Chapter 4
EXPERIMENTAL

4.1. **Isolation and identification of actinomycetes isolates from sample area.**

Soil samples were collected from agricultural fields of different parts of Jalpaiguri district of West Bengal. The area falls between 26°15'47'' & 26°59'34'' N Latitude and 88°23'2'' & 89°7'30''E Longitude of North Bengal in the State of West Bengal. The average rainfall is from 2500mm-3000mm, 80% of which falls during monsoon and have about 110 rain days. Soil is acidic to neutral in nature and low in fertility. Agricultural fields were chosen because of the fact that they have a diversity of soil microflora present in the root rhizosphere of the crop plants. These natural microflora boost the growth and disease resistance activity of the crop plants . Jalpaiguri being a prominent agriculture based district it is very important that microorganism based bioformulations and biofertilizers are developed for this region because of the fact that chemical formulations kills and changes not only the soil microflora but also changes the character of the soil and greatly affect the biodiversity. So a potent microorganism from the natural system will be of great importance which not only have positive effect on plant growth and health but also will maintain the balance of the ecosystem. The soil sample was collected from agricultural field from a minimal depth of 20cm to 25cm using sterile spatula. Initial soil layer was removed manually by hand and the samples were then transferred under sterile condition in Ziploc plastic bags and stored in airtight plastic containers (Cello) and transported to Laboratory. Samples were air dried and then heated in a incubator at 28ºC for two days to facilitate actinomycetes isolation. Warcup's serial dilution method was modified to some extent for the isolation process. A total number of 17actinomycetes were isolates from various sources, mainly from the potato growing fields.

Table 6. GIS position, code and colony characteristics of actinomycetes isolates

Soil sample code	GIS location of sampling	Colony colour
ARHS/PO/11	N26º44'54.08" E 88º48'14.53"	Reddish white
ARHS/PO/12	N26º44'54.88" E 88º48'14.04"	reddish
ARHS/PO/13	N26º44'53.18" E 88º48'13.24"	Whitish
ARHS/PO/14	N26º44'54.89" E 88º48'14.34"	Pinkish white
ARHS/PO/15	N26º44'57.23" E 88º48'15.24"	Reddish white
ARHS/PO/16	N26º47'09.44" E 88º22'06.53"	Pinkish
ARHS/PO/17	N26º44'54.08" E 88º48'14.53"	Reddish
ARHS/PO/18	N26º44'54.08" E 88º48'14.53"	Grayish white
ARHS/PO/20	N26º44'54.08" E 88º48'14.53"	Grayish
ARHS/PO/22	N26º44'54.08" E 88º48'14.53"	Whitish
ARHS/PO/23	N26º44'54.08" E 88º48'14.53"	Pinkish
ARHS/PO/24	N26º44'54.08" E 88º48'14.53"	Grayish
ARHS/PO/25	N26º44'54.08" E 88º48'14.53"	Grayish red periphery
ARHS/PO/26	N26º44'54.08" E 88º48'14.53"	Pinkish
ARHS/PO/27	N26º44'54.08" E 88º48'14.53"	Grayish
ARHS/PO/28	N26º44'54.08" E 88º48'14.53"	Whitish
ARHS/PO/29	N26º44'54.08" E 88º48'14.53"	Whitish

ARHS= Actinomycetes of Root rhizosphere, PO= Potato

4.1.1. Morphological characterization of isolates

Isolates were streaked on SCN media and incubated at 28ºC for 7 days. Morphological characterization of the isolates were done by observing colour of both aerial and substrate mycelium, production of aerial spore mass and by production of melanine pigment. Colours of the substrate mycelium in most isolates were insignificant. In most cases it was pale yellow. It was found that most of the isolates produce white or grey

colour spore mass with some of the isolates having different tints. Some of the isolates produced dark brown or chocolate coloured melanin pigments. Detail results have been presented in table 7.

Table 7. Morphological characteristic of isolates

Isolate code	Media	OptimumGrowth temperature	Colour of Aerial mycelium	Colour of Substrate mycelium	Melanoid pigment Production	Diffusible Pigments
ARHS/PO/11	SCN	28ºC	Reddish white	Pale yellow	-	-
ARHS/PO/12	SCN	28ºC	Reddish	Pale yellow	-	-
ARHS/PO/13	SCN	28ºC	Whitish	Pale yellow	-	-
ARHS/PO/14	SCN	28ºC	Pinkish white	Pale yellow	+	+
ARHS/PO/15	SCN	28ºC	Reddish white	Pale yellow	-	_
ARHS/PO/16	SCN	28ºC	Pinkish	purple	+	+
ARHS/PO/17	SCN	28ºC	reddish	Pale yellow	-	-
ARHS/PO/18	SCN	28ºC	Grayish white	purple	+	+
ARHS/PO/20	SCN	28ºC	grayish	Pale yellow	-	-
ARHS/PO/22	SCN	28ºC	whitish	Pale yellow	-	-
ARHS/PO/23	SCN	28ºC	Pinkish	purple	+	+
ARHS/PO/24	SCN	28ºC	Grayish	Pale yellow	-	_
ARHS/PO/25	SCN	28ºC	Grayish red periphery	Pale yellow	-	+
ARHS/PO/26	SCN	28ºC	Pinkish	purple	+	+
ARHS/PO/27	SCN	28ºC	Grayish	Pale yellow	-	_
ARHS/PO/28	SCN	28ºC	whitish	Pale yellow	-	_
ARHS/PO/29	SCN	28ºC	Whitish	Pale yellow	-	_

ARHS= Actinomycetes of Root rhizosphere, PO= Potato, SCN= Starch Casein Nitrate agar media

Figure 1: (A-Q) Growth Pattern of different actinomycetes isolates

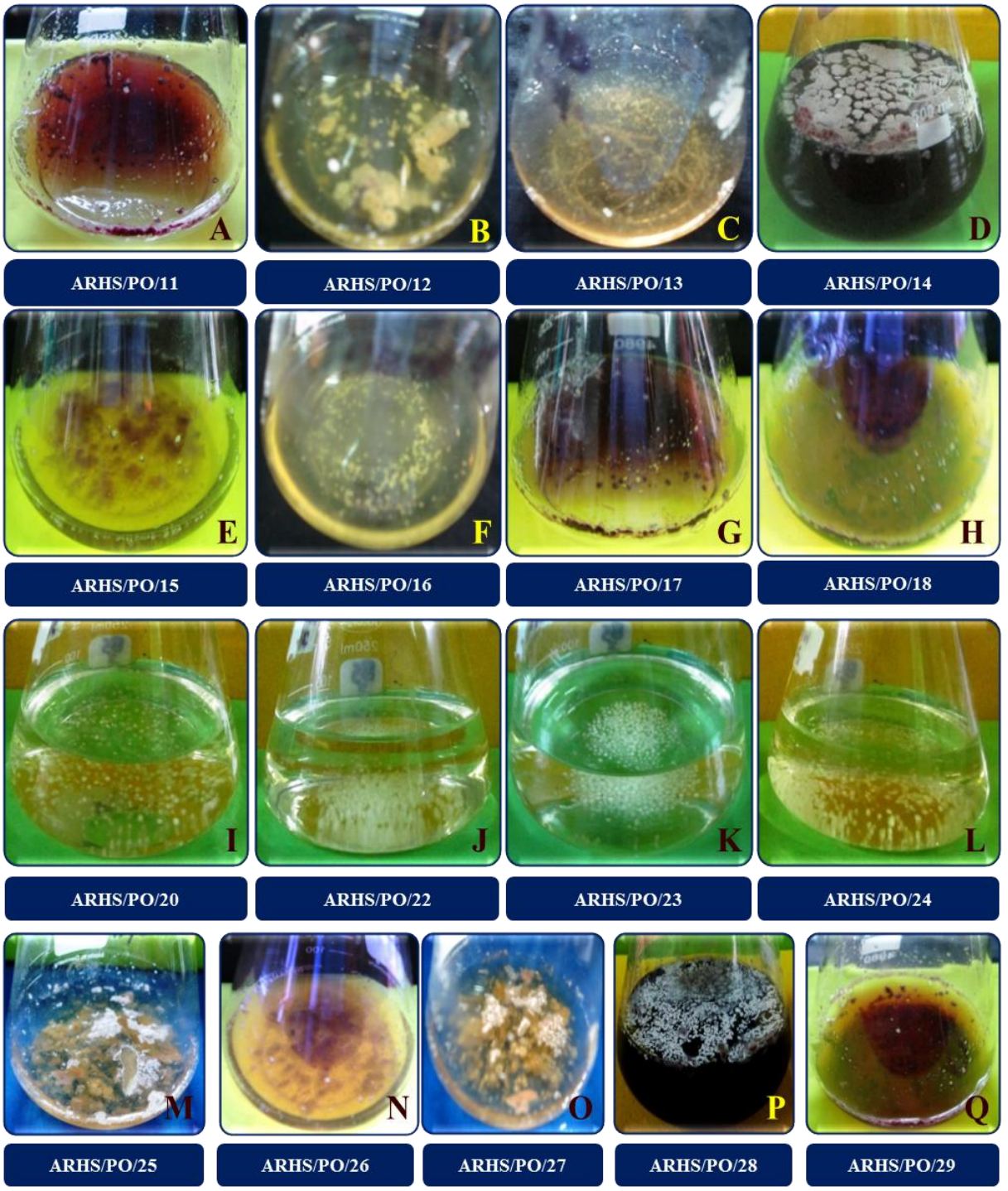

Figure 2: (A-Q) Screening for pigmentation by the Actinomycetes isolates.

4.1.2. Biochemical characterization of isolates.

All the isolatesobtained from different regions were first categorized as Gram positive organisms. Basic biochemical characterizations like Starch hydrolysis, H_2S production, catalase production, Indole production were characterized. The detail result is summarized in Table 8.All the isolates showed positive result for Gram staining, starch hydrolysis, catalase production (Fig. 3).

Table 8. Biochemical characterization of actinomycetes isolates

Isolates	Spore	Gram reaction	H_2S production	Phosphate solubilization	Starch hydrolysis	Casein hydrolysis	Chitin degrading	Siderophore production	Catalase production	Urase production	Cellulase Production	Indole Production	Identification
ARHS/PO/11	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/12	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/13	+	+	-	-	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/14	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/15	+	+	-	-	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/16	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/17	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/18	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/20	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/22	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/23	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/24	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/25	+	+	-	+	+	+	+	+	+	-	+	+	*Streptomyces sp.*
ARHS/PO/26	+	+	-	+	+	+	-	-	+	-	-	+	*Streptomyces sp.*
ARHS/PO/27	+	+	-	-	+	+	+	+	+	-	+	+	*Streptomyces sp*
ARHS/PO/28	+	+	-	+	+	+	-	-	+	-	+	+	*Streptomyces sp.*
ARHS/PO/29	+	+	-	+	+	+	-	-	+	-	+	+	*Streptomyces sp*

+= present, -= absent

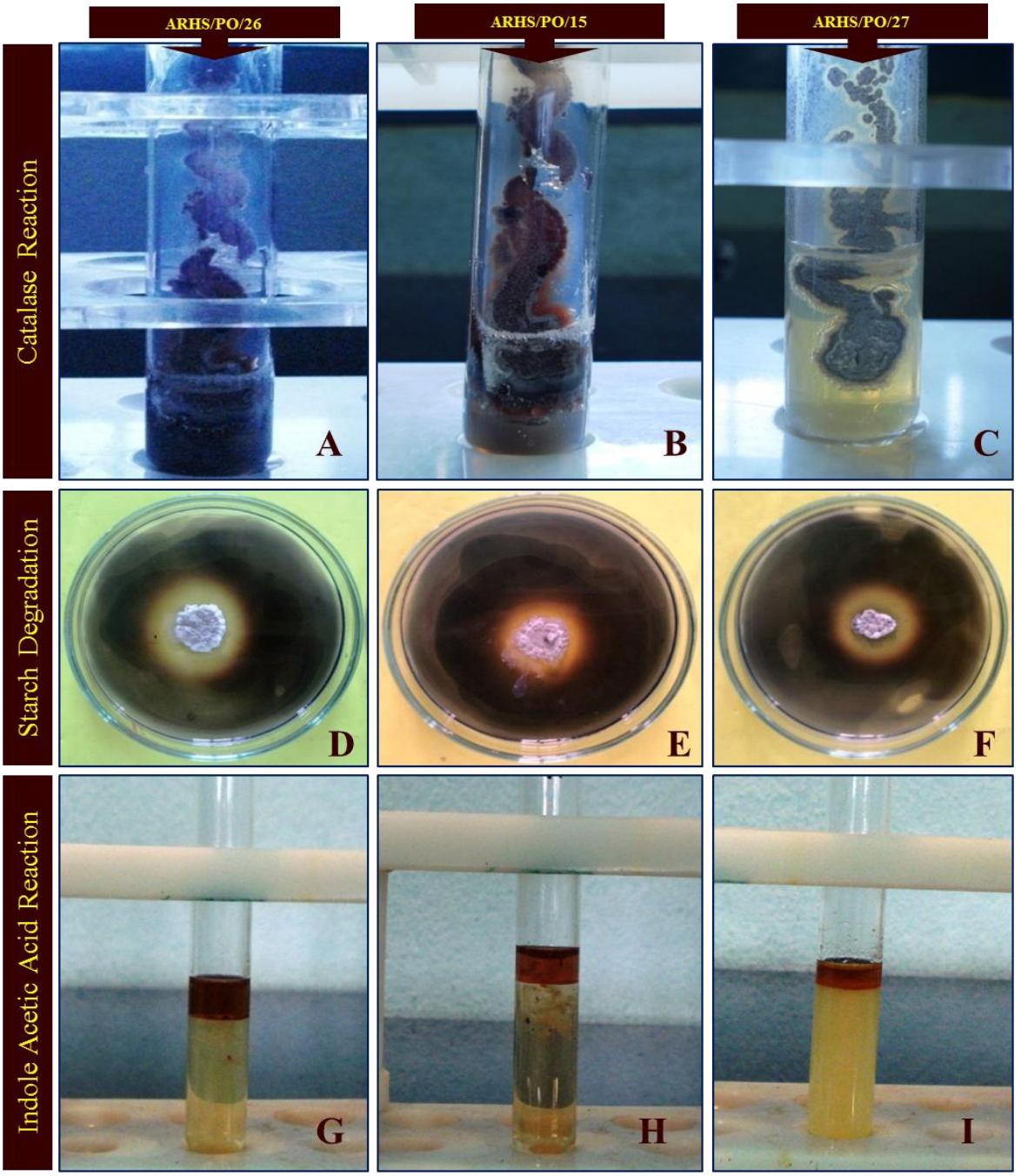

Fig. 3. (A-L) Biochemical characterization of actinomycetes isolates. (A-C) Catalase reaction; (D-F) Starch Degradation reaction;(G-I) Indole Acetic Acid (IAA) reaction.

4.1.3. Physiological characterization of isolates.

Physiological characterizations of the isolates were done by observing the growth of isolates in different media. Whereas the isolates grow vigorously in SCN media growth in NA is less. Sporulation in Oatmeal agar (ISP 3) was highest. Growth of the isolates in different temperature, different pH and salt concentration(1%, 2%, 5%, 8% and 10% salt concentration) were observed. The result showed that though in 1% salt concentration growth of the isolates were normal, growth was hampered in 5% salt concentration and in 10% salt concentration no growth was observed.(Fig. 5).Optimum temperature of the isolates for growth was in the range of 28ºC to35ºC. Resistance of isolates to different antibiotics were also characterized. Isolates were highly resistant to ampicillin followed by streptomycin and least resistant to kanamycin (Fig.4) Table 9.

Table 9 : Physiological characterization of actinomycetes isolates

Isolates	Growth on Media		Sporulation in ISP 3	NaCl Concentration			pH		Temperature			Resistance against		
	SCN	NA		1%	5%	10%	pH7	pH10	15ºC	28ºC	35ºC	Ampicillin (25µg/disc)	Streptomycin (25µg/disc)	Kanamycin (25µg/disc)
ARHS/PO/11	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/12	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/13	+++	+	+++	++	+	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/14	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/15	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/16	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/17	+++	+	+++	++	+	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/18	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/20	+++	+	+++	+	-	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/22	+++	+	+++	++	+	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/23	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/24	+++	+	+++	++	+	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/25	+++	+	+++	+	-	-	+++	+	+	+++	+++	+	+	-
ARHS/PO/26	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	+
ARHS/PO/27	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/28	+++	+	+++	++	+	-	+++	++	+	+++	+++	+	+	-
ARHS/PO/29	+++	+	+++	+	-	-	+++	+	+	+++	+++	+	+	-

+++= maximum growth, ++=moderate growth, +=less growth, - = no growthFor antibiotic resistance += resistance, - = susceptible

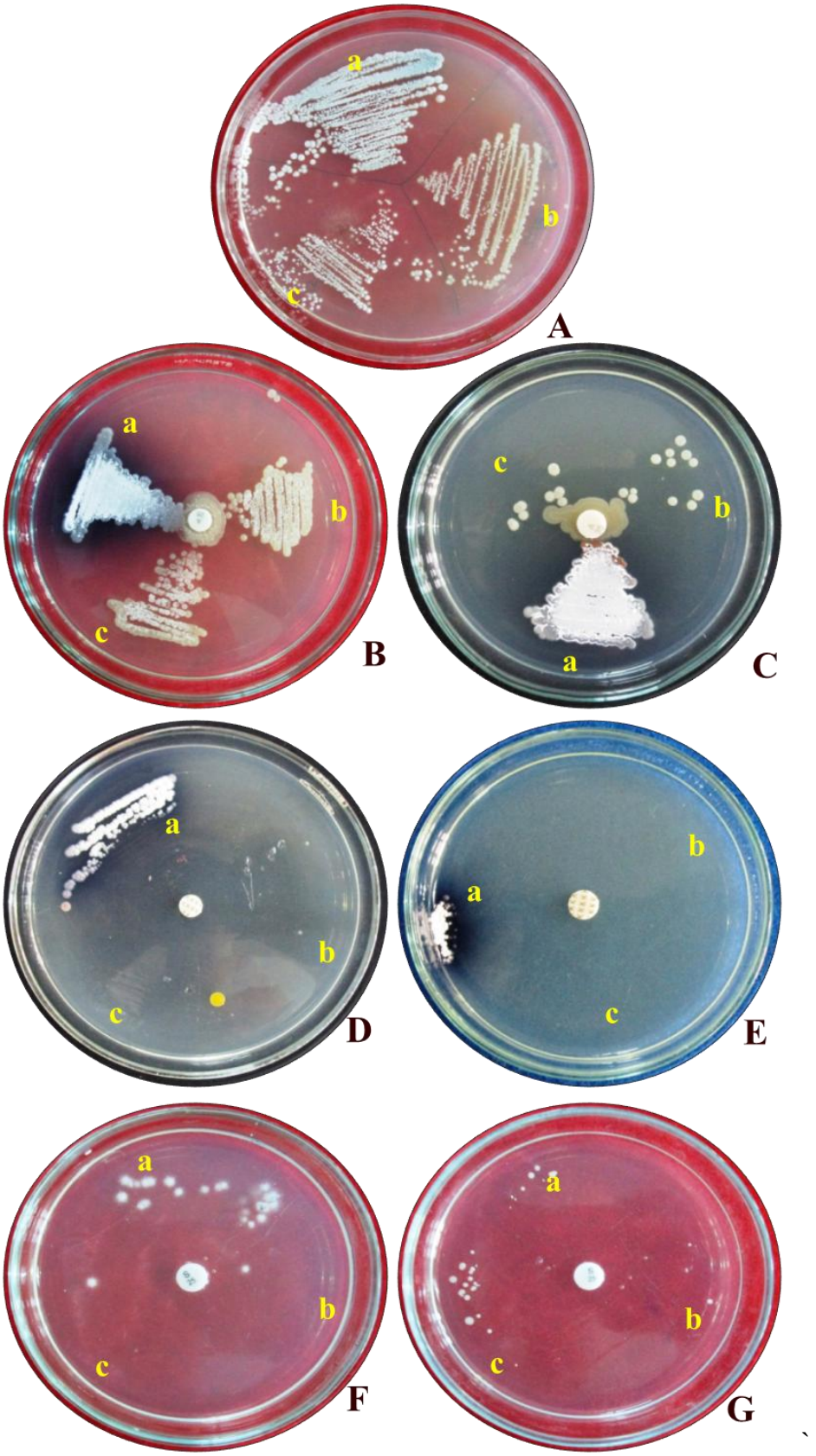

Fig. 4 . (A-G): Screening of Antibiotic Resistance of Selected Isolates; (a) ARHS/PO/26; (b) ARHS/PO/27; (c) ARHS/PO/15.A- Control; B-G Resistance to ; Amphicilin [B-C], Kanamycin [D-E], Streptomycin [F-G]

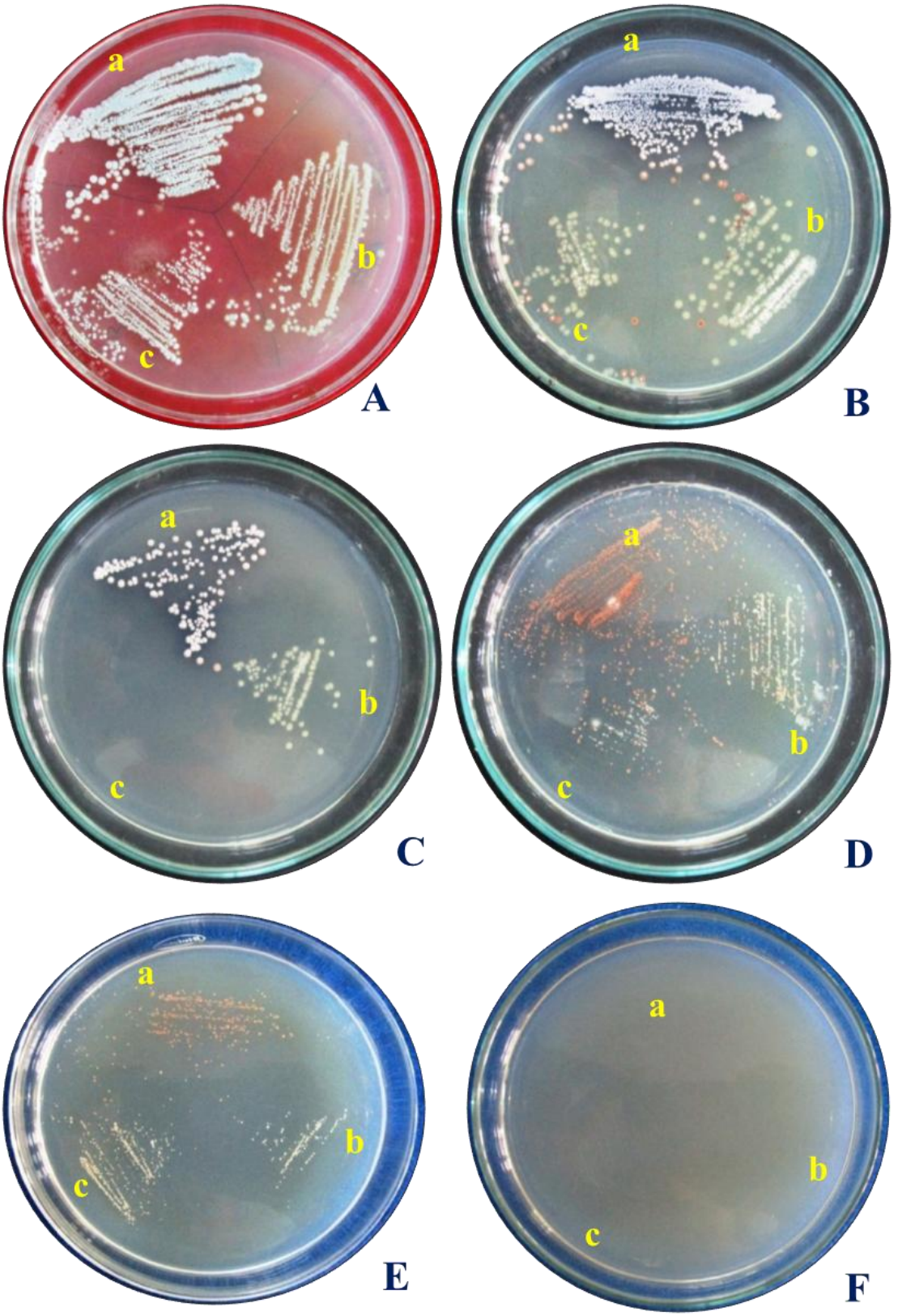

Figure 5: (A-F) Visual Assessment; Growth of Selected Isolates in different salt concentrations. . A- Control, B - 1% NaCl; C - 2% NaCl; D - 5% NaCl L; E -8 % NaCl ; F- 10% NaCl.

