19	11 ±0.12	5.38±0.005	-	-
TS13	3.68±0.25	-	-	-	-
TS14	29.2±0.28	-	29.37±0.06	38.2±0.3	-
TR15	10.86±0.14	10.6 ±0.41	-	50.7±0.2	42.1±0.2
TR16	41.94±0.35	-	11.4±0.005	-	-
TL18	16.52±0.25	12.5 ±0.02	9.65±0.005	70.2±0.3	25.4±0.2
TL19	5.88±0.34	-	8.15±0.27	-	-
TR20	42.89±0.21	9.4 ±0.32	2.52±0.006	-	-
TL21	7.51±0.17	5.8 ±0.01	23.91±0.03	26.4±0.4	-
TR22	54.24±0.23	-	19.73±0.03	-	-
TR23	9.94±0.54	-	3.95±0.003	38±0.3	-
TL24	7.43±0.08	-	4.73±0.09	-	-
TR25	3.33±0.11	-	1.84±0.001	-	-
TR26	24.23±0.40	-	6.35±0.003	16.8±0.2	-
TS28	28.96±0.20	-	-	-	-
TL29	5.4±0.09	-	21.75±0.005	-	-
TL30	11.55±0.13	-	8.44±0.01	-	-
TR31	2.45±0.19	-	18.38±0.03	-	-
TR32	4.5±0.19	-	21.60±0.026	-	-
TL33	51.5±0.59	13.8 ±0.01	8.44±0.01	46.2±0.2	-
TR34	56.66±0.71	-	18.38±0.03	-	-
TL35	40.73±0.26	4.2 ±0.02	21.60±0.02	-	-
TR36	2.65±0.12	-	12.10±0.01	5.1±0.5	-
TR39	27.81±0.4	-	2.31 ±0.008	15.4±0.1	-
TL38	-	-	-	12.6±0.1	-
TR43	60.31±0.07	-	-	-	-
TR40	-	-	-	11.2±0.1	-
TL45	22.69±0.27	-	3.06±0.006	-	-
TS46	10.69±0.27	-	-	52.4±0.2	-
TR49	51.73±0.34	-	-	-	-
TS50	41.93±0.38	20.2 ±0.15	19.05±0.01	-	9.3±0.07
TR52	35.83±0.36	-	-	-	-
TL56	53.8±0.25	-	17.71±0.02	15.4±0.09	2.8±0.2
TR58	6.02±0.37	8.2 ±0.1	18.76±0.003	-	-
TS59	10.54±0.040	-	-	-	-
TR60	124.37±0.26	32.42 ±0.2	27.05±0.19	134.5±0.09	92.5±0.2