4.2. Assessment of growth of isolates in media

4.2.1. In solid media

Pure culture of the isolates obtained from different sources was preserved in SCN slants. Morphological study, growth pattern, time of growth was observed in SCN plates for 7 days at 28ºC. Colour of aerial and substrate mycelia, Spore formation, melanin pigment production were observed in the isolates (Fig.1)

4.2.2. In liquid media

Isolates were inoculated in liquid SCN media (50/250 v/v) and kept in a rotary shaker at 28ºC for 7 days. Colonies were visible to the naked eye and diffusible pigment formation was also observed (Fig.2)

4.2.3.Microscopic observation

Microscopic observations of the isolates were made and photographs were taken with help of normal bright field microscope and Ocular attached stereo digital camera (Fig.6). Actinomycetes cells were found to be rod shaped in structure and they bind end to end to form chain like structure. By observing the characteristics of the spore bearing hyphae and the particular structures of the spore chains the isolates can be devided into three sections belonging to the group streptomycetes, namely rectiflexibiles (RF), Retinaculiapetri (RA) and Spirales(S). (table.10)

Table 10 . Grouping of isolates on the basis of spore chain morphology

Isolate	Spore chain morphology
ARHS/PO/11	RF
ARHS/PO/12	S
ARHS/PO/13	S
ARHS/PO/14	S
ARHS/PO/15	RF
ARHS/PO/16	S
ARHS/PO/17	RA
ARHS/PO/18	RF
ARHS/PO/20	S
ARHS/PO/22	RA
ARHS/PO/23	S
ARHS/PO/24	S
ARHS/PO/25	S
ARHS/PO/26	SRA
ARHS/PO/27	S
ARHS/PO/28	S
ARHS/PO/29	RA

Rectiflexibiles =RF, Retinaculiapetri =RA,Spirales=S

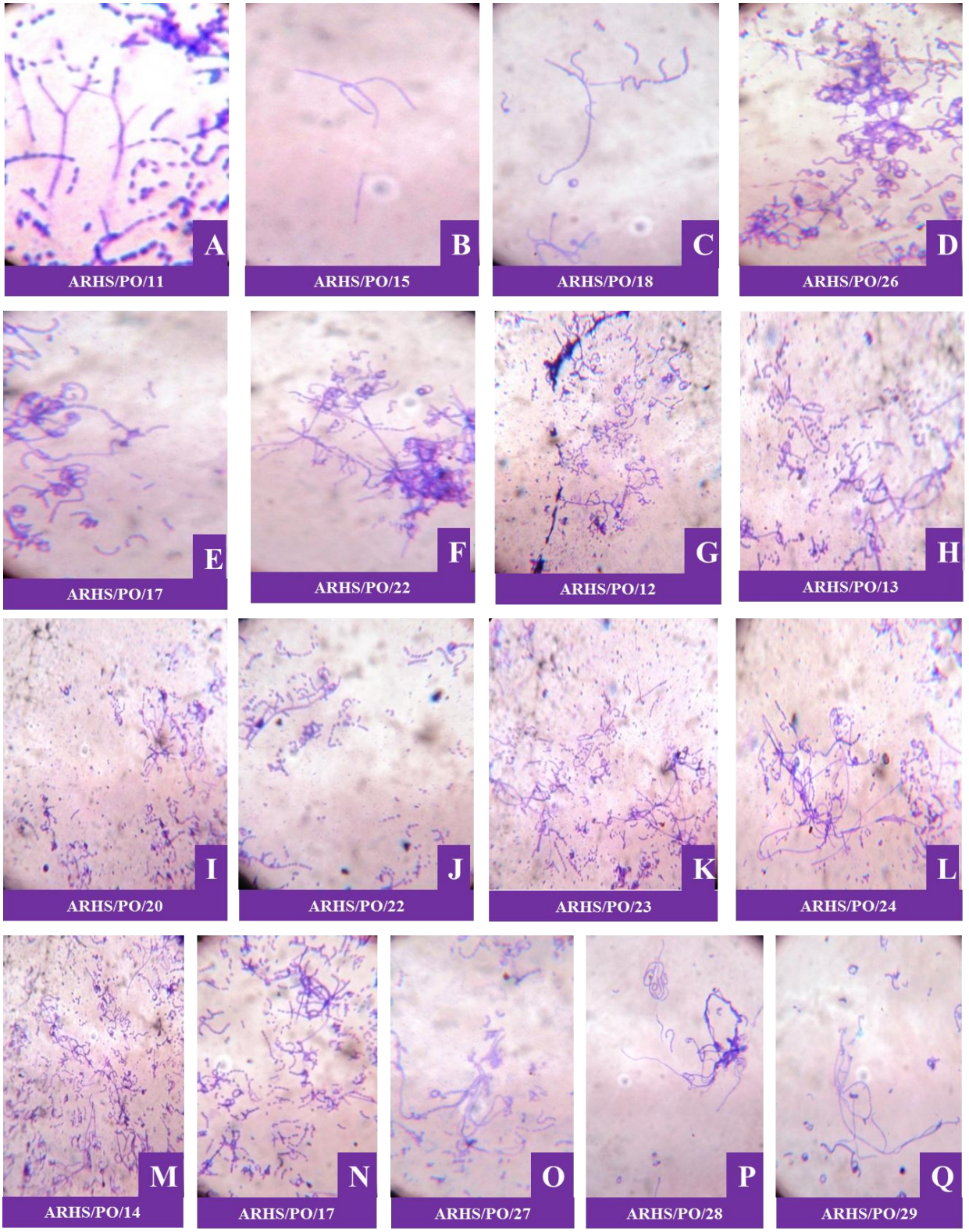

Figure 6: (A-Q) Microscopic identification of isolates based on morphological characters. (A-C) Rectiflexible, (D-E) Retinaculiapetri, (F-Q) Spirales.

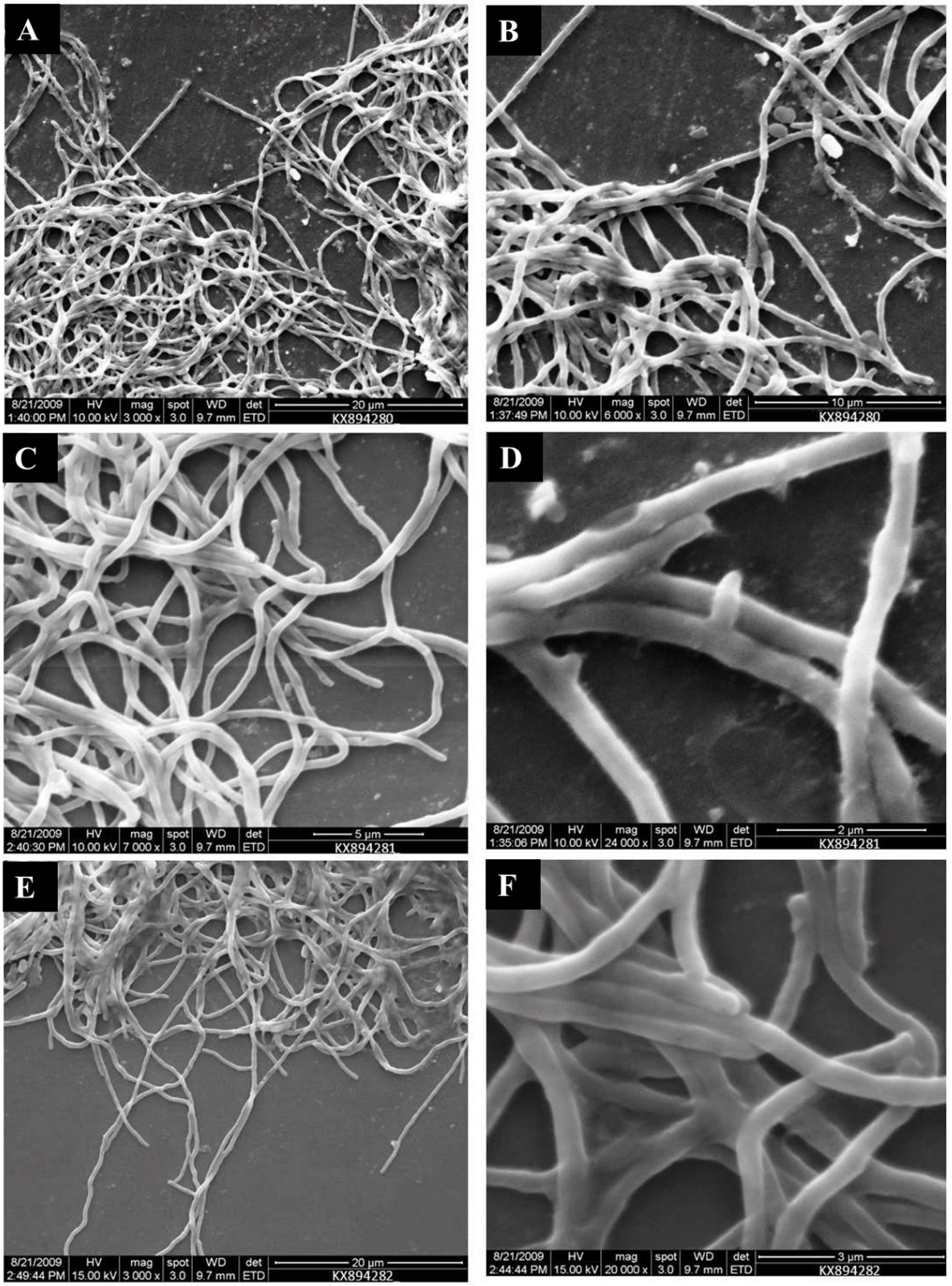

Figure7: (A-F) Scanning Electron Micrograph of different actinomycetes isolates. (A-B). *Streptomyces tricolor* (KX894280). (C-D). *Streptomyces flavogriseus* (KX894281). (E-F). *Streptomyces griseus*(KX894282).

4.2.4. Deposition of the Isolates to the National Agriculturally Important Culture Collection(NAIMCC)

Some of the isolates were initially identified on the basis of their morphological and biochemical properties By National Centre of Fungal Taxonomy, as *Streptomyces* spp. belonging to streptomycetes group of actinomycetes. These cultures were later submitted to the National Agriculturally Important Culture Collection (NAIMCC) of National bureau of Agriculturally Important Microorganisms, and their accession numbers are listed in Table 11.

Table 11.NAIMCC accession numbers of selected isolates

Isolate Code	NAIMCC acc no.
ARHS/PO/14	NAIMCC-B00913
ARHS/PO/15	NAIMCC-B00915
ARHS/PO/16	NAIMCC-B00917
ARHS/PO/17	NAIMCC-B00914
ARHS/PO/27	NAIMCC-B00916

4.3 *In vitro* Screening and evaluation of isolates for growth promoting attributes.

4.3.1. Phosphate solubilization

4.3.1.1. In solid medium

A total of 17 actinomycetes isolates were obtained from the agricultural fields. All the isolates were initially screened for their ability to solubilize phosphate *in vitro* by plating the isolates in Pikovskaya agar (PKV)medium. Formation of a clear halo zone around the colony indicate phosphate solubilizing property (Fig.8) The diameter of the halo zone formed was recorded to compare the efficacy of the isolates for phosphate solubilization (Table.12)

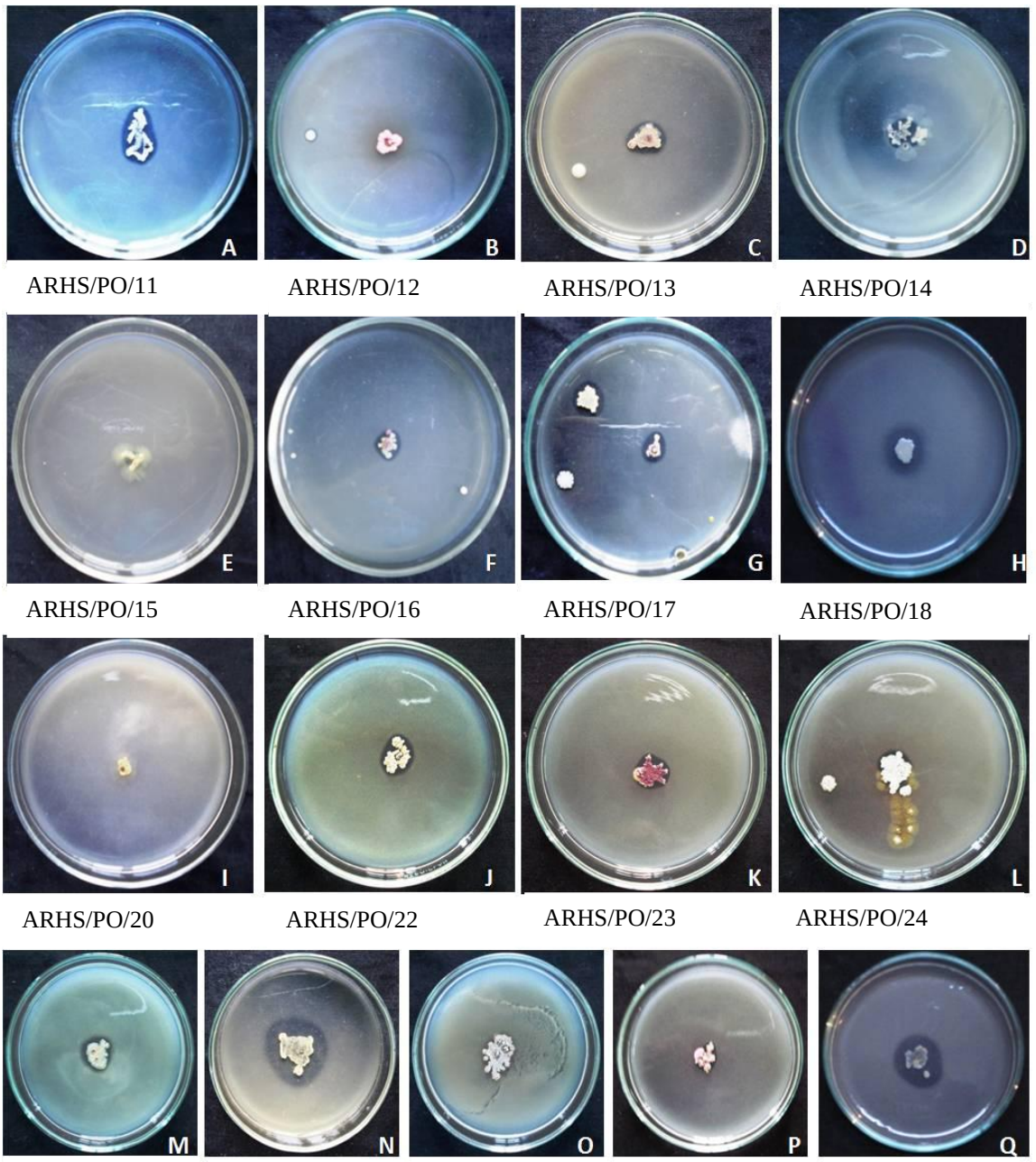

Figure 8: (A-Q) Screening for phosphate solubilizing activity by actinomycetes isolates in PVK solid media.

Table 12: Screening of Phosphate solubilization in the solid PKV medium

Isolates	Diameter of hallo zone(cm)	
	3 days	**7days**
ARHS/PO/11	0.10±0.091	0.25±0.008
ARHS/PO/12	0.16±0.02	0.34±0.025
ARHS/PO/14	0.21±0.014	0.45±0.06
ARHS/PO/16	0.20±0.035	0.43±0.012
ARHS/PO/17	0.13±0.071	0.31±0.032
ARHS/PO/18	0.17±0.050	0.35±0.061
ARHS/PO/20	0.11±0.001	0.27±0.055
ARHS/PO/22	0.18±0.062	0.38±0.023
ARHS/PO/23	0.24±0.021	0.51±0.01
ARHS/PO/24	0.12±0.011	0.29±0.06
ARHS/PO/25	0.18±0.006	0.39±0.1
ARHS/PO/26	0.28±0.071	0.57±0.21
ARHS/PO/28	0.22±0.023	0.49±0.
ARHS/PO/29	0.14±0.044	0.35±0.

Values are mean of three replicates, ± =standard error

4.3.1.2. In Liquid medium

The isolates which showed positive result for phosphate solubilization on PKV medium were further evaluated for phosphate solubilization capacity in liquid PKV media amended with Tricalcium phosphate and rock phosphate.The initial total phosphate content of Tricalcium phosphate was 920mg/L and of rock phosphate was 592mg/L. The amount of the total phosphate solubilized is presented in Table 13. The initial pH of the culture medium was 7. After 7 days of incubation pH in the control remained constant but dropped significantly in the broth culture containing samples.

Table13. *In vitro* quantification of phosphate solubilization by isolates in modified PKV broth culture

Isolates	TCP(µg/ml)	RP(µg/ml)
ARHS/PO/11	374.61 ± 1.21	244.86 ±0.53
ARHS/PO/12	478.23 ± 0.84	296.47 ±1.29
ARHS/PO/13	-	-
ARHS/PO/14	512.73 ± 0.56	392.39 ±0.68
ARHS/PO/15	-	-
ARHS/PO/16	536.99 ± 0.84	399.09 ±0.81
ARHS/PO/17	363.36 ± 1.23	279.79 ±1.05
ARHS/PO/18	417.52 ± 1.43	325.63 ±0.86
ARHS/PO/20	387.70 ± 0.95	211.59 ±0.72
ARHS/PO/22	476.38 ± 0.80	271.27 ±0.92
ARHS/PO/23	519.13 ± 0.38	281.58 ±0.45
ARHS/PO/24	347.53 ± 0.47	203.63 ±0.87
ARHS/PO/25	447.14 ±0.77	255.75 ±0.55
ARHS/PO/26	629.56 ±1.11	409.28 ±1.67
ARHS/PO/27	-	-
ARHS/PO/28	514.64 ±0.91	264.76 ±0.61
ARHS/PO/29	509.36 ±0.47	326.74 ±0.40

TCP=tricalcium phosphate RP= rock phosphate, Values are mean of three replicates, ± =standard error

4.3.2. IAA production

Isolates were grown for 48h in high C/N ratio medium. Trytophane (0.1mM) was added in order to enhance acetic acid (IAA) production by the isolates. Production of IAA in culture supernatant was assayed by Pillet- Chollet method. The detailed result is summarised in Table 14. The result shows that IAA production is highest in ARHS/PO/26, ARHS/PO/27 and ARHS/PO/15. ARHS/PO/26 produces highest amount of IAA.

Table14. Quantification of IAA production in actinomycetes isolates

Isolates	IAA(mg/L)
ARHS/PO/11	11.21 ±0.33
ARHS/PO/12	15.32 ±0.47
ARHS/PO/13	7.61 ±0.15
ARHS/PO/14	16.61 ±0.75
ARHS/PO/15	20.56 ±0.59
ARHS/PO/16	18.70 ±0.34
ARHS/PO/17	11.47 ±0.32
ARHS/PO/18	12.09 ±0.54
ARHS/PO/20	9.35 ±0.28
ARHS/PO/22	13.42 ±0.64
ARHS/PO/23	10.50 ±0.49
ARHS/PO/24	13.76 ±0.26
ARHS/PO/25	12.39 ±0.46
ARHS/PO/26	23.70 ±0.32
ARHS/PO/27	21.46 ±0.45
ARHS/PO/28	17.44 ±0.51
ARHS/PO/29	14.39 ±0.44

Values are mean of three replicates, ± =standard error

4.4. *In vitro* Screening and evaluation for antifungal activity

4.4.1.Inhibition in solid media

All the isolates were tested *in vitro* for inhibiting growth of the pathogen in dual culture using PDA and SCN media. Each actinomycetes isolate was placed at one side of the agar plate about 1cm away from the edge by streaking and 7mm diameter block of the pathogen(*Fusarium solani* and *Sclerotium rolfsii*) taken from growing edge of the fungal culture was inoculated at the other half of the Petri plate. In another experiment actinomycetes were placed circling the pathogen. After 7 days inhibition zone towards the fungus colony in individual plate was recorded.

Results of *in vitro* pairing experiments were recorded and enlisted in table (15, 16) Figs. (9.10) The result shows that maximum inhibition against *Fusarium solani* was exhibited by ARHS/PO/27, similarly ARHS/PO/26 showed maximum inhibition against *Sclerotium rolfsii.*

Table 15. *In vitro* pairing of the isolates with S*clerotium rolfsii* for evaluation of antifungal activities

Interacting microorganisms	Isolates	Radius of fungal colony after 7 days growth(cm)	% of Inhibition
Sclerotium rolfsii		2.6±0.09	-
S. rolfsii+Streptomyces sp.	ARHS/PO/11	1.99±0.08	41.66
S. rolfsii+Streptomyces sp.	ARHS/PO/12	1.72±0.11	56.18
S. rolfsii+Streptomyces sp.	ARHS/PO/13	1.91±0.09	46.66
S. rolfsii+Streptomyces sp.	ARHS/PO/14	1.77±0.04	53.72
S. rolfsii+Streptomyces sp.	ARHS/PO/15	1.81±0.33	51.81
S. rolfsii+Streptomyces sp.	ARHS/PO/16	2.00±0.67	40.95
S. rolfsii+Streptomyces sp.	ARHS/PO/17	1.93±0.86	44.90
S. rolfsii+Streptomyces sp.	ARHS/PO/18	1.93±0.41	40.90
S. rolfsii+Streptomyces sp.	ARHS/PO/20	1.9±0.01	46.66
S. rolfsii+Streptomyces sp.	ARHS/PO/22	1.68±0.32	58.22
S. rolfsii+Streptomyces sp.	ARHS/PO/23	1.92±0.04	45.45
S. rolfsii+Streptomyces sp.	ARHS/PO/24	1.92±0.78	45.77
S. rolfsii+Streptomyces sp.	ARHS/PO/25	1.87±0.41	48.25
S. rolfsii+Streptomyces sp.	ARHS/PO/26	1.41±0.04	70.26
S. rolfsii+Streptomyces sp.	ARHS/PO/27	1.55±0.56	64.86
S. rolfsii+Streptomyces sp.	ARHS/PO/28	1.76±0.07	54.05
S. rolfsii+Streptomyces sp.	ARHS/PO/29	1.72±0.02	56.45

Values are mean of three replicates, ± =standard error

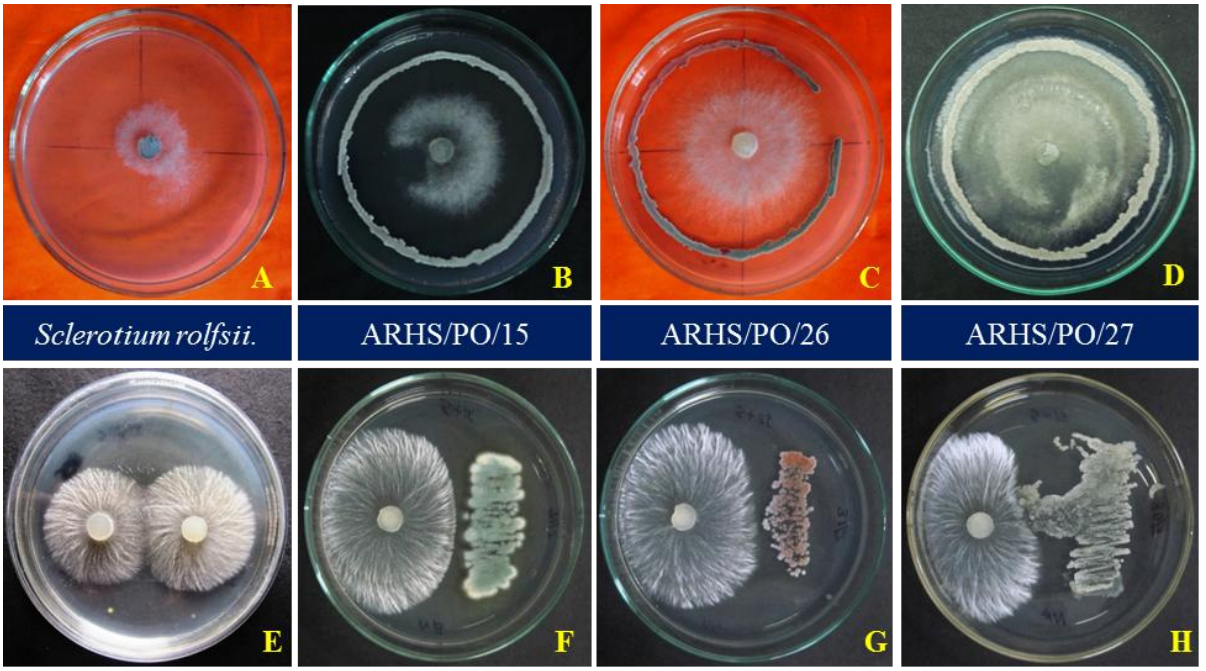

Figure 9: (A-H) *In vitro* antagonist activity assay of selected isolates (ARHS/PO/15, ARHS/PO/26 and ARHS/PO/27) with *Sclerotium rolfsii.;(*A &E) Control; (B-D) radial method ; (F-H) linear method

Table 16. *In vitro* pairing of the isolates with *Fusarium solani* for evaluation of antifungal activities

Interacting microorganisms	Isolates	Radius of fungal colony after 7 days growth(cm)	% of Inhibition
Fusarium solani		1.9±0.05	-
F.solani+Streptomyces sp.	ARHS/PO/11	1.07±0.01	67.8
F.solani+Streptomyces sp.	ARHS/PO/12	1.23±0.89	57.8
F.solani+Streptomyces sp.	ARHS/PO/13	1.07±0.63	67.8
F.solani+Streptomyces sp.	ARHS/PO/14	1.2±0.06	60.0
F.solani+Streptomyces sp.	ARHS/PO/15	1.17±0.07	62.2
F.solani+Streptomyces sp.	ARHS/PO/16	1.2±0.91	60.0
F.solani+Streptomyces sp.	ARHS/PO/17	1.2±0.51	60.0
F.solani+Streptomyces sp.	ARHS/PO/18	1.25±0.84	56.7
F.solani+Streptomyces sp.	ARHS/PO/20	1.09±0.07	66.7
F.solani+Streptomyces sp.	ARHS/PO/22	1.09±0.23	66.7
F.solani+Streptomyces sp.	ARHS/PO/23	1.2±0.44	60.0
F.solani+Streptomyces sp.	ARHS/PO/24	1.17±0.01	62.2
F.solani+Streptomyces sp.	ARHS/PO/25	1.07±0.03	67.8
F.solani+Streptomyces sp.	ARHS/PO/26	1.09±0.01	66.7
F.solani+Streptomyces sp.	ARHS/PO/27	0.64±0.03	88.5
F.solani+Streptomyces sp.	ARHS/PO/28	1.25±0.06	56.7
F.solani+Streptomyces sp.	ARHS/PO/29	1.15±0.31	63.4

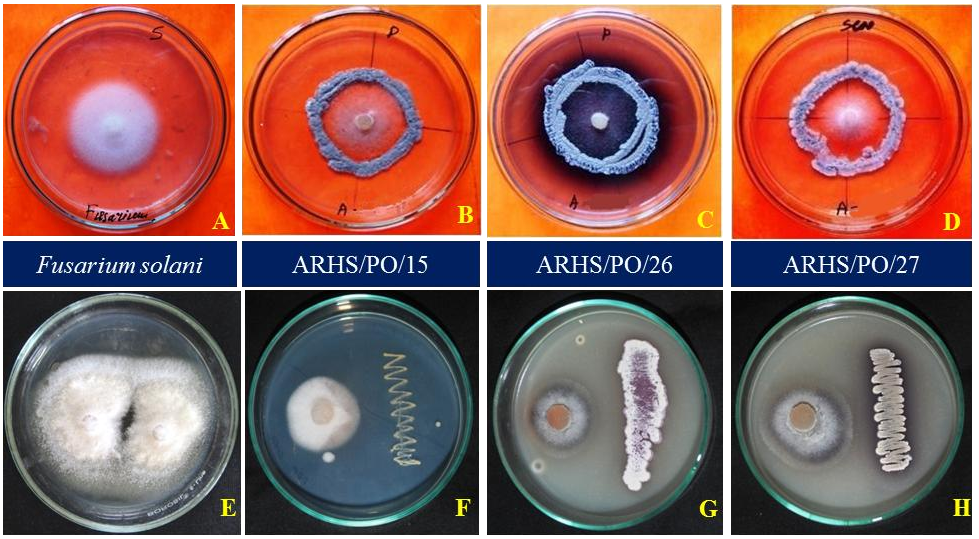

Figure 10: (A-H) *In vitro* antagonist activity assay of selected isolates (ARHS/PO/15, ARHS/PO/26, ARHS/PO/27) with *Fusarium solani*. (A &E) Control, (B-D) radial method ; F-H, linear method.

4.4.2. Inhibition of fungal growth by metabolites

4.4.2.1. Inhibition of spore germination

Fungal spores of *Fusarium solani* wereb ioassayed against metabolitesobtained from selected isolates . A drop of the test solution was placed on a clean, grease free grooved slide and following which a drop of spore suspension was placed over it. The slide was incubated in a moist Petri plate for 24 h at 30ºC ± 1 ºC. Finally one drop of lacto phenol - cotton blue solutionwas added to each spot to fix the germination of spore. The slides were observed under microscope and the percentage of germination was determined. The result showed that *Fusarium solani* spore germination was hampered in presence of metabolites suspension. ARHS/PO/27 had the highest germination inhibition rate with only 10.82% spore germination rate (Table 17, Fig 11).

Table 17. Effect of metabolites on spore germination of *Fusarium solani*

Treatment	% of spore germination
Control	89.7± 0.87
ARHS/PO/15	37.11± 0.65
ARHS/PO/26	32.6± 0.21
ARHS/PO/27	10.82± 0.86

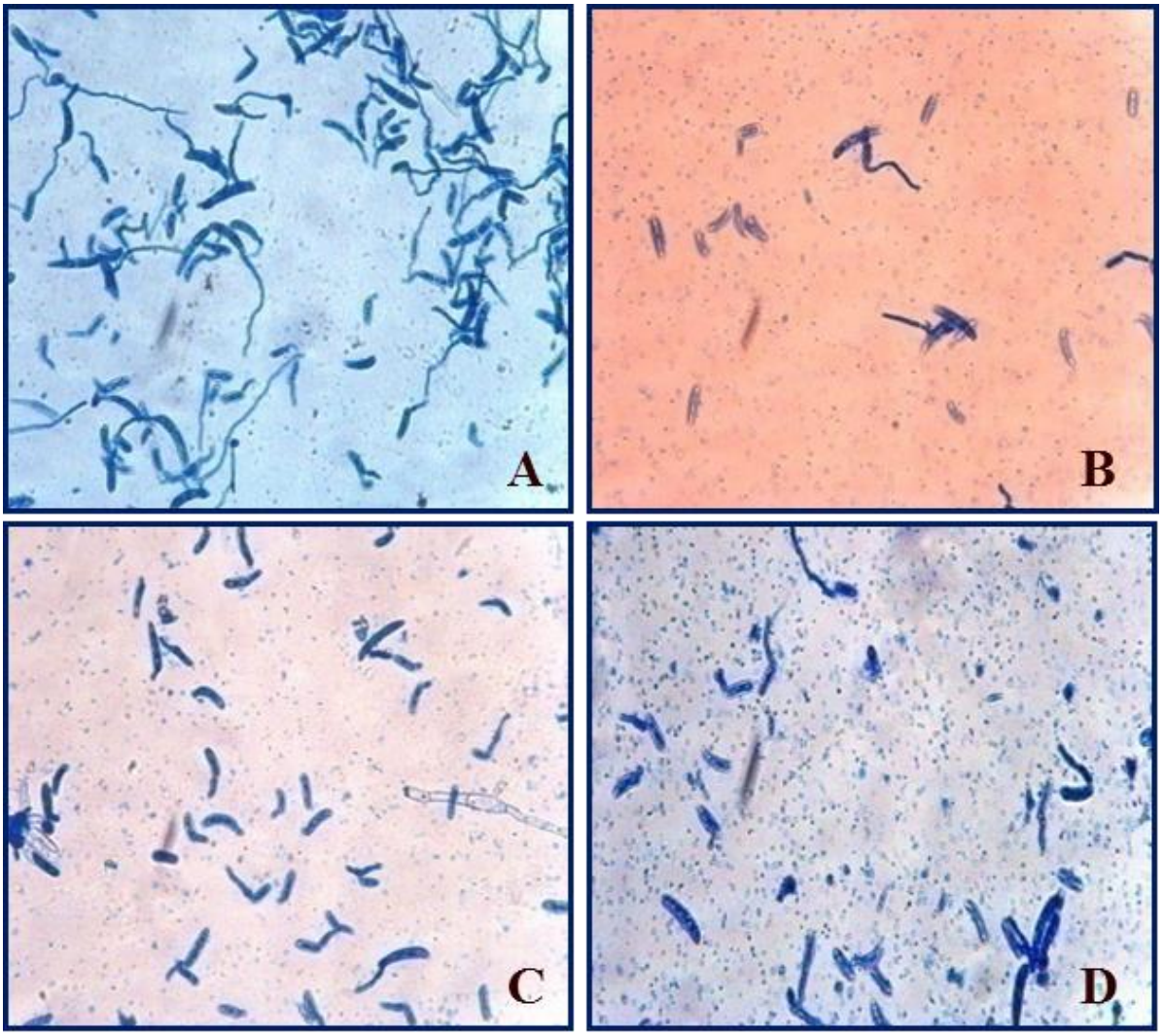

Figure 11: (A-D) Effect of metabolites of actinomycetes on conidial germination of *Fusarium solani.* A- Control ,B-ARHS/PO/15, C-ARHS/PO/26, D-ARHS/PO/27

4.4.2.2. Inhibition of mycelial growth

Autoclaved PDA medium (20ml) was mixed with 0.5ml of test compound solution and plated onto petriplates. After solidification, agar block from 4 days old cultures of *F.solani* and *Sclerotium rolfsii* was placed in the centre of each petriplate. The plates were incubated and radial growth of mycelium was measured.In case of *Fusarium solani* maximum inhibition was noted in metabolites of *Streptomyces flavogriseus* and in *Sclerotium rolfsii* maximum inhibition was noted in *S. tricolor* (Table 18,19)

Table 18. Effect of metabolites on radial growth of *Fusarium solani* after 48 hours incubation

Treatment	Diameter(mm)	% Inhibition
Control(*F. solani)*	8±0.98	-
F. solani+ S.griseus	6±0.70	43.74
F. solani+ S.tricolor	5±0.67	60.93
F. solani+ S.flavogriseus	2±0.03	93.75

Table 19. Effect of metabolites on radial growth of *Sclerotium rolfsii* after 72 hours incubation

Treatment	Diameter (mm)	% Inhibition
Control (*S.rolfsii)*	10±1.09	-
S. rolfsii+ S.griseus	3±0.07	91.0
S.rolfsii+ S.tricolor	2±0.08	96.0
S.rolfsii+ S.flavogriseus	7±0.99	51.0

4.4.2.3. Inhibition of sclerotia germination

For assessing the effect of the metabolites on the sclerotial germination of *S. rolfsii* the sclerotia were scrapped off from the culture growing in 7 days old PDA plates. The sclerotia were then soaked 1 hour in metabolites solution. After soaking the sclerotia were transferred asceptically to the petriplate containing sterile Black paper. These sterile filter papers were also soaked in metabolites solution for at least 30minutes and incubated at room temperature. Percent germination (Table 20) as well as the radial growth of the germinating sclerotia (Table 21), (Fig. 12) was measured.

Table 20. Effect of metaboliteson Sclerotia germination of phytopathogenic fungi

Treatment	**Germination % of Sclerotia**
Sclerotium rolfsii+ Streptomyces griseus	70.0
Sclerotium rolfsii + Streptomyces tricolor	40.0
Sclerotium rolfsii + Streptomyces flavogriseus	50.0

Table 21. Effect of metabolites on radial growth of Sclerotia after 72 hours incubation

Treatment	Diameter (mm)	% Inhibition
Control (*S rolfsii)*	15±1.8	-
S. rolfsii+ S.griseus	8±0.08	71.5
S.rolfsii+ S.tricolor	2±0.04	98.2
S.rolfsii+ S.flavogriseus	5±0.27	88.88

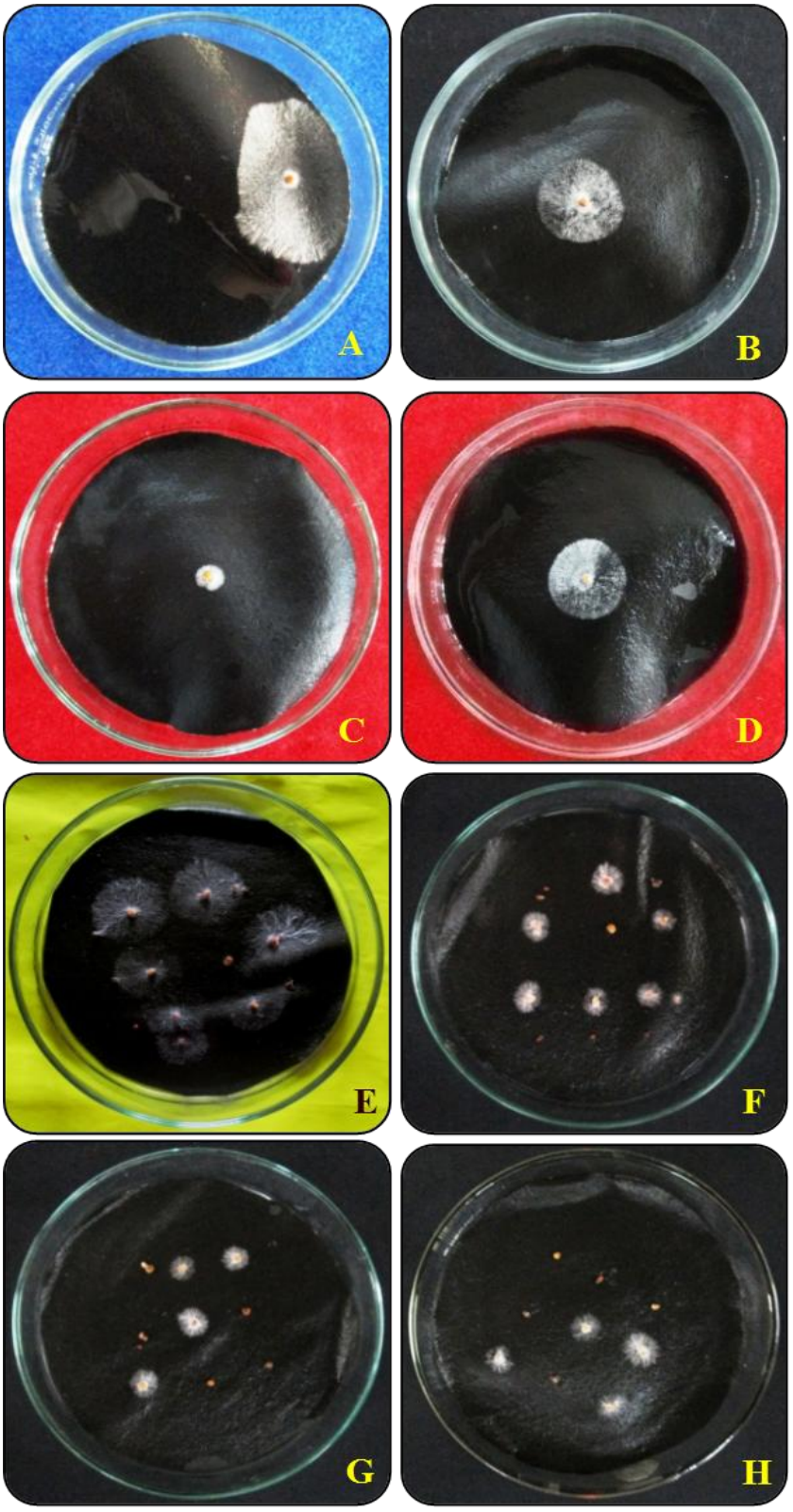

Figure12: (A-H): Effect of metabolites of actinomycetes on germination of sclerotia of *Sclerotium rolfsii*. (A–D) Single sclerotium; A- Control; B- ARHS/PO/15; C-ARHS/PO/26; D-ARHS/PO/27. (E-H) Germination of sclerotia; E-Control; F-ARHS/PO/15; G-ARHS/PO/26; H- ARHS/PO/27

4.5. *In vitro* Screening and evaluation for antibacterial activity.

In vitro screening and evaluation for antibacterial activities of the selected isolates were carried out with bacterial isolates of *Bacillus megaterium*, *Bacillus cereus* and *Escherichia coli*. *In vitro* pairing of the isolates with bacterial isolates shows that none of the isolates inhibit the growth of *Bacillus megaterium* which is a potent plant growth promoting rhizobacteria (Fig.13.)

Figure 13: *In vitro* screening for antibacterial activity.

4.6 Molecular detection

Genomic DNA of potent isolates ARHS PO 15, ARHS PO 26 and ARHS PO 27 were suspended in 100µl 1X TE buffer and stored in -20°C until further use. Agarose gel electrophoresis of genomic DNA revealed that they were RNA free. Purity of DNA evaluated in terms of the ratio between absorbance of A260 and A280 showed that genomic DNA was ~1.8. PCR amplification of ITS region of 16 S rDNA was carried out using primer pair, Forward primer:5'AGAGTTGATCMTYGCTWAC3' and reverse primer 5'CGYTAMCTTWTTACGRCT3'.

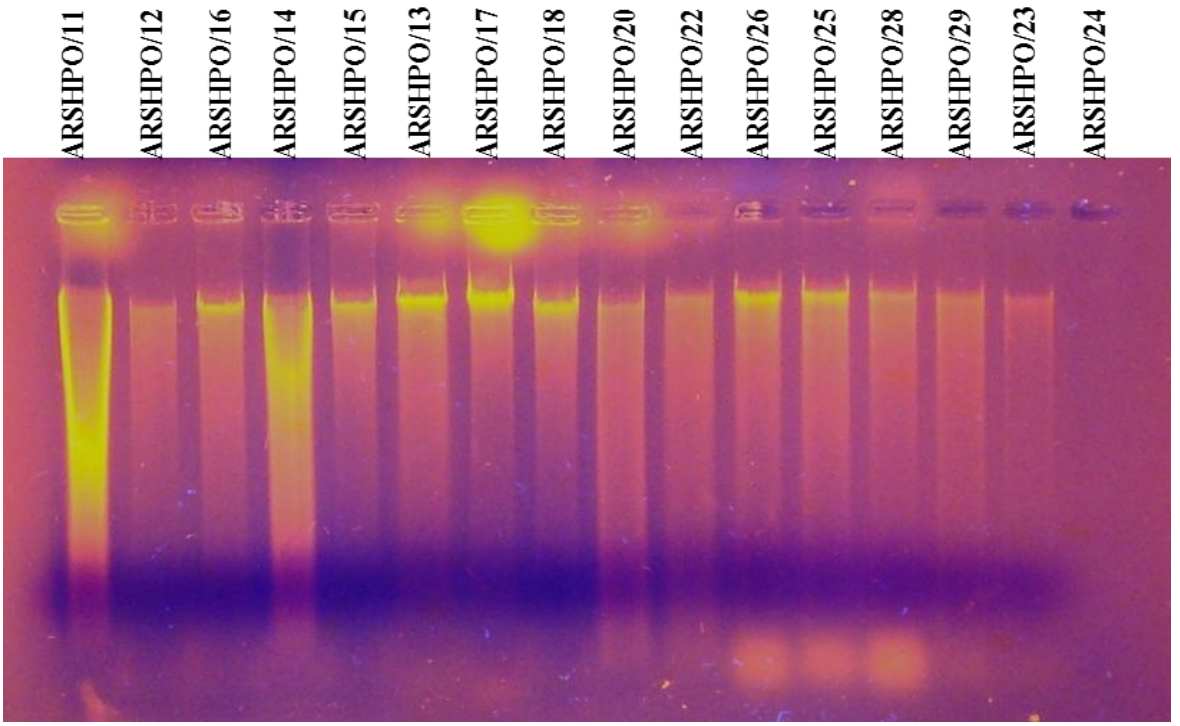

Figure14: Agarose Gel electrophoresis of genomic DNA of isolates from various sources.

4.6.1*Streptomyces griseus*

4.6.1.1. 16 S rDNA sequence analysis

The BLAST query of the 16S rDNA sequence of ARHS PO 15 against GenBank database confirmed that the isolate is *Streptomyces griseus.* The sequences have been deposited in NCBI, GenBank database under the accession no. KX894282.The sequence and chromatograms have been represented in Figure 15.

4.6.1.2 Multiple Sequence Alignment

A multiple sequence alignment of ITS gene sequences of *Streptomyces griseus* was conducted. Sequences of other strains obtained from NCBI GenBank database showing maximum homology with the strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 16).

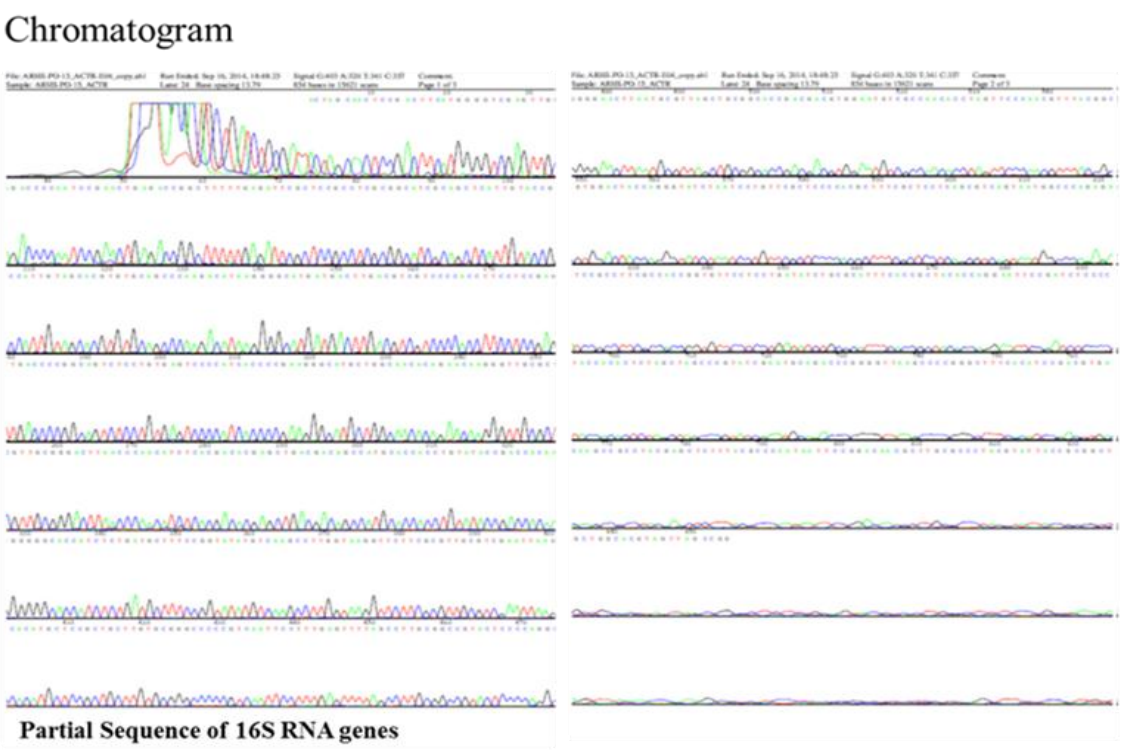

Fig.15 . Chromatogram and sequence deposit of 16S r RNA region *Streptomyces griseus* ARHS/PO/15 (NCBI-KX894282)

Figure16: 16S RNA gene sequence alignments of isolate *Streptomyces griseus* ARHS/PO/15 (NCBI-KX894282) with other extypes from NCBI GenBank Database.