CD@5%	0.90	0.33	0.17	0.22	0.53

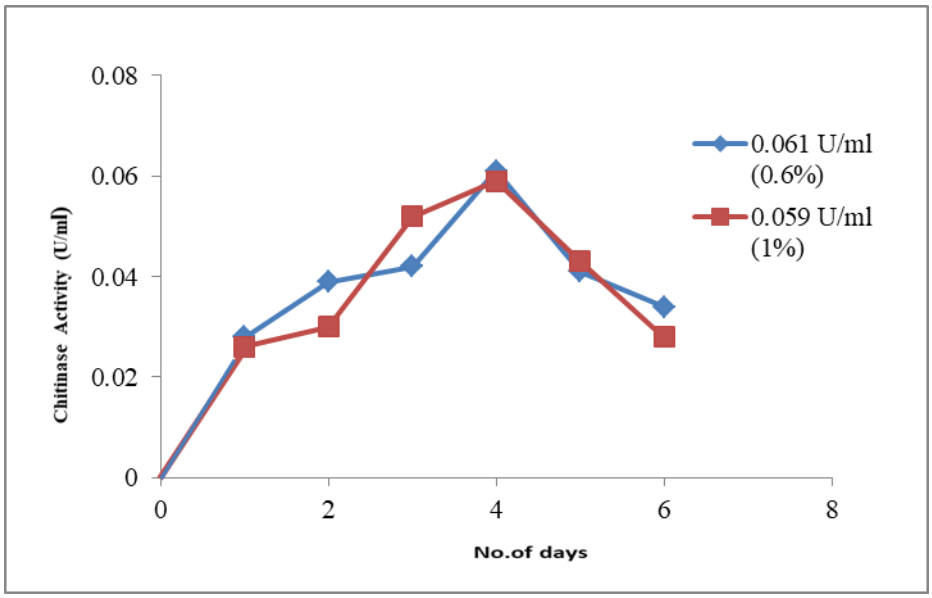

Fig 1:-Quantitative production of chitinase by TR60 isolate

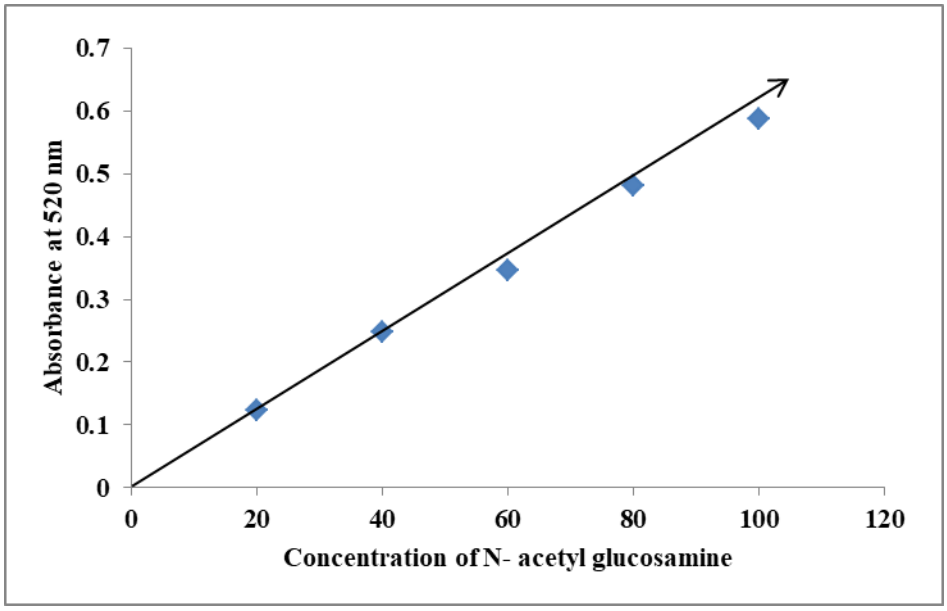

Fig1.2:-Standard curve of N-acetyl glucosamine

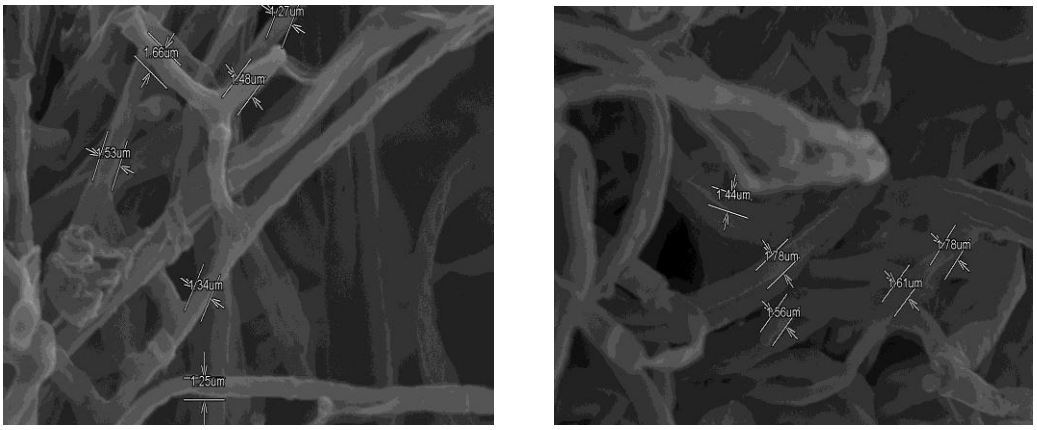

Fig 2:- (A) Rhizocotonia solani from pure culture (B) co-cultured Rhizocotonia solani with Streptomyces sp. (TR60)