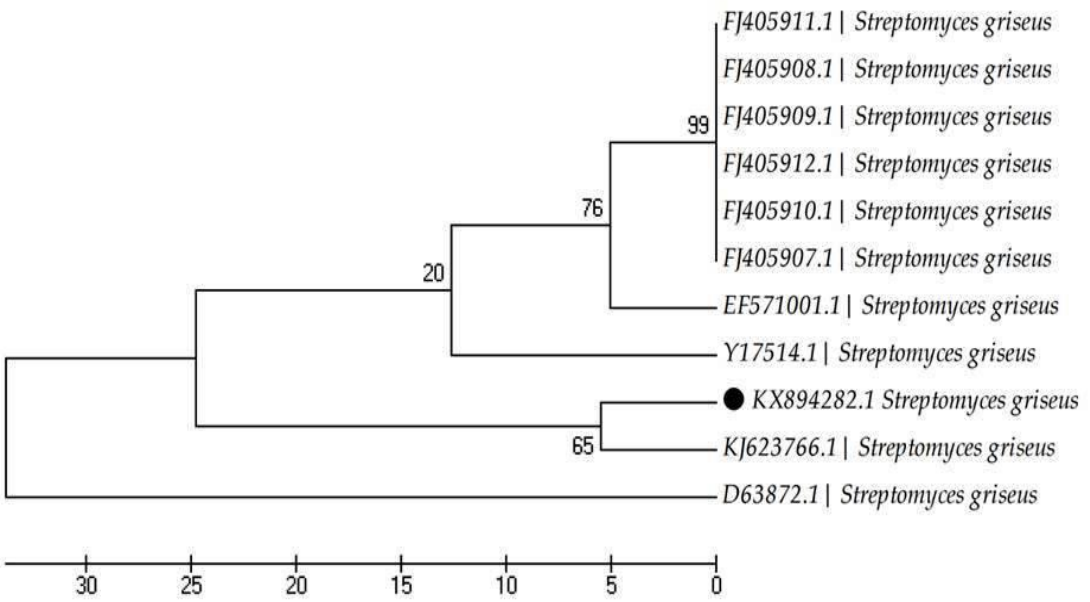

Figure17: Phylogenetic placement of *Streptomyces griseus*(KX894282) with other ex-type strain sequences obtained from NCBI GenBank Database.

4.6.1.3 Phylogenetic analysis

Phylogenetic analysis was carried out with extype strain sequences obtained from NCBI Genbank database which showed maximum homology with *Streptomyces griseus*(KJ623766) (Table 22).The evolutionary history was inferred using the UPGMA method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 435 positions in the final dataset. Phylogenetic analyses were conducted in MEGA 4(Fig. 17)

Table 22: GenBank accession numbers and geographic location of extype strains of *Streptomyces griseus* that showed homology with isolate ARHS/PO/15

Sl No	Accession No	Strain or Isolate	rDNA Sequence	Origin
1	FJ405911	D30	1365bp	China
2	FJ405908	FXJ124	1365bp	China
3	FJ405909	FXJ162	1365bp	China
4	FJ405912	E3	1365bp	China
5	FJ405910	FXJ175	1365bp	China
6	FJ405907	FXJ70	1365bp	China
7	EF571001	52-1	1487bp	Hungary
8	Y17514	10/ppi	435bp	USA
9	KJ623766	S131	1411bp	Egypt
10	D63872	ATCC25497	1532bp	Japan
11	**KX894282**	**ARSH/PO/15**	**852bp**	**India**

4.6.2. *Streptomyces tricolor*

4.6.2.1. 16 S rDNA sequence analysis

The BLAST query of the 16S rDNA sequence of ARHS PO 26against GenBank database confirmed that the isolate is *Streptomyces tricolor*. The sequences have been deposited in NCBI, GenBank database under the accession no. KX894280.The sequence and chromatograms have been represented in Figure 18.

4.6.2.2 Multiple Sequence Alignment

A multiple sequence alignment of ITS gene sequences of *Streptomyces tricolor* was conducted. Sequences of other strains obtained from NCBI GenBank database showing maximum homology with the strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 19).

Chromatogram

Partial Sequence of 16S RNA genes

GTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA
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GCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCTAACTCGACCCCATGAAGTCGGAGT
CGCTA GTAAT

Sequence Deposited: NCBI
Accession No: KX894280
Version : KX894280.1
DNA Linear: 1055bp

Title:
***Streptomyces tricolor* strain ARHS/PO/26 16S ribosomal RNA gene, partial sequence**

Origin

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```

Figure 18: Chromatogram and sequence deposit of 16S r RNA region of *Streptomyces tricolor* ARHS/PO/26 (NCBI-KX894280)

Figure 19: 16S r RNA gene sequence alignments of isolate *Streptomyces tricolor* ARHS/PO/26 (NCBI-KX894280) with other extypes from NCBI GenBank Database.

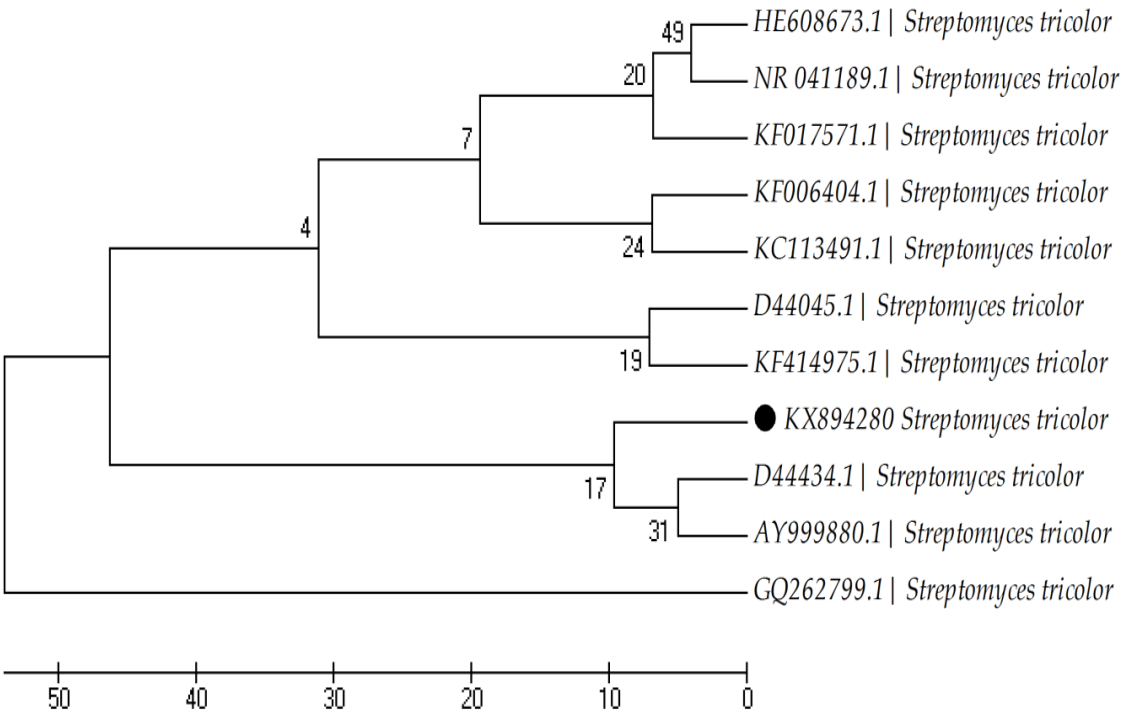


Figure20: Phylogenetic placement of *Streptomyces tricolor*(KX894280)with other ex-type strain sequences obtained from NCBI GenBank Database.

4.6.2.3 Phylogenetic analysis

Phylogenetic analysis was carried out with extype strain sequences obtained from NCBI GenBank database which showed maximum homology with *Streptomyces tricolor*(AY999880) (Fig.20) (Table 23).The evolutionary history was inferred using the UPGMA method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 119 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 .

Table 23: GenBank accession numbers and geographical location of ex-type strains of *Streptomyces* species that showed homology with isolate ARHS/PO/26

Sl No	Accession No	Strain or Isolate	rDNA Sequence	Origin
1	HE608673	D0710T2_2B_S3	784bp	Spain
2	NR041189	NBRC15461	1450bp	Japan
3	KF017571	ICN14	1314bp	India
4	KF006404	ERINLG	1030bp	India
5	KC113491	Vh85	1339bp	India
6	D44045	JCM4295	120bp	Japan
7	KF41975	Mhce0811	788bp	India
8	D44434	JCM5065	121bp	Japan
9	AY999880	AS4.1867/CSSP401	1406bp	USA
10	GQ262799	Vh85	1341bp	India
11	**KX894280**	**ARSH/PO/26**	**1055bp**	**India**

4.6.3 *Streptomyces flavogriseus*

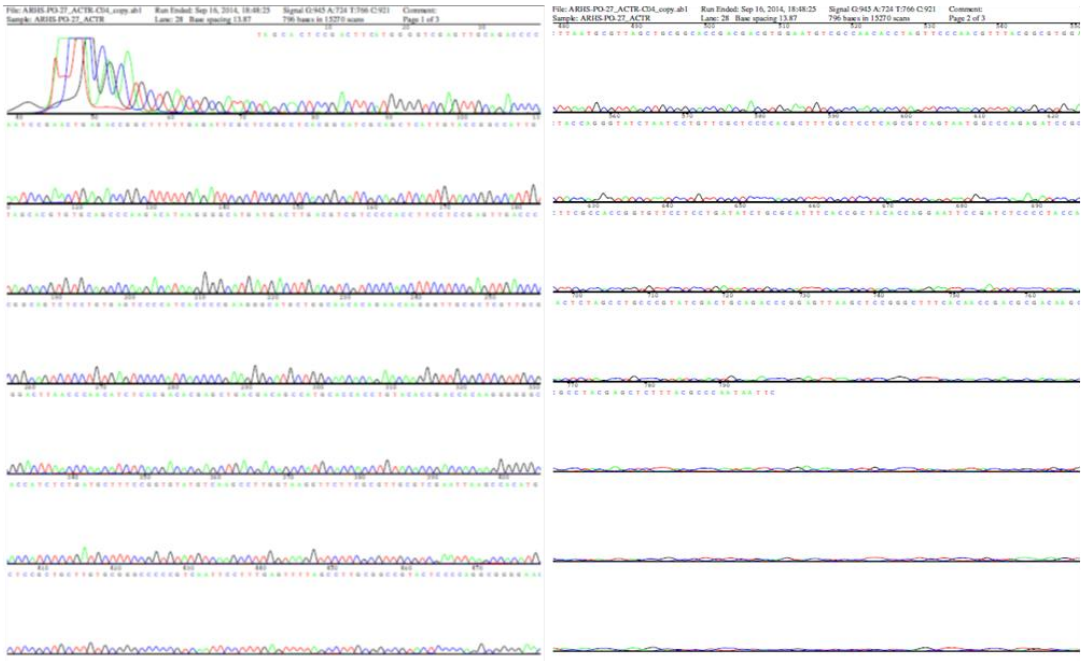
4.6.3.1. 16 S rDNA sequence analysis

The BLAST query of the 16S rDNA sequence of ARHS PO 27against GenBank database confirmed that the isolate is *Streptomyces flavogriseus*. The sequences have been deposited in NCBI, GenBank database under the accession no. KX894281.The sequence and chromatograms have been represented in Figure 21 .

4.6.3.2. Multiple Sequence Alignment

A multiple sequence alignment of ITS gene sequences of *Streptomyces flavogriseus* was conducted. Sequences of other strains obtained from NCBI GenBank database showing maximum homology with the strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 22).

Chromatogram



Partial Sequence of 16S RNA genes

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Origin

Sequence Deposited: NCBI
Accession No: KX894281
Version : KX894281.1
DNA Linear: 1045bp

Title:
***Streptomyces flavogriseus* strain ARHS/PO/26**
16S ribosomal RNA gene, partial sequence

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Figure 21: Chromatogram and sequence deposit of 16S r RNA region *Streptomyces flavogriseus* ARHS/PO/27(NCBI-KX894281)

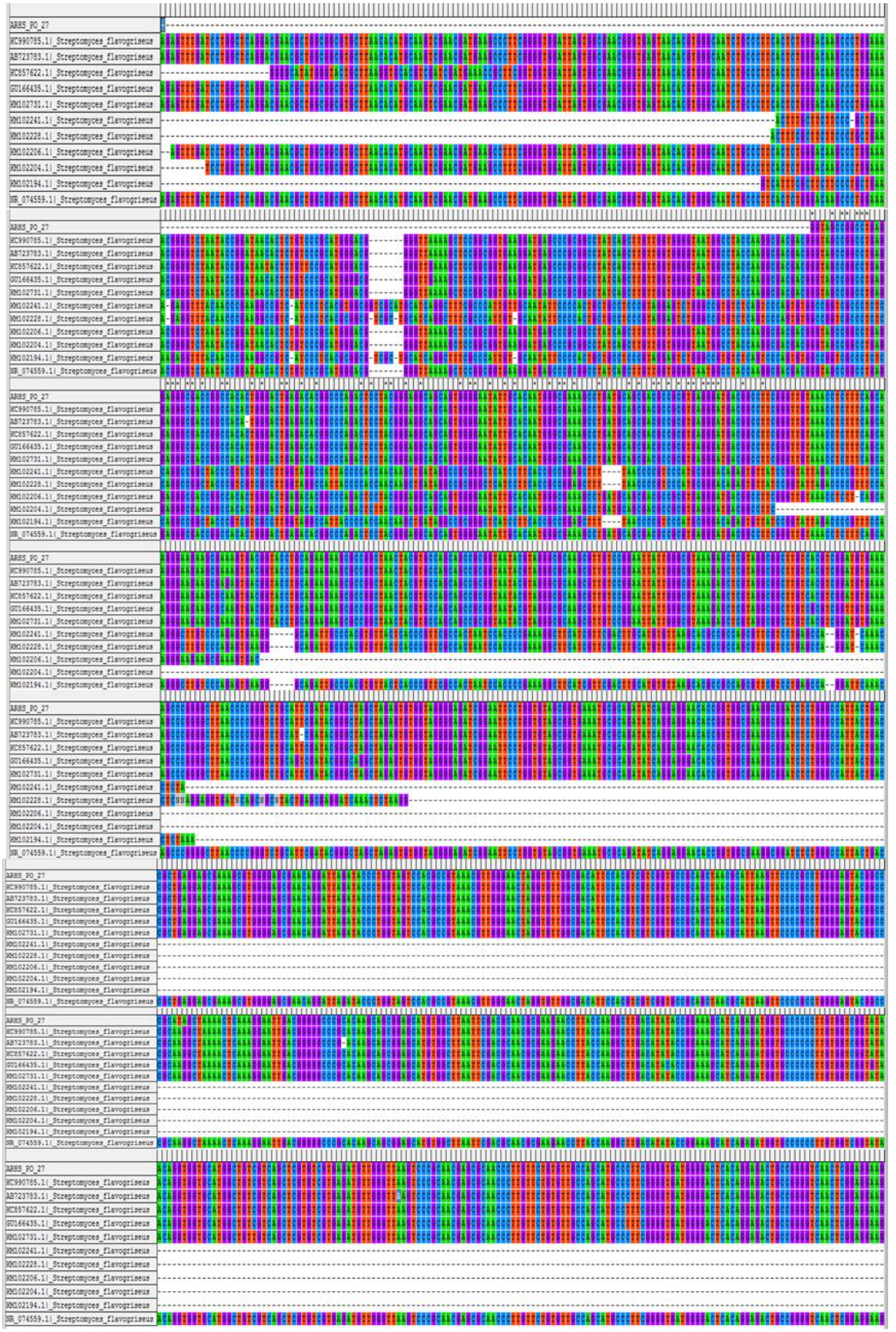


Figure22: 16S rRNA gene sequence alignments of isolate *Streptomyces flavogriseus* ARHS/PO/ 27 (NCBI-KX894281) with other extypes from NCBI GenBank Database.

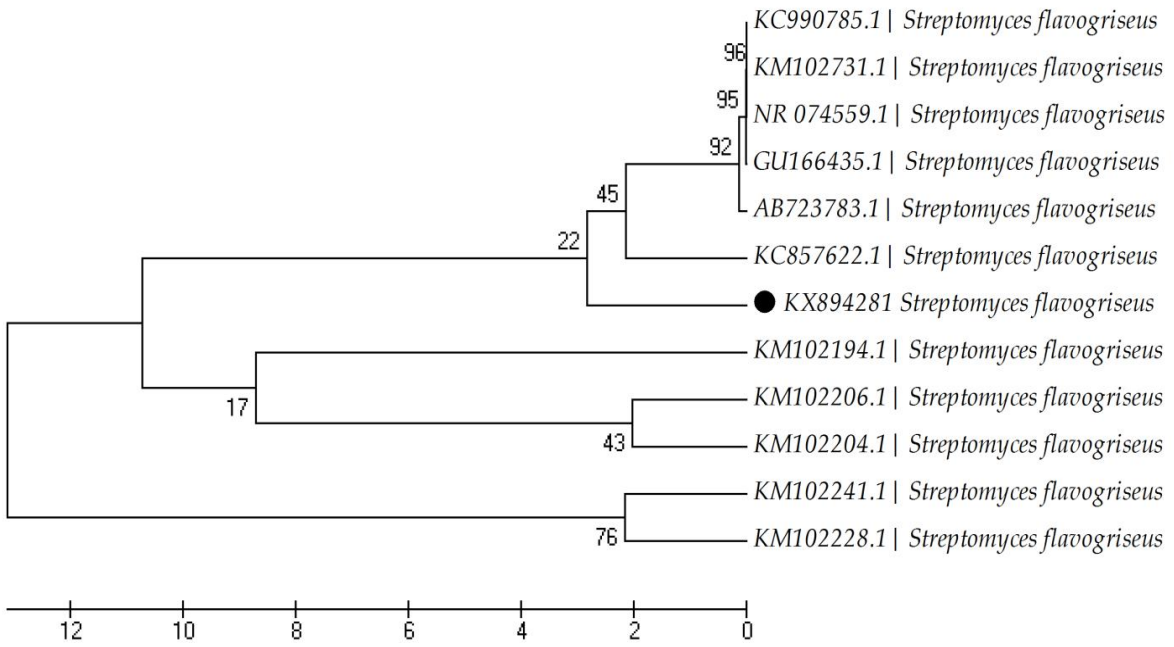


Figure 23: Phylogenetic placement of *Streptomyces flavogriseus*(KX894281)with other ex-type strain sequences obtained from NCBI GenBank Database

4.6.3.3 Phylogenetic analysis

Phylogenetic analysis was carried out with extype strain sequences obtained from NCBI GenBank database which showed maximum homology with *Streptomyces flavogriseus* (KC857622) (Fig.23).The evolutionary history was inferred using the UPGMA method . The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 398 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4.(Fig 23)

Table 24: GenBank accession numbers and geographical location of extype strains of *Streptomyces* species that showed homology with isolate ARHS/PO/27

Sl No	Accession No	Strain or Isolate	rDNA Sequence	Country
1	KC990785	ACTK2	1483bp	India
2	KM102731	NJ-4	1484bp	China
3	NR074559	ATCC33331	1514bp	USA
4	GU166435	030	1489bp	Korea
5	AB723783	NRC2012	1197bp	Egypt
6	KC857622	NRC10	1350bp	Saudi Arabia
7	KM102194	2LA10	451bp	Canada
8	KM102206	3LD2	445bp	Canada
9	KM102204	5LA3	398bp	Canada
10	KM102241	8LC11	446bp	Canada
11	KM102228	6LE2	490bp	Canada
12	**KX894281**	**ARSH/PO/27**	**1045bp**	**India**

4.6.4. Multiple sequence alignment *of Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

A multiple sequence alignment of ITS gene sequences of *Streptomyces griseus Streptomyces tricolor, Streptomyces flavogriseus* was conducted. Sequences of other strains obtained from NCBI GenBank database showing maximum homology with the strains was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 24).

Figure24: Multiple sequence alignment of *Streptomyces griseus* (KX894282*), Streptomyces tricolor* (KX894280) and *Streptomyces flavogriseus* (KX894281) with other extypes from NCBI

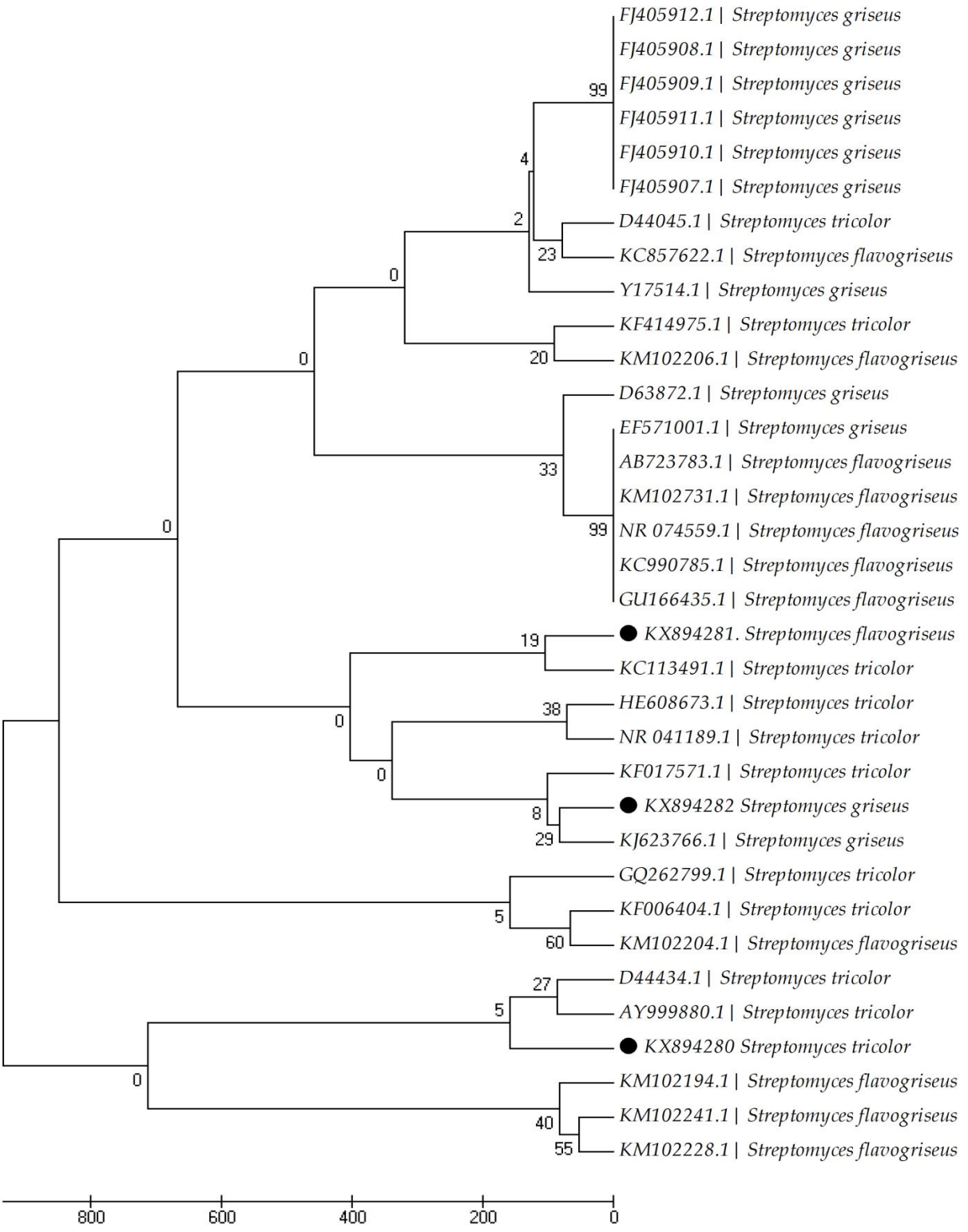


Figure25: UPGMA based Phylogenetic placement of *Streptomyces griseus* (KX894282), *Streptomyces tricolor* (KX894280) and *Streptomyces flavogriseus* (KX894281) with other extype strain sequences obtained from NCBI GenBank Database.

Table 24: GenBank accession numbers and geographic location of extype strains of *Streptomyces griseus, Streptomyces tricolor and Streptomyces flavogriseus* that showed homology with isolate ARHS/PO/15, ARHS/PO/26, ARHS/PO/27

Sl No	Accession No	Strain or Isolate	rDNA Sequence	Country
1	FJ405911	D30	1365bp	China
2	FJ405908	FXJ124	1365bp	China
3	FJ405909	FXJ162	1365bp	China
4	FJ405912	E3	1365bp	China
5	FJ405910	FXJ175	1365bp	China
6	FJ405907	FXJ70	1365bp	China
7	EF571001	52-1	1487bp	Hungary
8	Y17514	10/ppi	435bp	USA
9	KJ623766	S131	1411bp	Egypt
10	D63872	ATCC25497	1532bp	Japan
11	**KX894282**	**ARSH/PO/15**	**852bp**	**India**
12	HE608673	D0710T2_2B_S3	784bp	Spain
13	NR041189	NBRC15461	1450bp	Japan
14	KF017571	ICN14	1314bp	India
15	KF006404	ERINLG	1030bp	India
16	KC113491	Vh85	1339bp	India
17	D44045	JCM4295	120bp	Japan
18	KF41975	Mhce0811	788bp	India
19	D44434	JCM5065	121bp	Japan
20	AY999880	AS4.1867/CSSP401	1406bp	USA
21	GQ262799	Vh85	1341bp	India
22	**KX894280**	**ARSH/PO/26**	**1055bp**	**India**
23	KC990785	ACTK2	1483bp	India
24	KM102731	NJ-4	1484bp	China
25	NR074559	ATCC33331	1514bp	USA
26	GU166435	030	1489bp	Korea
27	AB723783	NRC2012	1197bp	Egypt
28	KC857622	NRC10	1350bp	Saudi Arabia
29	KM102194	2LA10	451bp	Canada
30	KM102206	3LD2	445bp	Canada
31	KM102204	5LA3	398bp	Canada
32	KM102241	8LC11	446bp	Canada
33	KM102228	6LE2	490bp	Canada
34	**KX894281**	**ARSH/PO/27**	**1045bp**	**India**

4.6.5. Phylogenetic analysis

Phylogenetic analysis was carried out with extype strain sequences obtained from NCBI GenBank database which showed maximum homology with *Streptomyces griseus* (KX894282), *Streptomyces tricolor* (KX894280) and *Streptomyces flavogriseus*(KX894281)(Table 25). The evolutionary history was inferred using the

UPGMA method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 119 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Figure 25).

4.7. Evaluation of potent Actinomycetes isolates on plant growth promotion

On the basis of initial screening of the antagonism of the isolates against the plant pathogenic fungi, phosphate solubilizing activity, IAA production three isolates were selected for enhancement of growth of two crop plant, *Phaseolus vulgaris* and *Vigna radiata*. Growth promotions in seedlings were evaluated in terms of increase in height, shoot and root length, number of leaf, leaf area, root shoot fresh and dry weight of the treated as well as untreated control plants. The result shows that the isolates have significant positive effect on the growth of the two crop plants, *Phaseolus vulgaris* and *Vigna radiata* in pot condition. Enhancement of nodule formation was also taken into account and it was found that nodulation frequency increased in treated plants in comparison to the untreated plants though Nodulation Index did not vary that much.

4.7.1. Evaluation of potential Growth promotion of *Phaseolus vulgaris* upon treatment

The Isolates *Streptomyces griseus* (KX894282), *S. tricolor* (KX894280) and *S.flavogriseus* (KX894280) have positive effect in *Phaseolus vulgaris* growth promotion. Treatments were done in form of seed coating, foliar spray and soil drench in regular intervals. The final result showed that *S. tricolor* (KX894280) is the most effective in plant growth promotion.Growth promotion in *Phaseolus vulgaris* after treatment with three potent isolates is summarized in the Table (26 and 27) and Fig(26 and 27).

Table 26: Growth promotion in *Phaseolus vulgaris* following treatment with actinomycetes

Treatment		Shoot length(cm)			Root length(cm)		
		7 days	**15 days**	**30days**	**7 days**	**15 days**	**30days**
CV2	Untreated Healthy	9.5 ±1.02	10.00±0.12	20.00±1.82	2.1±0.02	3.1±0.02	8.80±0.42
	Treated with *Streptomyces griseus*	9.7±0.98	12.00±0.18	30.00±3.18	2.9±0.20	3.9±1.52	17.00±1.32
	Treated with *S. tricolor*	10.6±1.20	15.15±0.75	33.75±1.70	2.5±0.07	4.7±1.71	15.38±1.07
	Treated with *S. flavogriseus*	11.7±1.03	14.00±0.53	30.25±1.93	3.0±.08	4.1±0.87	20.25±4.87
CV3	Untreated Healthy	8.9±1.15	11.2±0.51	23.25±1.65	2.8±0.04	3.4±1.12	10.80±1.64
	Treated with *S. griseus*	9.2±1.21	13.00±0.27	28.50±2.21	2.3±0.05	3.2±0.92	14.75±3.52
	Treated with *S. tricolor*	10.3±1.10	14.9±0.10	35.50±2.10	3.1±.08	4.8±0.08	17.80±1.08
	Treated with *S. flavogriseus*	9.7±1.11	12.50±1.19	31.50±1.19	2.8±0.14	4.7±0.14	14.83±0.44
CD (P=0.05)	Treatments	1.10	2.81	4.46	1.41	1.25	8.38
	Varieties	0.78	1.99	3.15	1.00	0.88	5.92

Values are mean of 10 plants. ± denote standard errorCV2=Cultivar 2(Jwala), CV3=Cultivar3(Kholar). Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

Table 27: Growth promotion in *Phaseolus vulgaris* after treatment with actinomycetes isolates.

Treatment		Leaf number			Leaf area(cm^2)		
		7 days	**15 days**	**30days**	**7 days**	**15 days**	**30days**
C2	Untreated Healthy	2±1.02	3±0.41	11±0.12	15±0.04	23.5±0.1	28.40±4.04
	Treated with *Streptomyces griseus*	3±0.98	5±1.09	13±0.32	18±0.05	29.8±1.51	35.06±0.85
	Treated with *S. tricolor*	4±1.20	8±1.34	15±0.37	21±1.17	45.2±0.97	79.50±4.17
	Treated with *S. flavogriseus*	2±1.03	6±0.44	13±0.77	16.5±0.24	37.9±0.34	62.70±3.64
C3	Untreated Healthy	2±1.15	3±1.14	10±0.61	19.2±1.87	29.2±1.36	49.63±8.37
	Treated with *Streptomyces griseus*	3±1.21	4±0.52	14±1.22	20.5±0.36	35.6±0.16	72.48±4.66
	Treated with *S. tricolor*	2±1.10	4±1.78	17±0.18	23.7±2.32	46.8±2.22	87.18±9.32
	Treated with *S. flavogriseus*	3±1.11	5±1.04	14±0.74	22.01±1.64	41.6±0.64	78.90±5.84
CD (P=0.05)	Treatments	2.83	3.89	2.83	3.17	4.46	28.16
	Varieties	2.00	2.75	2.00	2.24	3.15	19.91

Values are mean of 10 plants. ± denote standard errorC2=Cultivar 2(Jwala), C3=Cultivar 3(Kholar), Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

4.7.2. Evaluation of potential Growth promotion of *Vigna radiata* upon treatment

The isolates *Streptomyces griseus* (ARHS/PO/15), *S. tricolor* (ARHS/PO/26) and *S. flavogriseus* (ARHS/PO/27) have positive effect in *Vigna radiata* growth promotion. Treatments were done in form of seed coating, foliar spray and soil drench in regular intervals. The final result showed that *S.tricolor* (ARHS/PO/26) is the most effective in plant growth promotion.Growth promotion in *Vigna radiata* after treatment with three potent isolates is summarized in the Table (28, 29) and Figure (28,29).

Table 28: Growth promotion in *Vigna radiata* following treatment with Actinomycetes

Treatment	**Shoot length(cm)**			**Root length(cm)**		
	7 days	**15 days**	**30days**	**7 days**	**15 days**	**30days**
Untreated Healthy	09.0±01.73	14.0±0.21	19±1.21	2.0±0.67	2.85±0.55	4.52±0.16
Treated with *Streptomyces griseus*	15.0±01.14	19.0±1.63	26±1.73	2.6±0.93	4.7±1.12	9.05±1.74
Treated with *S. tricolor*	13.0±01.05	18.0±1.75	27±1.04	2.5±0.46	4.5±1.22	9.72±0.36
Treated with *S. flavogriseus*	14.0±0.1.73	17.0±1.54	23±1.11	2.3±1.22	4.6±1.33	9.51±0.54

Values are mean of 10 plants. ± denote standard error. Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

Table 29: Growth promotion in *Vigna radiata* after treatment with actinomycetes

Treatment	**Leaf number**			**Leaf area(cm^2)**		
	7 days	**15 days**	**30days**	**7 days**	**15 days**	**30days**
Untreated Healthy	4±0.67	6±0.67	16±0.67	6.85±0.25	6.25±1.24	7.78±0.33
Treated with *Streptomyces griseus*	6±0.93	15±0.93	21±0.93	8.8± 1.15	6.3± 1.14	9.49±0.41
Treated with *S. tricolor*	6±0.46	10±0.46	23±0.46	8.55±1.24	6.45±1.22	9.77±0.33
Treated with *S. flavogriseus*	7±1.22	12±1.22	20±1.22	8.6±0.54	6.5±0.73	8.98±0.41

Values are mean of 10 plants. ± denote standard error . Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

4.7.3. Effect of treatment upon nodule formation

4.7.3.1. Effect on nodule formation in *Phaseolus vulgaris*

Effect of the potent isolates upon nodulation was evaluated by comparing the nodulation frequency of the isolates in pot condition. Though the nodulation frequency revealed positive effect of treatments on nodule formation, nodulation Index among the treatments do not have much deviation from the control (Figs. 30, 32) and Table 30.

Table 30: Nodulation frequency and Nodulation Index of pot grown one month old *Phaseolus* plants among different treatments

Cultivar	Treatments	Nodule frequency	Nodulation Index
Jwala(CV2)	Untreated Healthy	16.6±1.02	8.5±0.66
	Treated with *Streptomyces griseus*	18.2±0.98	9.7±0.49
	Treated with *S. tricolor*	23.3±1.23	11.5±0.42
	Treated with *S. flavogriseus*	20.9±2.05	10.31±0.01
Kholar (CV3)	Untreated Healthy	17.9±0.74	9.78±0.2
	Treated with *Streptomyces griseus*	19.5±0.23	9.93±1.06
	Treated with *S. tricolor*	19.7±0.02	12.04±1.22
	Treated with *S. flavogriseus*	21.3±0.03	10.55±1.09
CD(P=0.05)	Treatments	5.26	1.10
	Varieties	3.72	0.78

Values are mean of 10 plants. ± denote standard error CV2=Cultivar 2(Jwala), CV3=Cultivar 3(Kholar), Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*

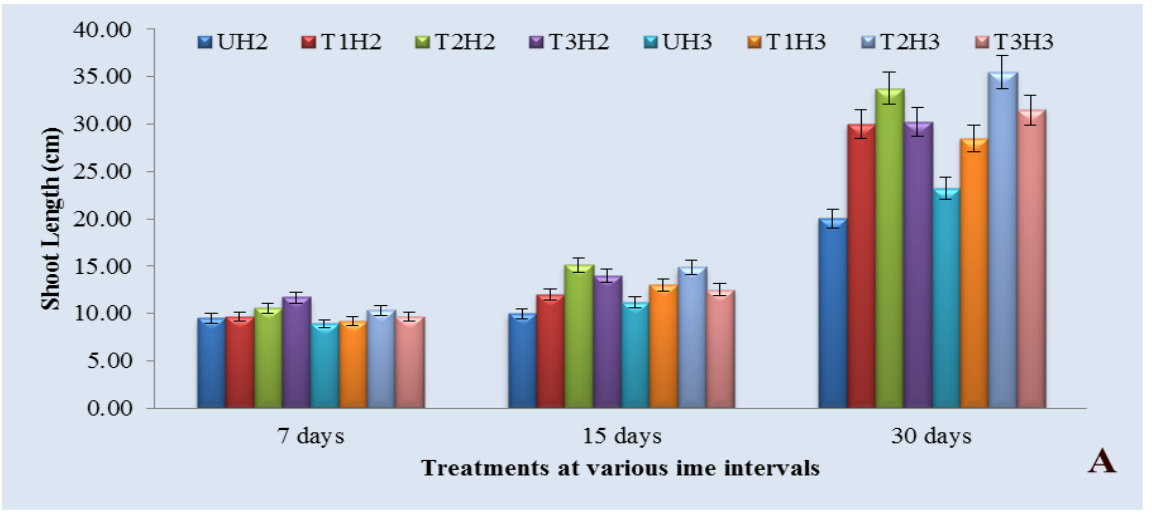
4.7.3.2. Effect on nodule formation in *Vigna radiata*

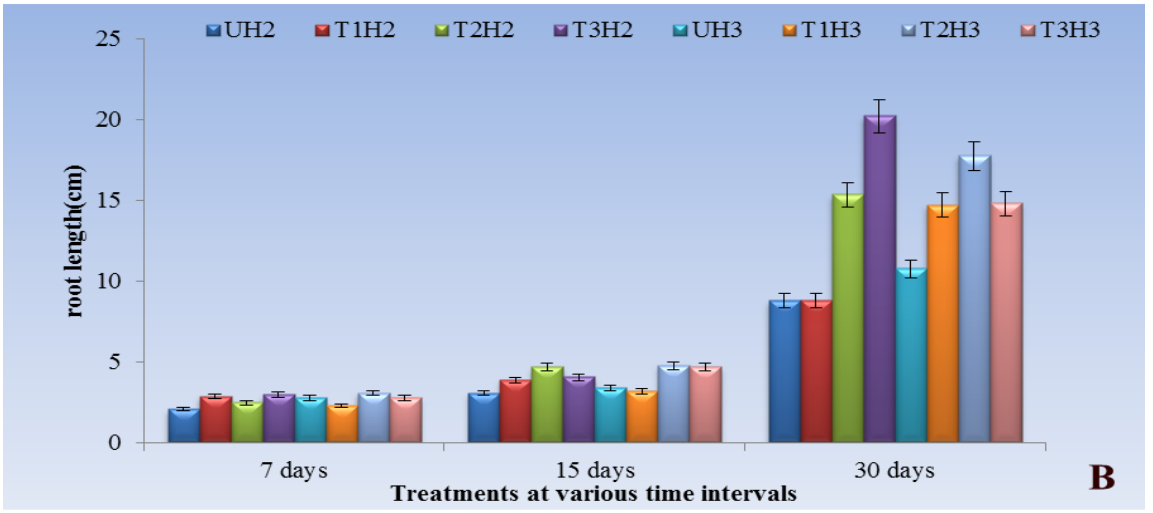
Effect of the potent isolates upon nodulation was evaluated by comparing the nodulation frequency of the isolates in pot condition. Though the nodulation frequency revealed positive effect of treatments on nodule formation, nodulation Index among the treatments do not have much deviation from the control (Fig 31, 33) table 31.

Table 31: Nodulation frequency and Nodulation Index of pot grown one month old *Vigna* plants among different treatments

Treatment	Nodule frequency	Nodulation Index
Untreated Healthy	4.57±0.67	6.0±0.03
Treated with *Streptomyces griseus*	5.87±0.25	7.0±0.05
Treated with *S. tricolor*	8.25±0.09	8.5±0.97
Treated with *S. flavogriseus*	7.25±0.11	6.25±1.05

Values are mean of 10 plants. ± denote standard error. Treatment with *Streptomyces griseus, Streptomyces tricolor, Streptomyces flavogriseus*





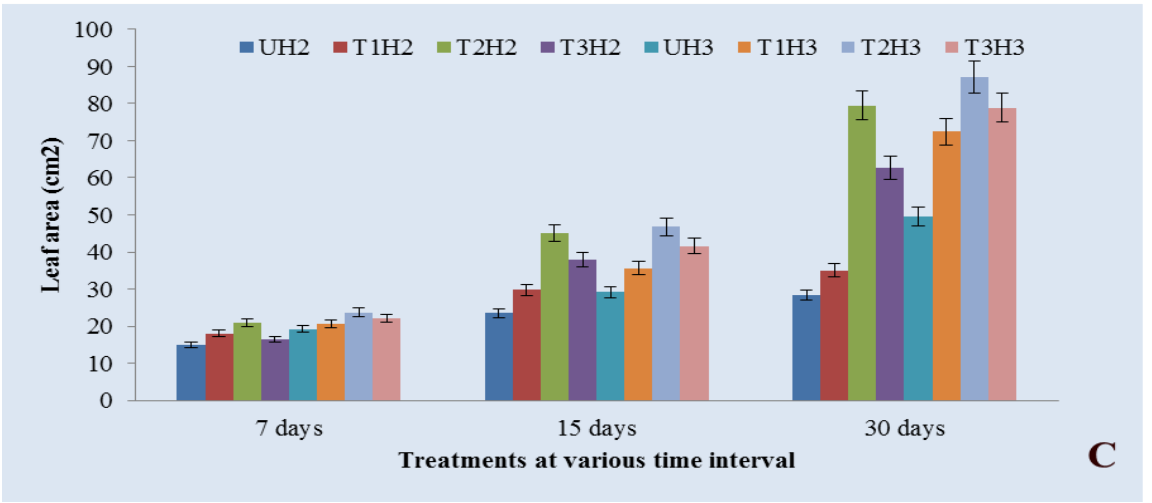


Figure 26: Evaluation of growth promotion (A) Shoot Length , (B) root Length & (C) Leaf Area of varieties of *P vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani***.** UH2= Untreated Healthy (Jwala/CV2), UH3= Untreated Healthy (Kholar/CV3) T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3= Treated with *Streptomyces flavogriseus.*

Figure 27: (A-L) *Phaseolus vulgaris* in open field and pot condition ; (A-D) *Phaseolus vulgaris* in open field condition ; (E-H) *Phaseolus vulgaris* Cultivar Jwala (CV2) in pot condition,(I-L) *Phaseolus vulgaris* Cultivar Kholar (CV3) in pot condition,(E & I) Control, (F&J) T1, (G&K) T2,(H&L) T3.T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor*,T3= Treated with *Streptomyces flavogriseus.*

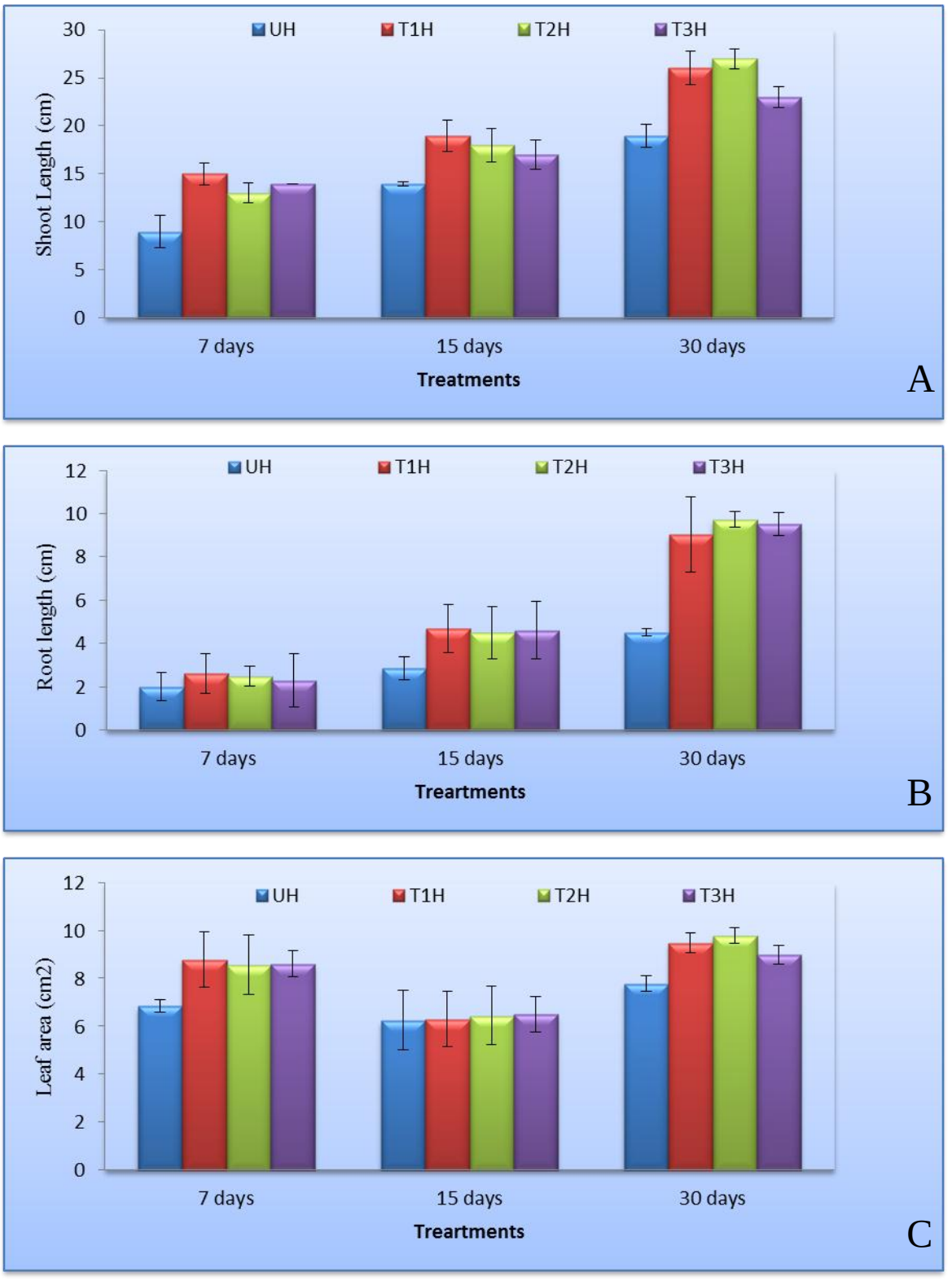


Figure 28: Evaluation of growth promotion (A) Shoot Length , (B) root Length & (C) Leaf Area of *Vigna radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii.* T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3= Treated with *Streptomyces flavogriseus.*