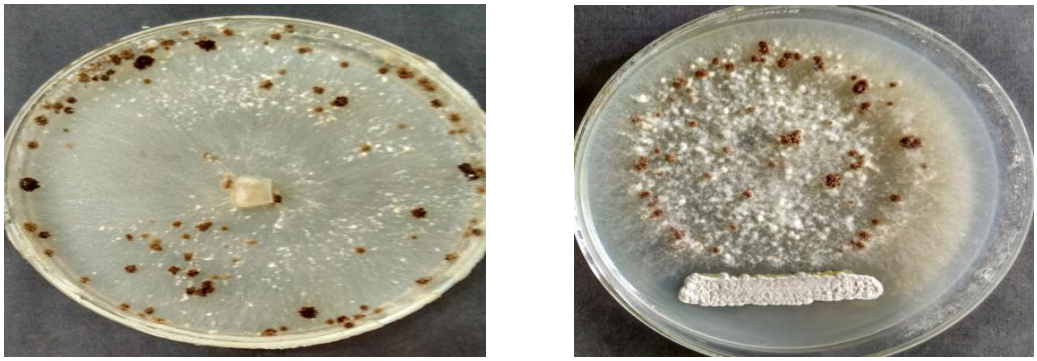

Fig 3:- (A) Rhizoctonia solani (B) Rhizoctonia solani + TR60

Table 2:-Effect of various treatments on fungal wilt in tomato

Treatment	Wilt incidence (%)	Days to flowering	Number of shoots			shoot length (cm)			Root length (cm)		
			30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T0	-	40.67	8.33	10.66	14.55	8.57	10.67	21.71	5.55	6.21	12.06
T1	92.30	43.33	7.66	7.15	5.89	7.83	8.33	10.21	4.51	4.68	4.60
T2	53.62	36.66	11.33	12.11	18.55	11.45	15.21	19.60	6.75	6.35	10.23
T3	-	31.67	12.33	14.33	27.43	11.78	18.85	23.97	6.55	6.11	12.80
CD at 5%		0.87	1.49	0.79	0.35	1.53	1.25	2.47	NS	0.93	0.92

T0 - Absolute Control (no pathogen and no actionmycete)
T1 - Control (pathogen only)
T2 - Seeds treated with filtrate of potential actinomycete isolate + fungal spore suspension
T3 - Seeds treated with filtrate of potential actinomycete isolate TR60

Table 3:-Sequence producing significant alignments (TR60)

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces fulvissimus strain FHM275 16S ribosomal RNA gene, partial sequence	1146	1146	99%	0.0	99%	KM438035.1
Uncultured bacterium clone MONS_IW0306_11 16S ribosomal RNA gene, partial sequence	1118	1118	98%	0.0	99%	FJ432349.1
Variovorax sp. strain MAK8 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	KX665557.1
Variovorax boronicumulans partial 16S rRNA gene, isolate 1011MAR4D42.1	1116	1116	98%	0.0	99%	LN867144.1
Variovorax sp. i25s gene for 16S rRNA, partial sequence	1116	1116	98%	0.0	99%	AB974279.1
Uncultured bacterium clone HJ3E05 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	JX644348.1
Variovorax sp. SMX332 partial 16S rRNA gene, strain SMX332	1116	1116	98%	0.0	99%	HF571534.1
Variovorax sp. LY1 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	JQ360172.1
Variovorax sp. CRF3-Va-1 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	JQ010855.1
Uncultured Variovorax sp. partial 16S rRNA gene, clone H319-9	1116	1116	98%	0.0	99%	HE585190.1
Uncultured Comamonadaceae bacterium clone G-19 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	JF703396.1
Uncultured bacterium clone POME_T37_B18 16S ribosomal RNA gene, partial sequence	1116	1116	98%	0.0	99%	HM440296.1