Figure 29: (A-D)*Vigna radiata* in open field condition; A- Control; B- T1; C- T 2; D-T3 (E-H) *Vigna radiata* in pot condition, E- Control; F- T1;G- T 2; H-T3. T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.*

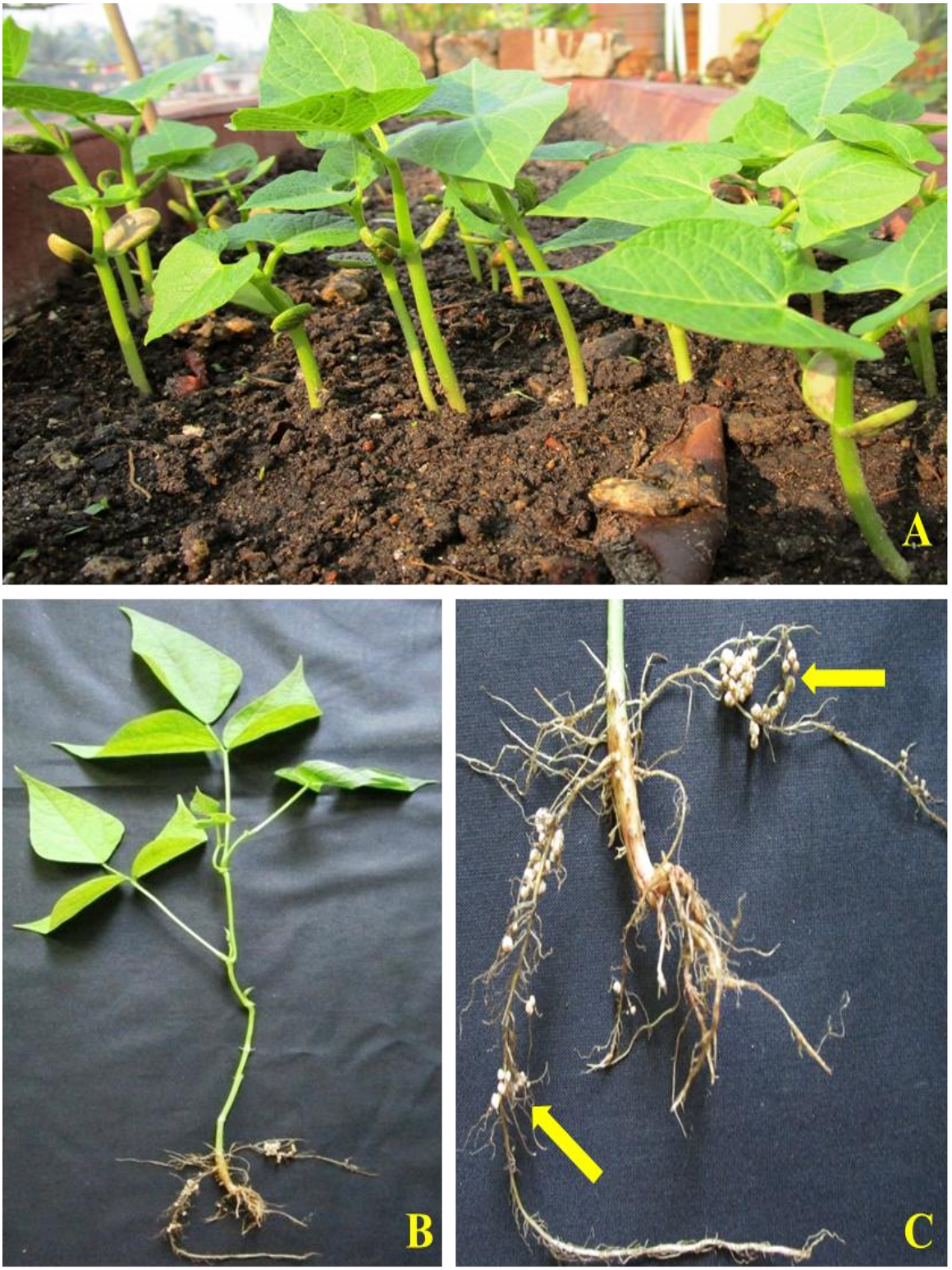


Figure 30: Nodulation of *Phaseolus vulgaris* and the effect following application with actinomycetes. A- Field grown plant ; B-Uprooted plant, C- Root nodulation

Figure 31: Nodulation of *Vigna radiata* and the effect following application with actinomycetes; A- Field grown plant ; B-Uprooted plant; C-Root nodulation

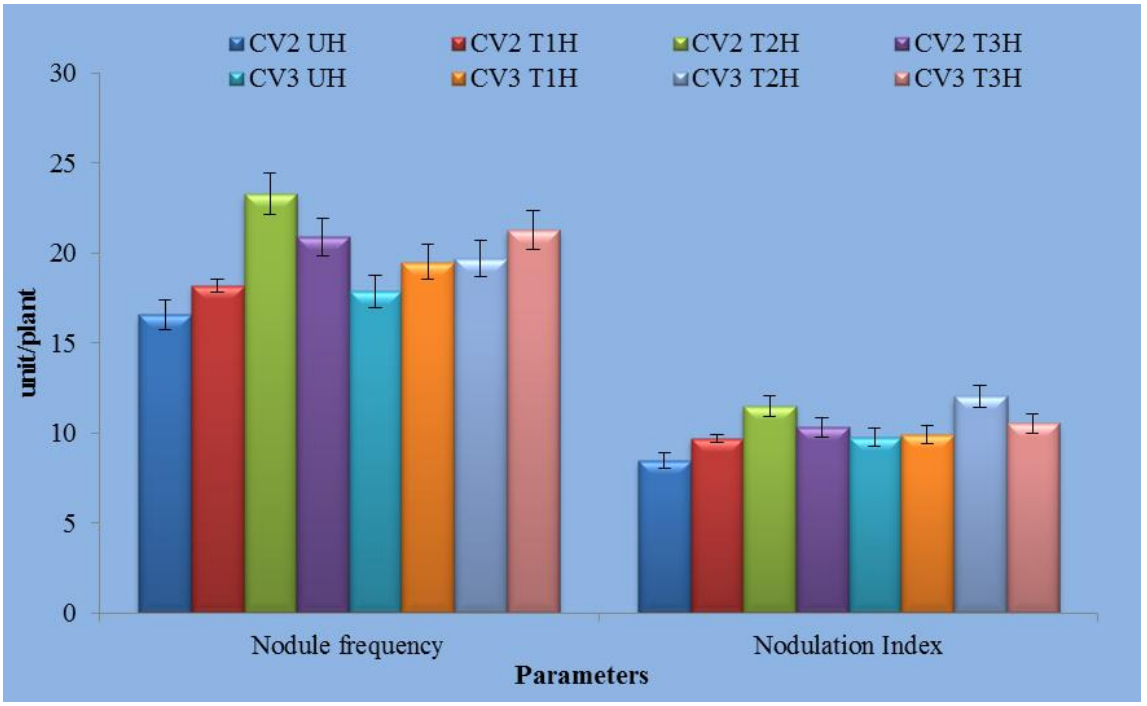


Figure 32: Nodulation frequency and Nodulation Index in *Phaseolus vulgaris* under various treatments in selected cultivars CV2(Jwala) and CV3(Kholar). UH= Untreated Healthy, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3= Treated with *Streptomyces flavogriseus.*

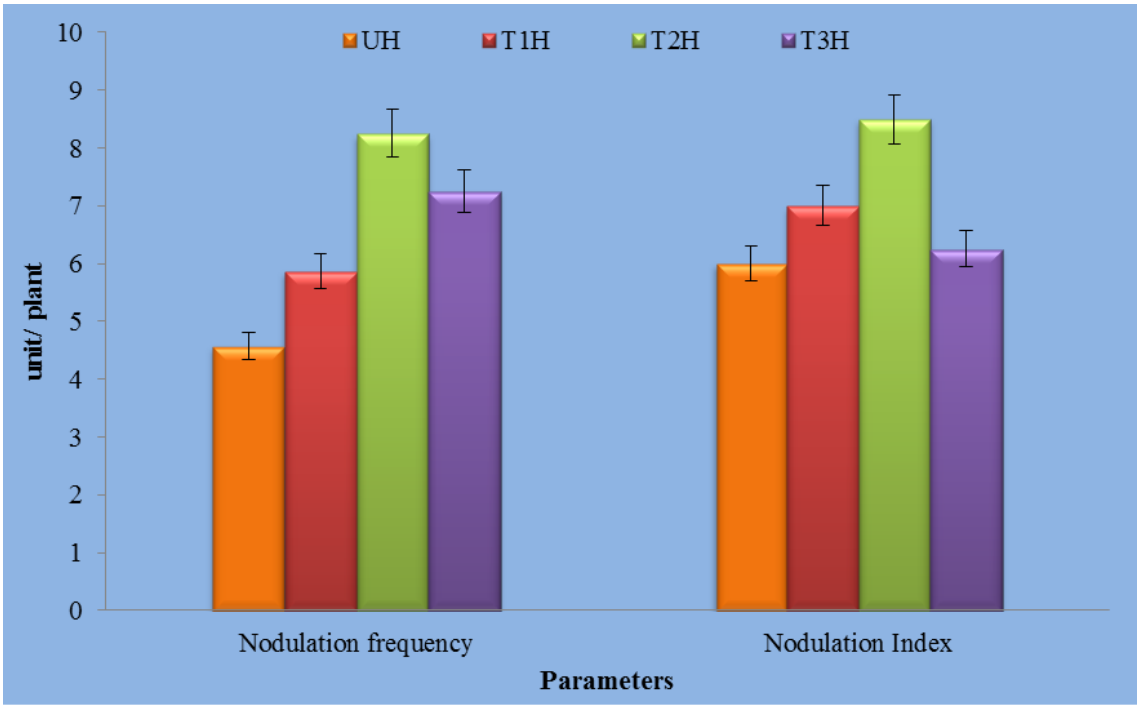


Figure 33: Nodulation frequency and Nodulation Index under various treatments in *Vigna radiata*. UH= Untreated Healthy, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3= Treated with *Streptomyces flavogriseus.*

4.8. Fungal pathogen

Fungal pathogens were collected from Immuno-phytopathology laboratory, Department of Botany NBU, West Bengal and from National Fungal Collection Centre of India, (NFCCI) Pune ,Maharastra**.**

4.8.1. Fusarium solani

Virulent strain of *Fusarium solani* was initially collected from germplasm of Immuno-phytopathology lab, Dept of Botany, NBU. *In vitro* tests were conducted to prove the Koch's postulates. Another potent virulent strain with accession number NFCCI 606 was collected from NFCCI. Upon further investigation NFCCI Accession number 606 was found to be more active and further experiments were carried out with the same strain.

4.8.2. Sclerotium rolfsii

Virulent strain of *Sclerotium rolfsii* was initially collected from germplasm of Immuno-phytopathology lab, Dept of Botany ,NBU. *In vitro* tests were conducted to prove the Koch's postulates. Another potent virulent strain with accession number NFCCI 1002 was collected from NFCCI. Upon further investigation Accession number 1002 was found to be more active and further experiments were carried out with the same strain.

4.9. Histopathological study of roots of *Phaseolus vulgaris*, before and after pathogen inoculation

To understand and investigate the interaction of the pathogen with the host plant in different conditions, anatomical study with conventional fixations and staining was carried out under bright field microscopy. Root samples were collected prior to challenge inoculation by uprooting plants from the fields and light washing under Jet stream water and surface dried with tissue paper.

Dissection of root sample was done with a blade, various dimensions of transverse sections of samples were obtained and temporarily stored in watch glass. Visual parameters with a fine brush were used to select sections with optimum edge and visibility to open eyes. Differential staining with saffranine and light green and simple staining with lactophenol cotton blue both were applied for study of the healthy as well as the diseased plant tissue(Fig.34) Fungal hyphae penetrating the host tissue were seen in the sections.

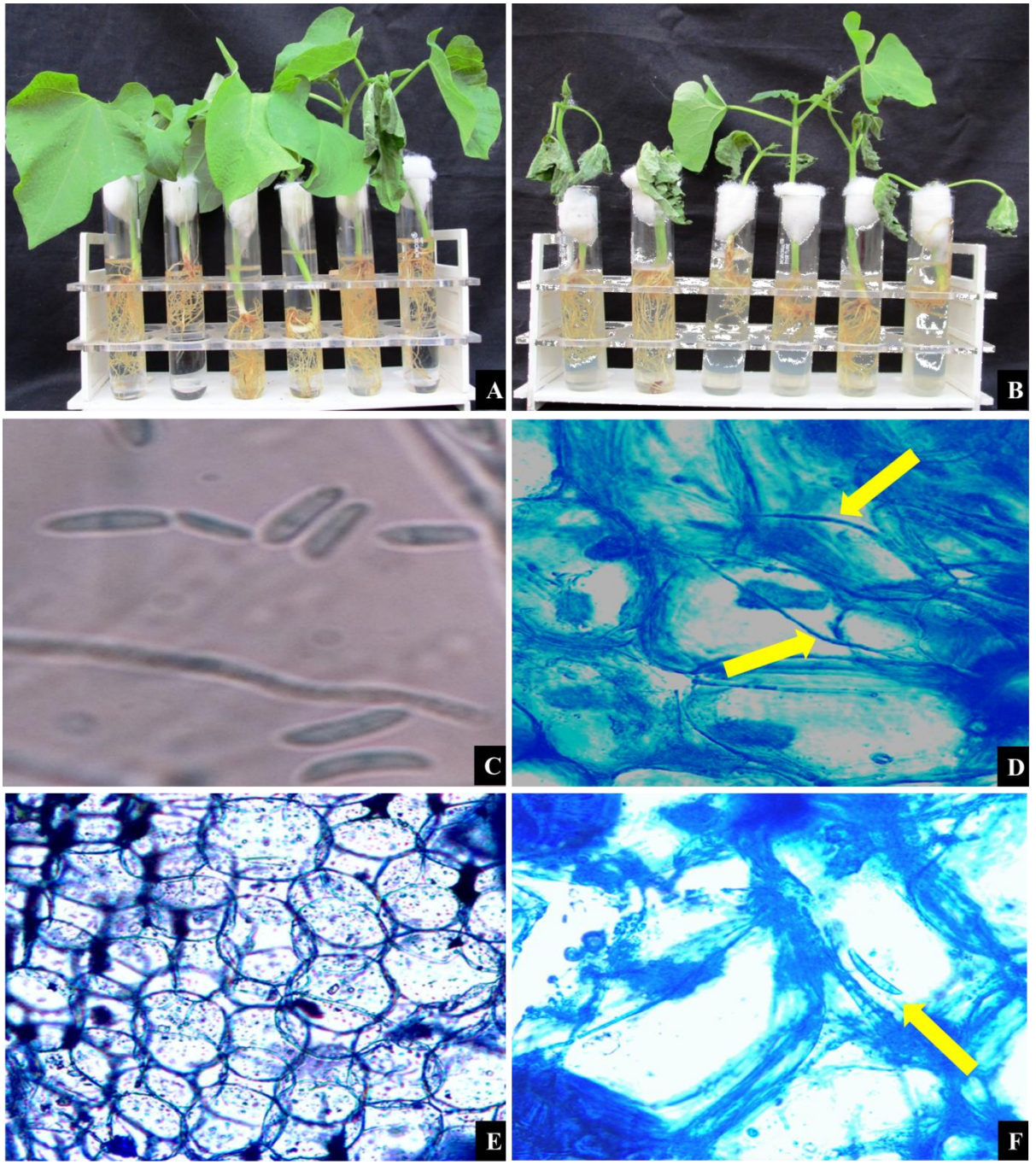


Figure34: Histopathological interaction of host and pathogen in *Phaseolus vulgaris.* A- Control in sterile water, B- Direct contact method with target pathogen, *Fusarium solani* C- Conidia of *F. solani* observed under microscopic field. (D & F)- Hyphal invasion of pathogen inside host tissue ;E-Control tissue section.

4.10. Histopathological study of roots of *Vigna radiata*, before and after pathogen inoculation

To understand and investigate the interaction of the pathogen with the host plant in different conditions, anatomical study with conventional fixations and staining was

carried out under light microscopy. Root samples were collected prior to challange inoculation by uprooting plants from the fields and light washing under steady stream water and surface dried with tissue paper.

Dissection of root sample was done with a blade; various dimensions of transverse sections of samples were obtained and temporarily stored in watch glass. Visual parameter with a fine brush was used to select sections with optimum edge and visibility to open eyes. Differential staining with safranin and light green and simple staining with lactophenol cotton blue both were applied for study of the healthy as well as the diseased plant tissue (Fig .35)

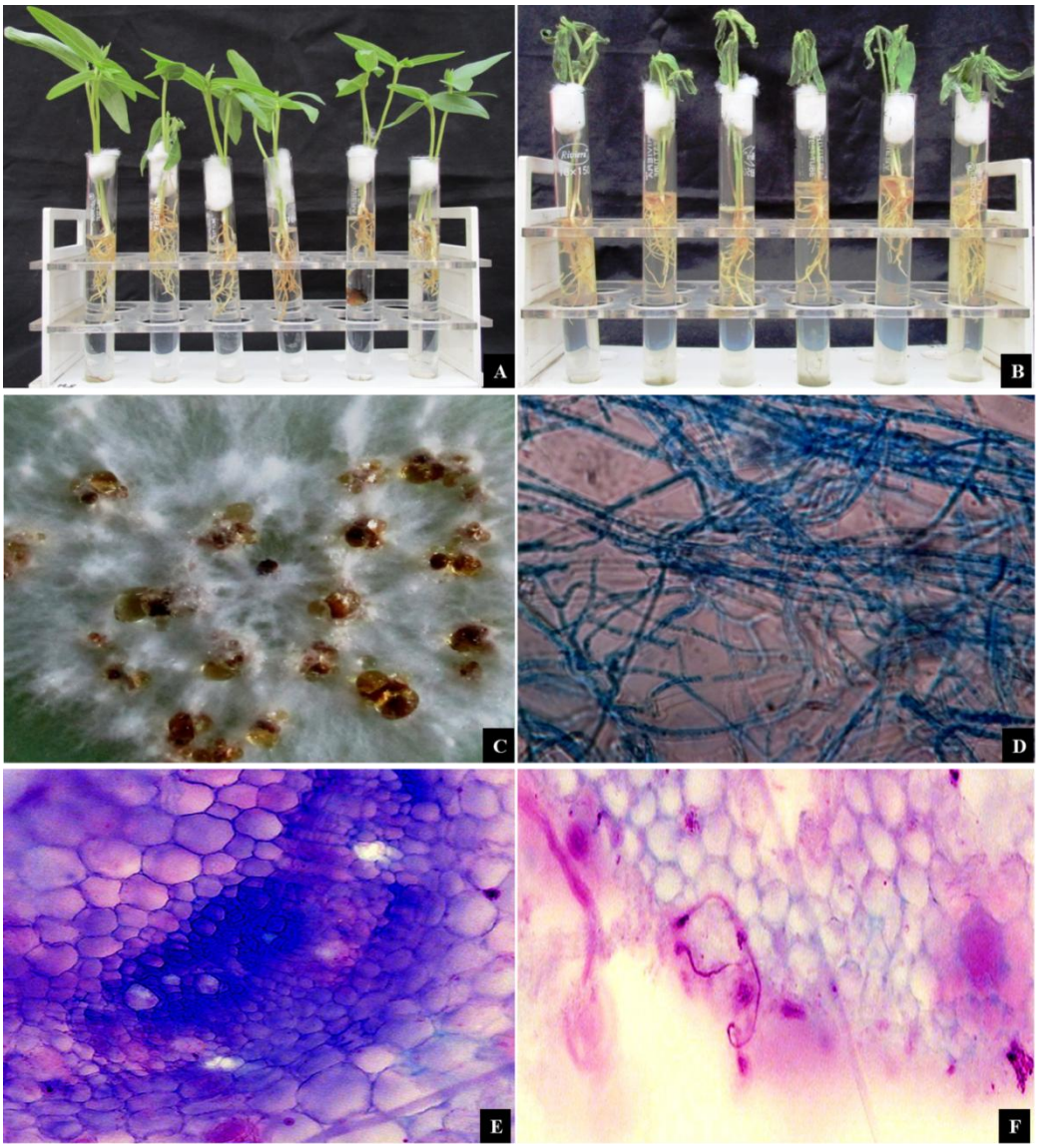


Figure 35: (A-F) . Histopathological interaction of Host & Pathogen in *Vigna radiata* .A- Control in sterile water, B- Direct contact method with target pathogen, *Sclerotium rolfsii* C- sclerotia of *S.rolfsii* observed under microscopic field. (D & F)-Hyphal invasion of pathogen inside host tissue ; E-Control tissue section.

4.11. Influence of Actinomycetes isolates on Fusarial root rot of *Phaseolus vulgaris*

4.11.1. Disease development

Effect of three actinomycetes isolates *Strptomyces griseus, S. tricolor, S. flavogriseus* in development of fusarial root rot of *Phaseolus vulgaris* caused by *Fusarium solani* was determined (Fig 36). 7 days old seedlings of *Phaseolus* was inoculated with *F. solani* and disease assessment was done after 7, 14, 21 and 28 days of inoculation. The disease index of the plants was recorded. The disease severity increased with time reaching the highest pick at the end of 28 days. It was observed that when the plants were pretreated with *Strptomyces griseus, S. tricolor,* and *S. flavogriseus* the disease severity was lower than the untreated control. *Strepomyces flavogriseus* was most effective in reducing the disease followed by *S. tricolor* and *S. griseus*.

4.11.2. Percent Disease Index (PDI%)

The disease severity in *Phaseolus vulgaris* inoculated with the pathogen *Fusarium solani* increased with time reaching a maximum value after 28 days (Table 32). But when the soil was pretreated with Actinomycetes isolates the maximum disease severity was reduced. *Streptonyces flavogriseus* (ARHS/PO/27) was the most effective to inhibit the root rot disease followed by *S. tricolor* (ARHS/PO/26) and *S. griseus* (ARHS/PO/15) (Fig 42, 43).

Table 32: Fusarial root rot development in the roots of *Phaseolus vulgaris* in presence and absence of actinomycetes isolates in pot condition

Treatments		**7 d**	**14d**	**21d**	**28d**
Jwala (CV2)	Control	20	35	53	90
	T1	8.3	23.33	41.66	66.66
	T2	6.6	15	33.33	53.33
	T3	5	10	20	30
Kholar (CV3)	Control	20	40	53.33	75
	T1	5	12	23.33	53.33
	T2	3.3	7.5	16.6	35
	T3	1.6	8.3	15	23.33

4.11.3. Associated Biochemical changes

Application of *Streptomyces griseus, S. tricolor, S. flavogriseus* to soil was found to affect the biochemical properties of plants. Disease establishment also affect the

biochemical characters. So the biochemical response of both the root and leaves of plants following application of actinomycetes isolates and challenge inoculated with the pathogen *Fusarium solani* was determined. The conference of resistance towards the pathogen was evaluated in terms of enhancement of key defence enzymes- PAL, POX,GLU and CHT in both the root and leaves of *Phaseolus vulgaris* (Figs. 37, 39, 40, 41). The results showed that the level of defence enzyme increases in plants treated with actinomycetes isolates and challenge inoculated with the pathogen followed by only treated plants. Amount of defence enzyme was higher in pathogen challenged plants than control plants. Total phenol content of the roots and leaves of plants were also evaluated which showed significant increase in treated plants (Fig 38).

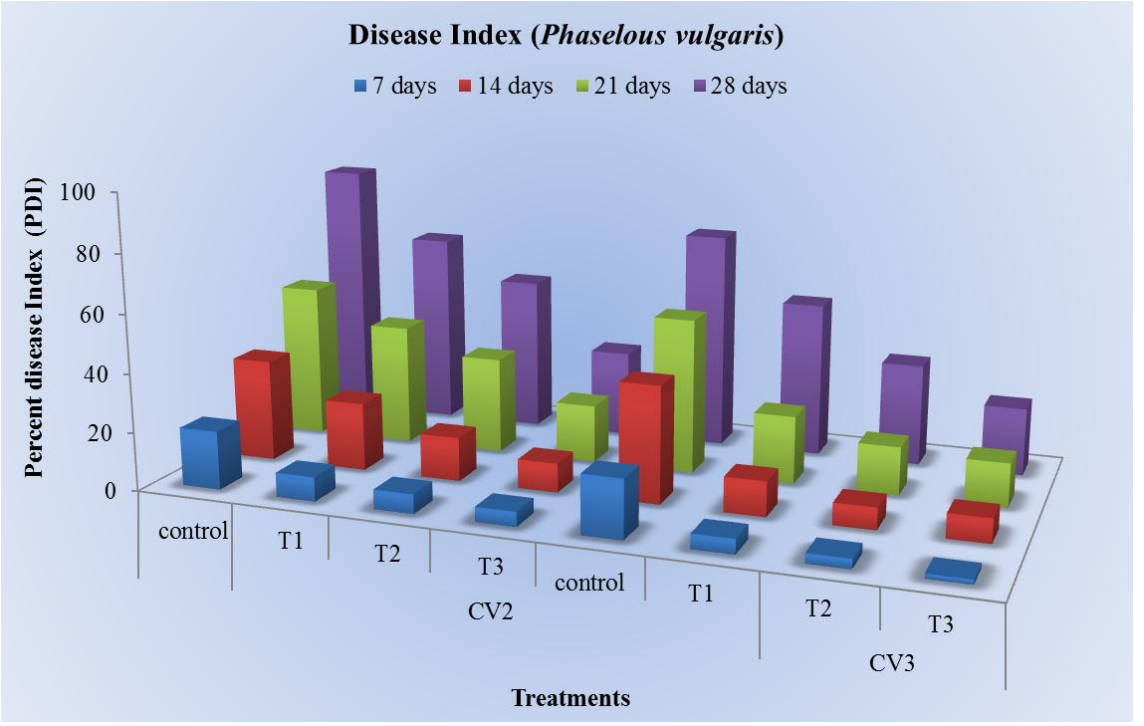


Figure36: Disease index (Evident symptom of Disease) after challenge inculcation with target pathogen (*Fusarium solani*) and host response upon respective isolate treatments in two cultivars of *Phaseolus vulgaris.* T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus*

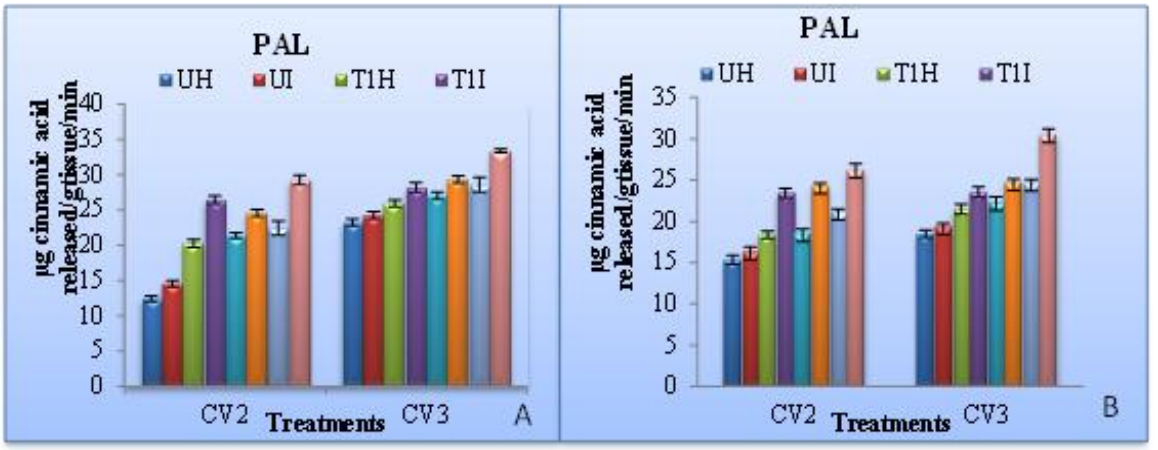


Figure 37: PAL activity in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani;* UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.*CV2=Jwala, CV3=Kholar

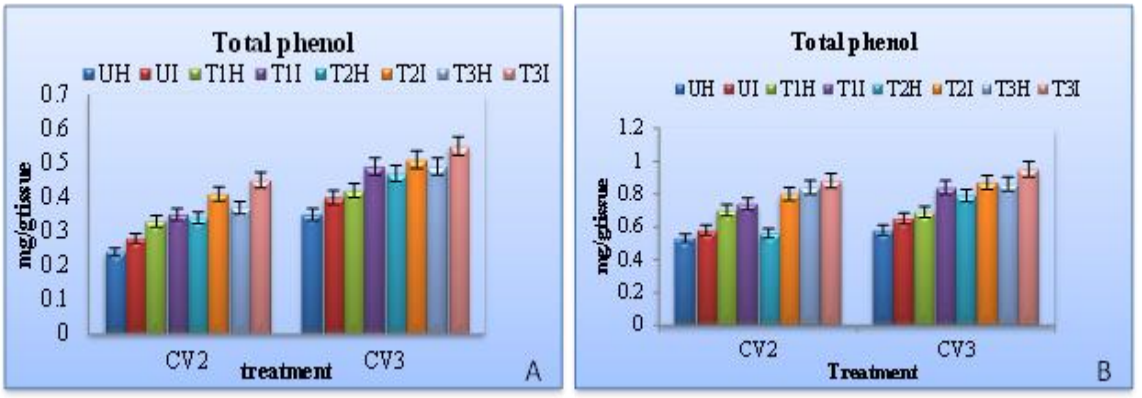


Figure 38: Total phenol content in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.* CV2=Jwala, CV3=Kholar

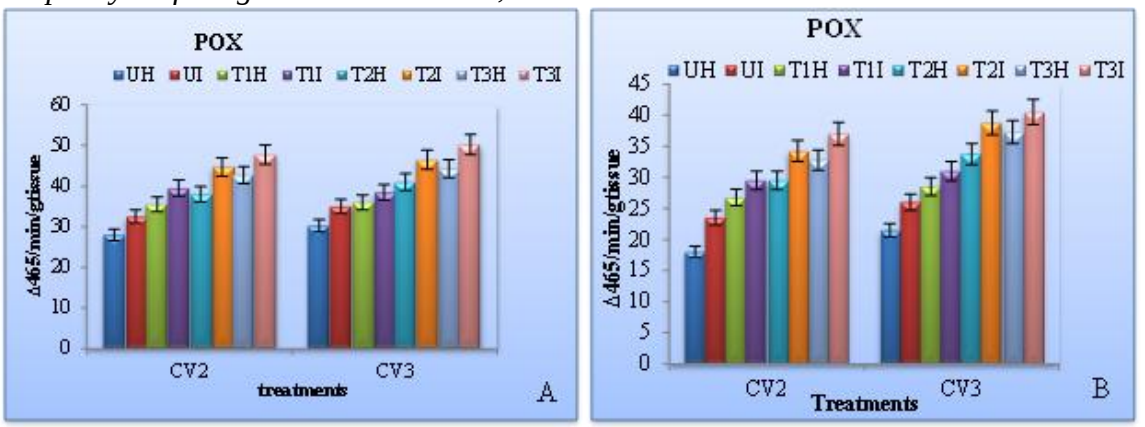


Figure 39: Peroxidase activity in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.* CV2=Jwala, CV3=Kholar

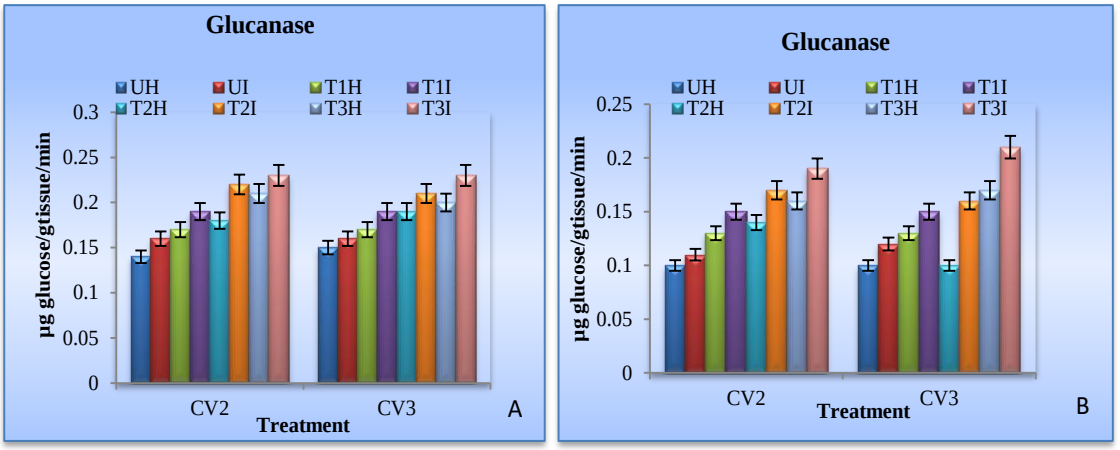


Figure 40: β-1,3 Glucanase activity in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with pathogen *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus* CV2=Jwala, CV3=Kholar

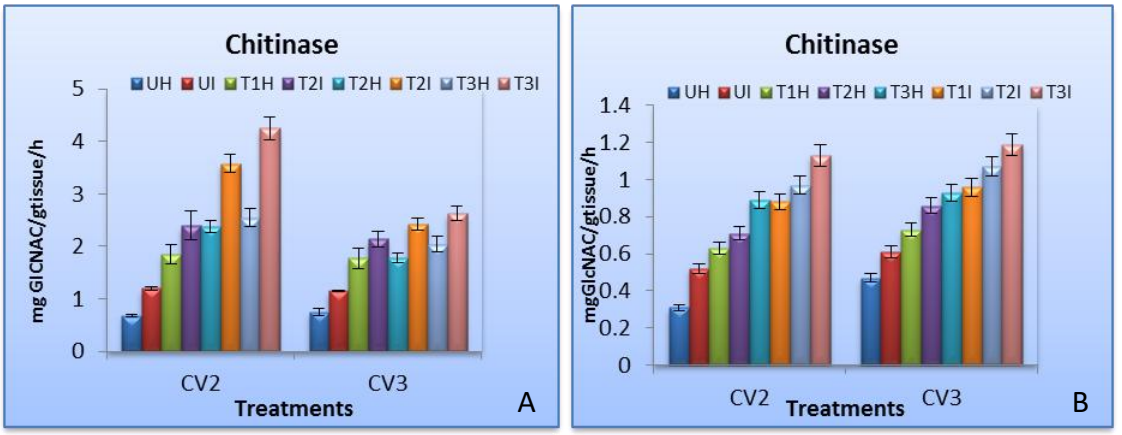


Figure41: Chitinase activity in (A) leaf & (B) roots of varieties of *P vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus.* CV2=Jwala, CV3=Kholar

Figure 42: (A-D) *Phaseolus vulgaris* (Cultivar 2, Jwala), in pot condition, A- Control, Untreated Healthy (UH). B- Control, Untreated Inoculated (UI). C- T1, Treated Healthy (T1H). D- T1 Treated Inoculated (T1I), E- T2 Treated Healthy (T2H), F-T2, Treated Inoculated (T2I), G- T3, Treated Healthy (T3H). H-T3, Treated Inoculated (T3I)

T1= treated with *Streptomyces griseus*, *T2=Streptomyces tricolor,* T3= *Streptomyces flavogriseus,* Inoculated with *Fusarium solani*

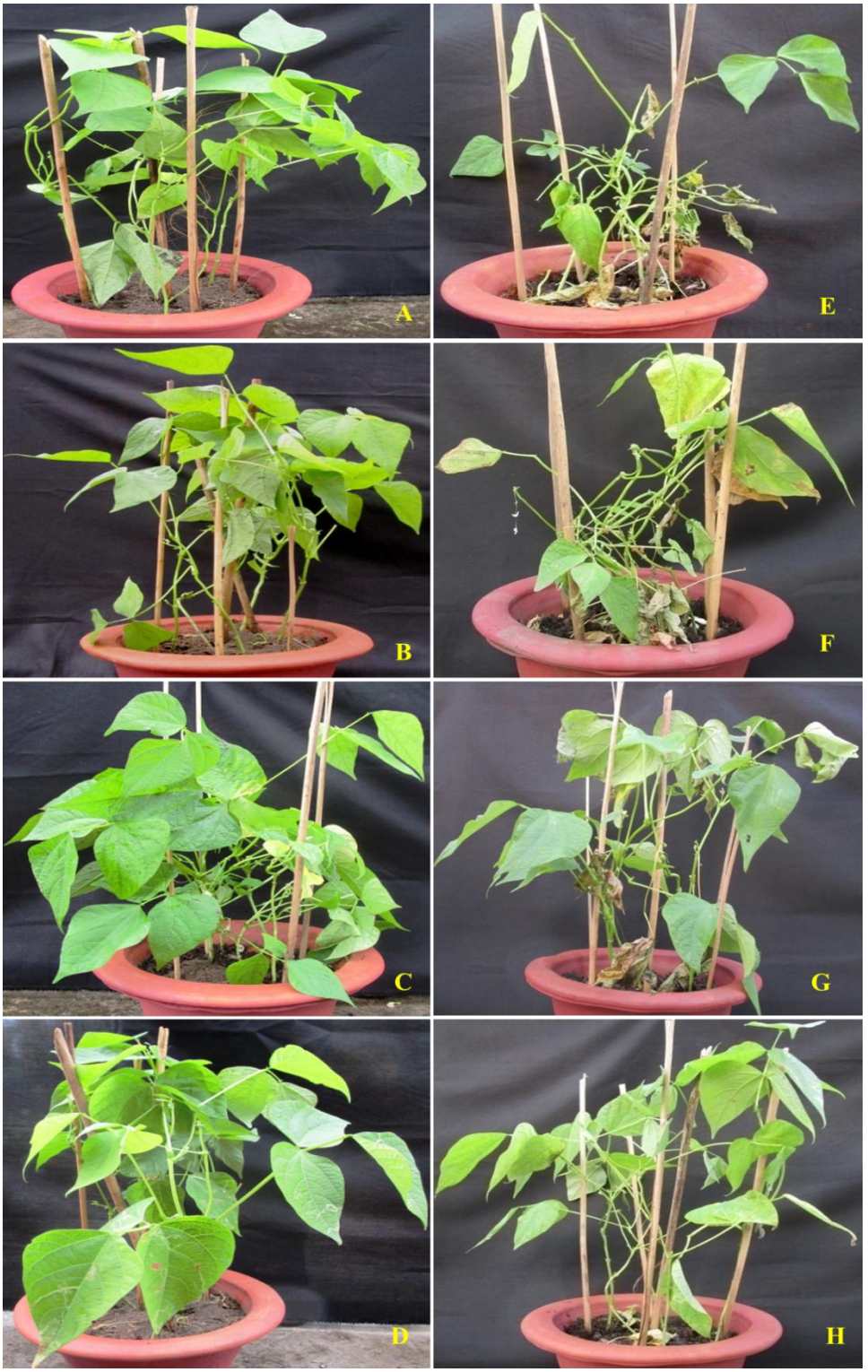


Figure 43: (E-H) *Phaseolus vulgaris* (Cultiver 3, Kholar), in pot condition, A- Control, Untreated Healthy (UH). B- Control, Untreated Inoculated (UI). C- T1, Treated Healthy (T1H). D- T1 Treated Inoculated (T1I), E- T2 Treated Healthy (T2H), F-T2, Treated Inoculated (T2I), G- T3, Treated Healthy (T3H). H-T3, Treated Inoculated (T3I)

T1= treated with *Streptomyces griseus*, *T2=Streptomyces tricolor*, T3= *Streptomyces flavogriseus*, Inoculated with *Fusarium solani.*

4.12. Influence of Actinomycetes isolates on sclerotial root rot of *Vigna radiata*

4.12.1. Disease development

Effect of three actinomycetes isolates *Streptomyces griseus, S. tricolor, S. flavogriseus* in development of sclerotial root rot of *Vigna radiata* caused by *Sclerotium rolfsii* was determined (Fig. 44).7 days old seedlings of *Vigna* was inoculated with *S. rolfsii* and disease assessment was done after 5, 10,15,21 and 28 days of inoculation. The disease index of the plants was recorded. The disease severity increased with time reaching the highest pick at the end of 30 days. It was observed that when the plants were pretreated with *Strptomyces griseus, S. tricolor,* and *S. flavogriseus* the disease severity was lower than the untreated control. *Strepomyces tricolor* was most effective in reducing the disease followed by *S. flavogriseus* and *S. griseus*.

4.12.2. Percent Disease Index (PDI%)

The disease severity in *Vigna radiata* inoculated with the pathogen *Sclerotium rolfsii* increased with time reaching a maximum value after 30 days. But when the soil was pre-treated with actinomycetes isolates the maximum disease severity was reduced to 53.3% in *Streptomyces tricolor* treated plants (Table 33) (Fig 50).

Table33: Sclerotal root rot development in the roots of *Vigna radiata* in presence and absence of actinomycetes isolates in pot condition

Treatments	7d	15d	21d	28d
Control	15	35	60	90
T1	8.3	20	46.66	66.66
T2	6.6	15	35	53.3
T3	7.8	16.6	40	60

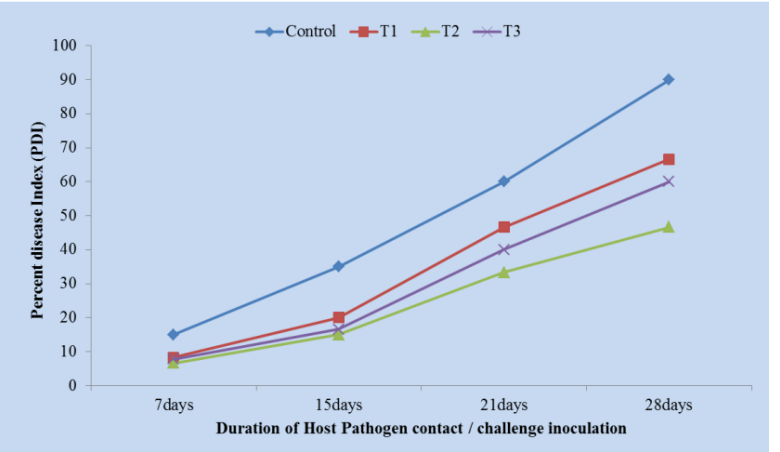


Figure 44: Disease index (Evident symptom of Disease) after challenge inculcation with target pathogen(*Sclerotium rolfsii*) and host response upon respective isolate treatments in *Vigna radiata.* T1= treated with *Streptomyces griseus, T2=Streptomyces tricolor,* T3= *Streptomyces flavogriseus*

4.12.3. Biochemical changes

Application of *S. griseus*, *S.tricolor*, *S. flavogriseus* to soil was found to affect the biochemical properties of plants. Disease establishment also affect the biochemical characters. So the biochemical response of both the root and leaves of plants following application of actinomycetes isolates and challenge inoculated with the pathogen *Sclerotium rolfsii* was determined. The conference of resistance towards the pathogen was evaluated in terms of enhancement of key defence enzymes- PAL, POX, GLU and CHT in both the root and leaves of *Vigna radiata*. The results showed that the level of defence enzyme increases in plants treated with actinomycetes isolates and challenge inoculated with the pathogen followed by only treated plants. Amount of defence enzyme was higher in pathogen challenged plants than control plants (Figs. 45, 47, 48, 49). Total phenol content of the roots and leaves of plants were also evaluated which showed significant increase in treated plants (Fig 46).

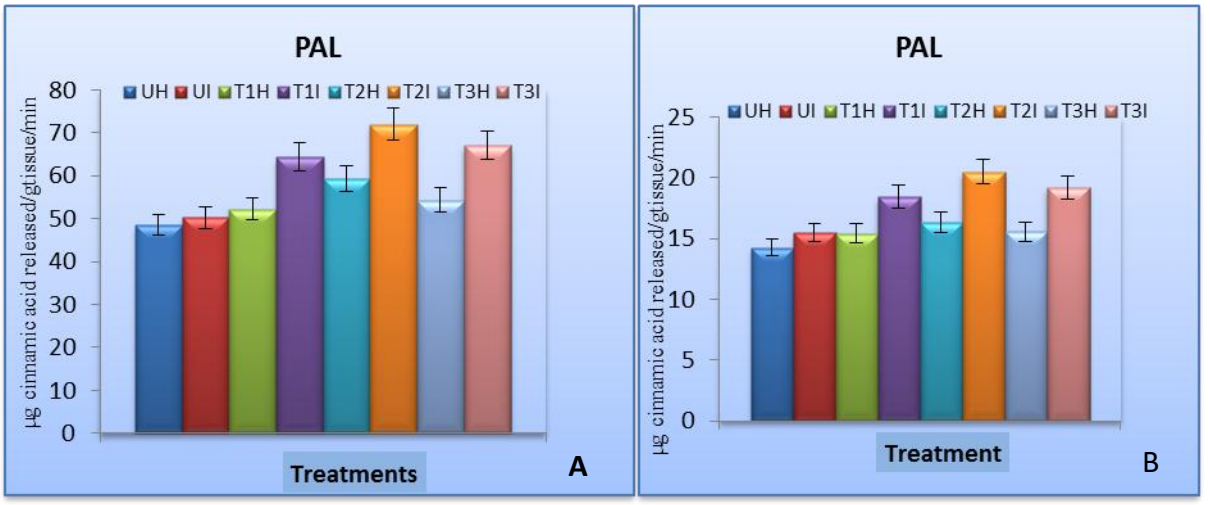


Figure 45: PAL activity in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus.*

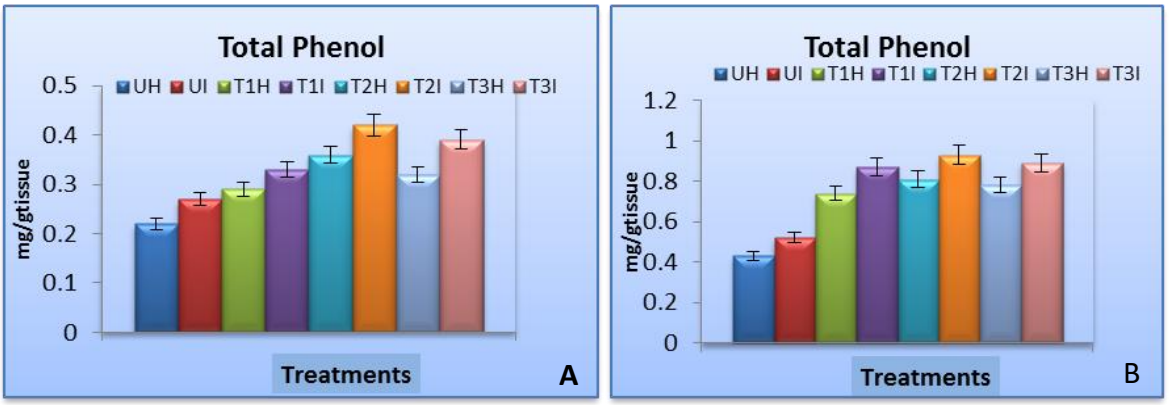


Figure 46: Total phenol content in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus.*