References

1. Sreeja, S.J. and Gopal K. S. (2013) Bio-efficacy of endophytic actinomycetes for plant growth promotion and management of bacterial wilt in tomato. *Pest Management in Horticultural Ecosystems* **19 (1):**63-66.
2. National Sample Survey Office (NSSO), Ministry of Statistics and Programme Implementation, government of India (2016-17).
3. Youssef, S.A., Kamel, A.T., Ghada, A. A. (2016) Evaluation of Trichoderma harzianum and Serratia proteamaculans effect on disease suppression, stimulation of ROS-scavenging enzymes and improving tomato growth infected by *Rhizoctonia solani*. *Journal of Biological Control* **100:** 79-86.
4. Parissa Taheri and Saeed Tarighi (2012) The Role of Pathogenesis-Related Proteins in the Tomato-Rhizoctonia solani Interaction. Journal of Botany pp 1-6.
5. Romanazzi, G., Smilanick, J.,Feliziani, E., Droby, S. (2016) Integrated management of postharvest gray mold on fruit crops. *Postharvest Biology and Technology* **113:**69–76.
6. Kachhawa D (2017) Microorganisms as a biopesticides. *Journal of Entomology and Zoology Studies* 2017; 5(3): 468-473.
7. Gangwar, M., Kaur, N., Saini, P. and Kalia, A. (2015) The diversity, plant growth promoting and anti-microbial activities of endophytic actinomycetes isolated from Emblica officinalis Gaertn. *International Journal of Advance Research* **3**: 1062-71.
8. Vurukonda, S.S., Prasad K., Davide G. and Emilio, S. (2018) Plant Growth Promoting and Biocontrol Activity of Streptomyces spp. as Endophytes. *International Journal of Molicular Science* **19:** 952.
9. Gangwar, M., Dogra, S., Gupta, U.P., Kharwar, R.N. (2014) Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. *African Journal of Microbiology* **8(2):** 184-191.
10. Passari, A. K., Mishra, V. K., Singh, G., Singh, P., Kumar, B., Gupta, V. K., Sarma, R. K., Donovan, A. O. and Singh, B. P. (2017) Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. *Science Reports* **7:**1-17.
11. Olsen, S. and Sommers, L.E. (1982) Phosphorus In: Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties. *American Society of Agronomy* pp 403-427.
12. Gordon, S.A.,Weber, R.P. (1951) Colorimetric estimation of indole acetic acid. *Journal of Plant Physiology* **26:** 192–195.
13. Cappucino, J.C., Sherman N. (1992) Microbiology: a laboratory manual. New York, Benjamin: Cummings Publishing Company; p. 125-179.

14. Schwyn, B., Neilands, J,B. (1987) Universal chemical assay for the detection and determination of siderophores. Anals of Biochemistry **160:** 47-56.
15. Arnow, L.E. (1937) Calorimetric estimation of components of 3, 4 dihydroxy phenylalanine tyrosine mixtures. *Journal of Biological Chemistry* **118:**531-535.
16. Csaky, T.Z. (1948) On the estimation of bound hydroxylamine in biological materials. *Acta Chemistry Scand* **2:** 450-54.
17. Taechowisan, T., Peberdy, J.F. and Lumyong, S. (2003) Chitinase production by endophytic *Streptomyces aureofaciens* CMUAc130 and its antagonism against phytopathogenic fungi. *Annals of Microbiology* **53**: 447-61.
18. Tang-um, J. and Niamsup, H. (2012a) Chitinase production and antifungal potential of endophytic *Streptomyces* strain P4. *Maejo International Journal of Science and Technolnology* **6**:95-104.
19. Hsu, S. C. and Lockwood, J. L., 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Journal of Applied Microbiology*. **29**:422-26.
20. Green, F., Clausen, C.A. and Highley, T.L. (1989) Adaptation of the Nelson-Somogyi reducing-sugar assay to a microassay using microtiter plate. *Annals of Biochemistry* **12**:197-99.
21. Gangwar M, Khushboo, Saini1 P (2014) Diversity of endophytic actinomycetes in *Musa acuminata* and their plant growth promoting activity. *Journal of Biological and Chemical Sciences* **1(1):**13-23
22. Khushboo, Kaur N (2019) Screening of Endophytic Actinomycetes for their Plant Growth Promoting Activity and for Biocontrol in Chilli (*Capsicum annum*) against *Sclerotium Rolfsii. International Journal of Science and Research* **8(8)**:1683-1690
23. Bhosale . H.J. and Kadam T.A. (2015) Generic diversity and a comparative account on plant growth promoting characterstics of actinomycetes in roots and rizosphere of *Saccharum officinarum. International journal of Current Microbiology and Applied Science* **4(1):**230-244.
24. Young,M.E. and Bell, R.L.(1985) Kinetics of chitinase production.II Relationship between bacterial growth, chitin hydrolysis and enzyme synthesis. *Journal of Biotechnology* **21:**679-82.
25. Neugebour, E., Gamache, B., Dery, C. and Brzezinski, R., 1991. Chitinolytic properties of *Streptomyces lividans. Archieves of Microbiology* 156: 192-97.
26. Passari, A. K., Mishra, V. K., Gupta, V. K., Yadav, M. K. and Saikia, R. (2015) *In vitro* and *in vivo* plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS One* **10(9):** e0139468.
27. Rawlinson, L. B., Ryan, S. M., Mantovani, G., Syrett, J. A., Haddleton, D. M. and Brayden, D. J., 2010. Antibacterial effects of poly (2-(dimethylamino ethyl) methacrylate) against selected Gram-positive and Gram-negative bacteria. *Biomacromolecules* 11: 443-453.