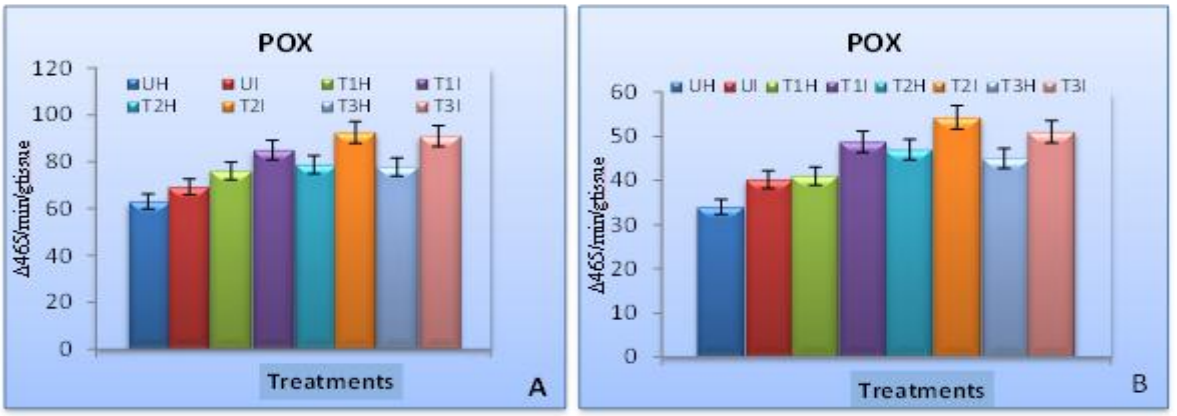


Figure 47: Peroxidase activity in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus.*

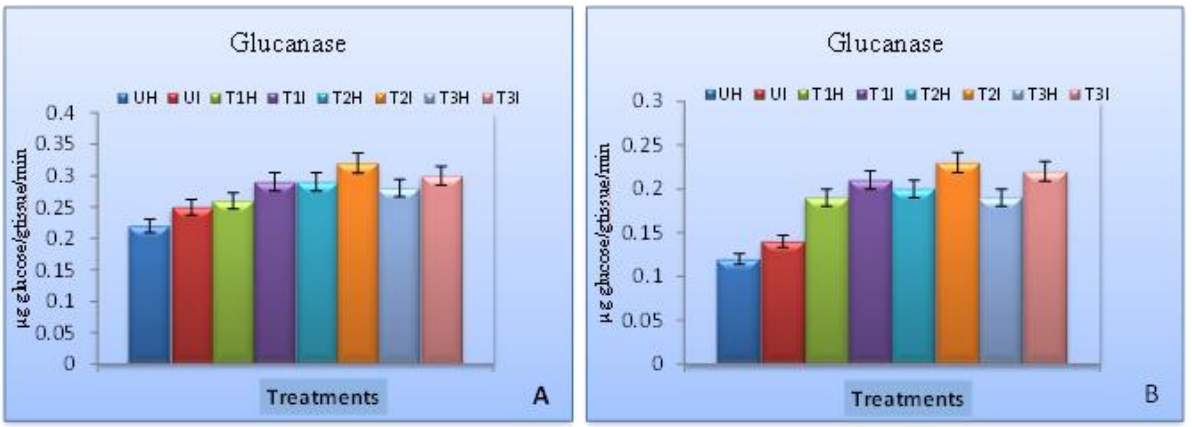


Figure 48: . β 1-3 Glucanase activity in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with pathogen *Sclerotium rolfsii*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus*, T2= Treated with *Streptomyces tricolor*, T3=Treated with *Streptomyces flavogriseus.*

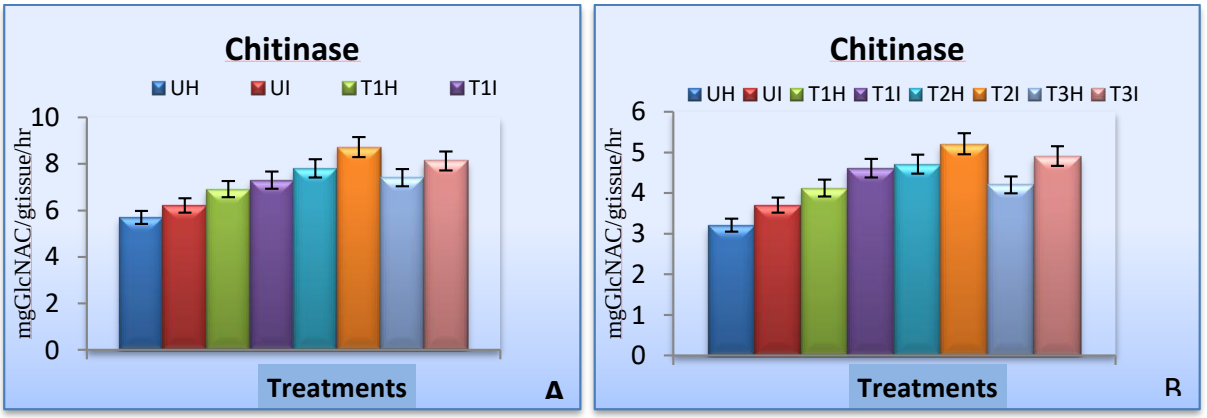


Figure 49: Chitinase activity in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.*

4.13. Artificial inoculation of *Phaseolus vulgaris* and *Vigna radiata* with actinomycetes formulation and associated changes in protein content before and after fungal inoculation

4.13.1. Total protein in *Phaseolus vulgaris*

Total protein was extracted from root and leaf tissue of *Phaseolus vulgaris* plants. The plants were artificially inoculated with *Fusarium solani* and treated with actinomycetes formulation. Result showed that protein content was higher in treated plants in comparison to the untreated healthy plant. The increase was highest in case of plants challenge inoculated with the pathogen, *Fusarium solani*. Protein content in the leaves was higher than the root. Detailed information can be obtained from the Table 34 and Table 35.

Table34. Protein content in Leaf of varieties of *P. vulgaris* treated with actinomycetes formulation before and after inoculation with pathogen

Cultiver	Treatments	Activity (mg/gm tissue)
Jwala (CV2)	UH	22.04±0.02
	UI	31.11±0.09
	T1H	33.12±0.09
	T2H	37.23±0.13
	T3H	40.25±0.13
	T1I	49.28±0.17
	T2I	53.13±0.09
	T3I	61.31±0.18
Kholar(CV3)	UH	19.39±0.12
	UI	23.55±0.18
	T1H	26.46±0.18
	T2H	37.08±0.02
	T3H	36.63±0.20
	T1I	61.29±0.17
	T2I	69.06±0.02
	T3I	67.37±0.17
CD(P=0.05)	Treatments	14.60
	Varieties	7.30

UH=Untreated healthy, UI= Untreated Inoculated, T1=treated with *Streptomyces griseus*, T2= treated with *S. tricolor*, T3= treated with *S. flavogriseus*

Table 34 a. ANOVA of the data in table 34 (Total Protein content)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3786.5	7	540.9285	14.17963	0.0012	3.78704354
Columns	11.1556	1	11.1556	0.292427	0.60544	5.591447848
Error	267.038	7	38.14829			
Total	4064.693	15				

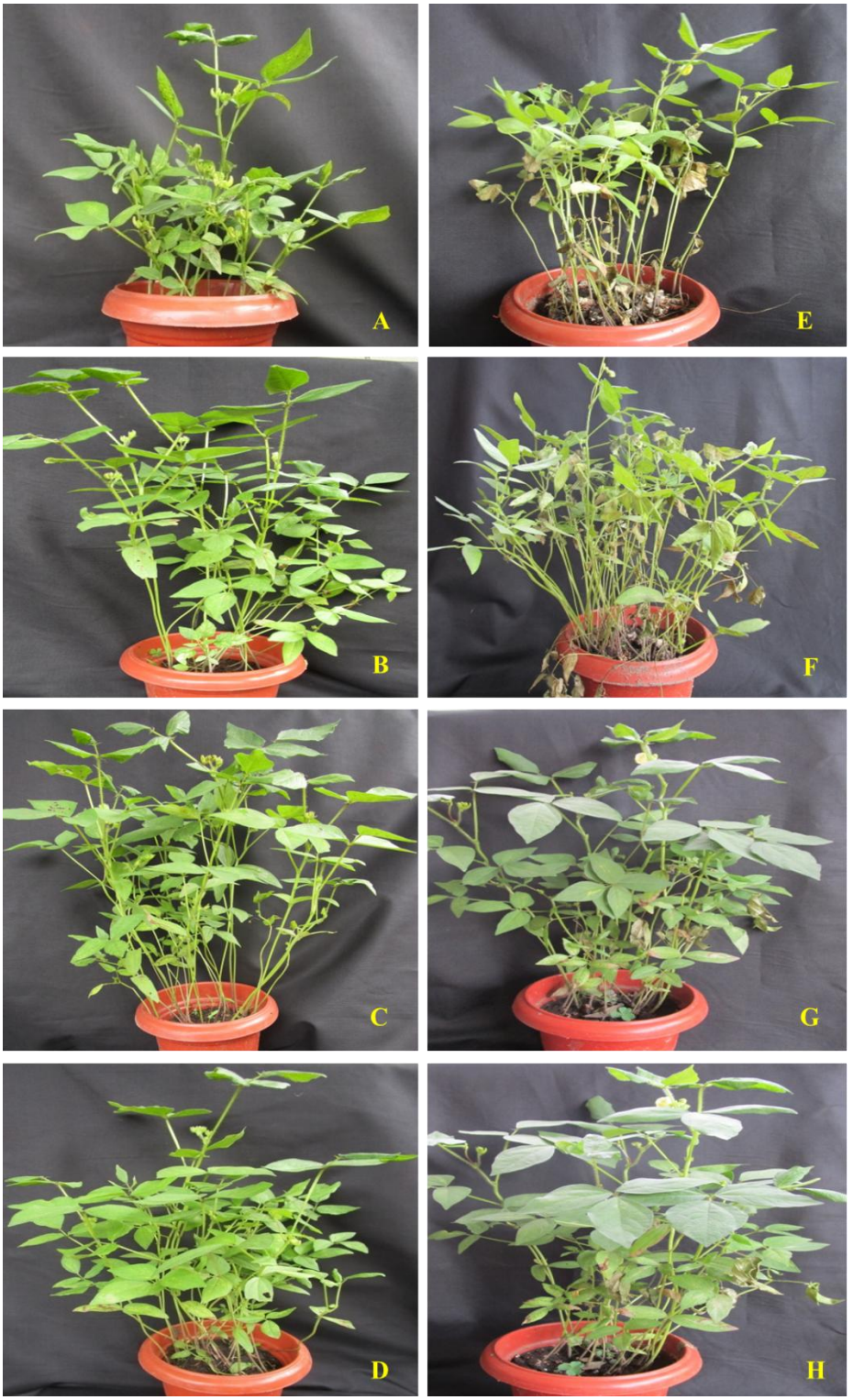


Figure 50: (A-D) *Vigna radiata*, in pot condition, A- Control Untreated Healthy (UH), B- T1 Treated Healthy (T1H), C- T2 Treated Healthy (T2H), D- T3 Treated Healthy (T3H). T1= treated with *Streptomyces griseus*, *T2=Streptomyces tricolor*, T3= *Streptomyces flavogriseus*. (E-H) *Vigna radiata*, in pot condition, E- Control Untreated Inoculated (UI), F- T1 Treated Inoculated (T1I), G- T2 Treated Inoculated (T2I), H- T3 Treated Inoculated (T3I), challenge pathogen *Sclerotium rolfsii* after 7 days under glass house condition.

Table 35. Protein content in root of varieties of *P. vulgaris* treated with actinomycetes formulation before and after inoculation with pathogen.

Cultiver	Treatments	Activity (mg/gm tissue)
Jwala (CV2)	UH	6.06±0.02
	UI	9.15±0.25
	T1H	11.33±0.11
	T2H	16.25±0.03
	T3H	18.20±0.11
	T1I	11.83±0.25
	T2I	18.25±0.18
	T3I	22.33±0.13
Kholar(CV3)	UH	11.48±0.05
	UI	12.10±0.13
	T1H	14.36±0.16
	T2H	15.19±0.14
	T3H	14.48±0.16
	T1I	19.34±0.18
	T2I	24.72±0.04
	T3I	20.43±0.11
CD(P=0.05)	Treatments	4.76
	Varieties	2.38

UH=Untreated healthy, UI= Untreated Inoculated, T1=treated with *Streptomyces griseus*, T2= treated with *S. tricolor*, T3= treated with *S. flavogriseus*

Table 35 a. ANOVA of the data in table 35 (Total Protein content)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	321.5544	7	45.93634	11.30142	0.002424	3.78704354
Columns	29.43063	1	29.43063	7.240623	0.031048	5.591447848
Error	28.45258	7	4.064654			
Total	379.4376	15				

4.13.2. Total protein in *Vigna radiata*

Total protein was extracted from root and leaf tissue of *Vigna radiata* plants. Result showed that protein content was higher in treated plants in comparison to the untreated healthy plant. The increase was highest in case of plants challenge inoculated with the pathogen *Sclerotium rolfsii*. Protein content in the leaves was higher than the root. Detailed information can be obtained from the Figure 51.

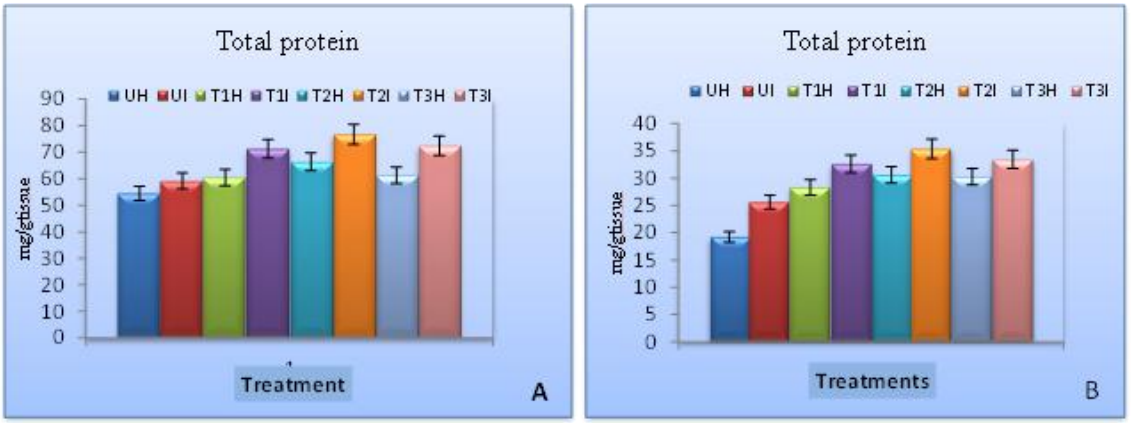


Figure 51: Total protein content in (A) leaf & (B) roots of varieties of *V radiata* treated with actinomycetes formulation before and after challenge inoculation with *Sclerotium rolfsii* **.** UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.*

4.14. Tissue and cellular location of Chitinase and Glucanase enzyme by FITC labelling in *Phaseolus vulgaris*

Apart from the quantitative assessment of plant defence enzyme ,localisation of chitinase enzyme in the plant tissue was also observed by FITC labelling. Root and leaf samples were collected from treated and inoculated plants. Samples from healthy plants without any treatment were also collected for immunofluorescence study. Main objective of the study was to localize chitinaseat the cellular level in the root and leaf tissue of *Phaseolus vulgaris*. Cross section of the root and leaf tissue were treated with normal antisera and PAb of Chitinase and labeled with FITC. The treated root and leaves showed bright apple green fluorescence in the epidermal and cortical tissue which is indication of localization of Chitinase in this region. So, strong reaction with FITC in plant tissue indicated the induction of chitinase (PR 3) in *Phaseolus* (Figs. 52-54).

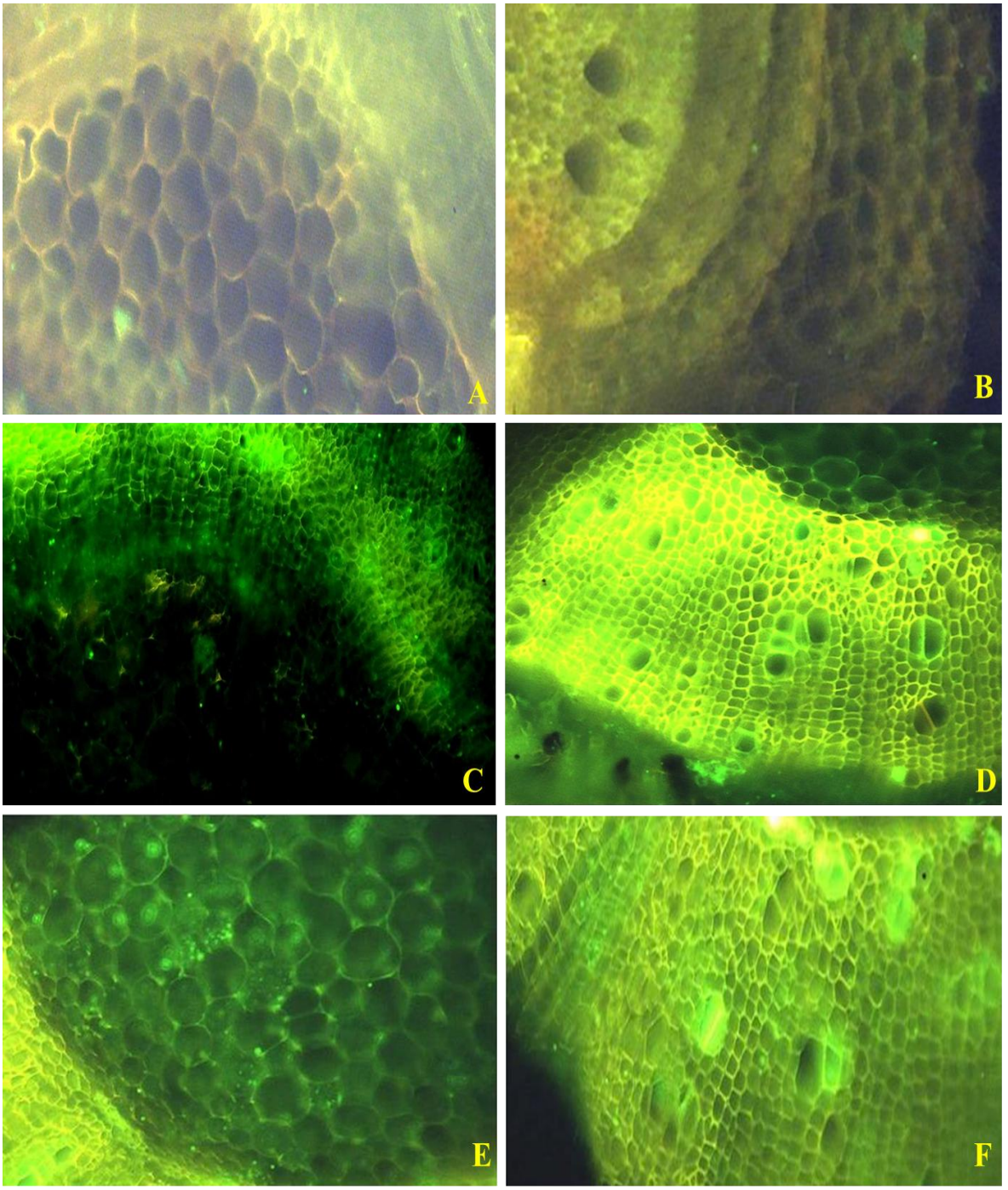


Figure 52: (A-F) FITC labeling of root tissue of *Phaseolus vulgaris* with Pab of Chitinsae enzyme after treatment with *S.flavogriseus* (KX894281) and pathogen challenge. A. transverse section (TS) of cortical tissue of control; B-TS of vascular tissue of control; (C & D) TS of cortical & vascular tissue of root of CV2 plants;(E&F) TS of cortical & vascular tissue of root of CV3 plants

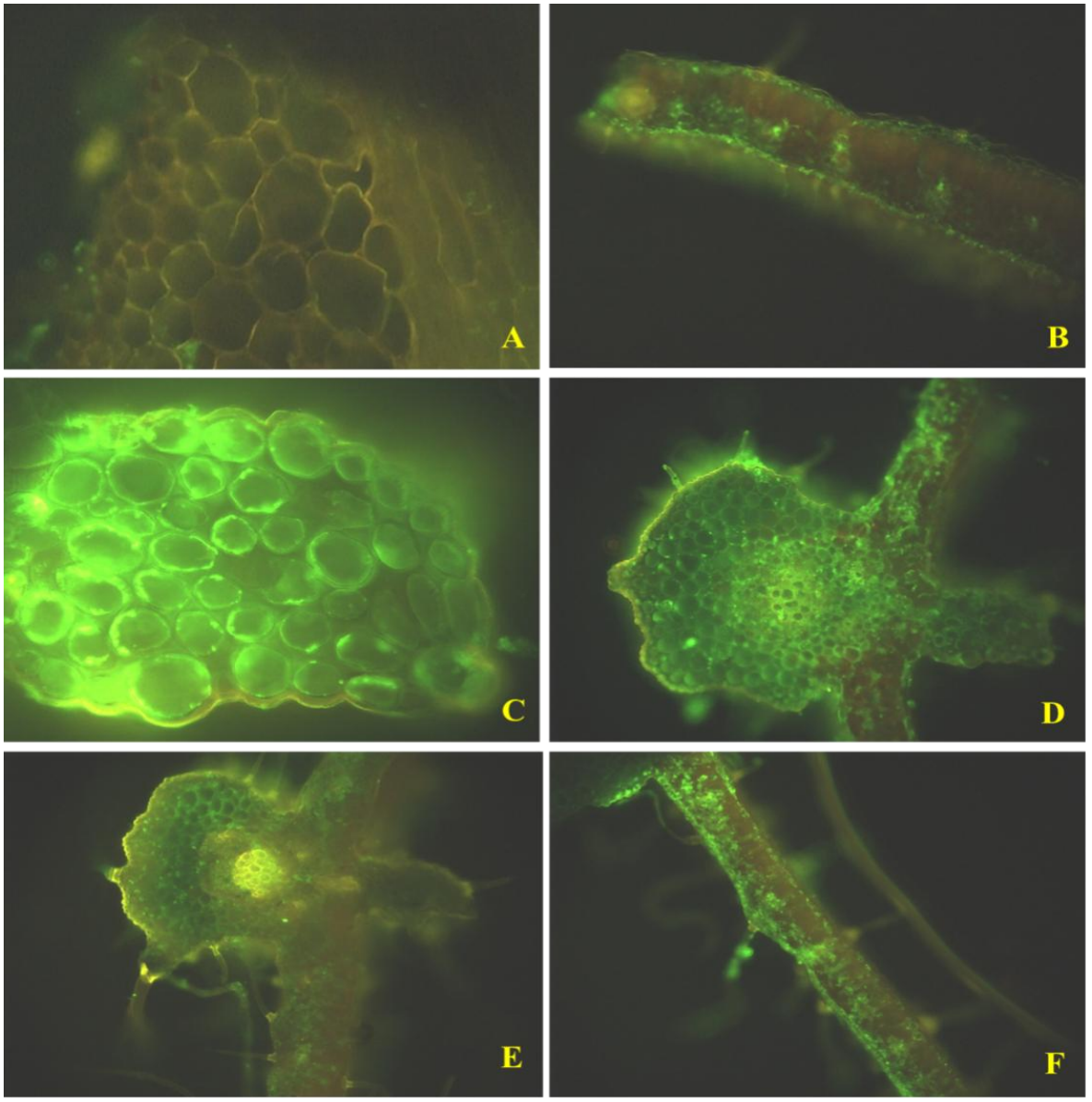


Figure 53: . (A-F) FITC labeling of leaf tissue of *Phaseolus vulgaris* with Pab of Chitinsae enzyme after treatment with *S.flavogriseus* (KX894281) and pathogen challenge . (A&B) TS of leaf tissue in untreated control; (C&D) TS of leaf tissue in CV2; (E&F) TS of leaf tissue in CV3, showing localization of chitinase enzyme.

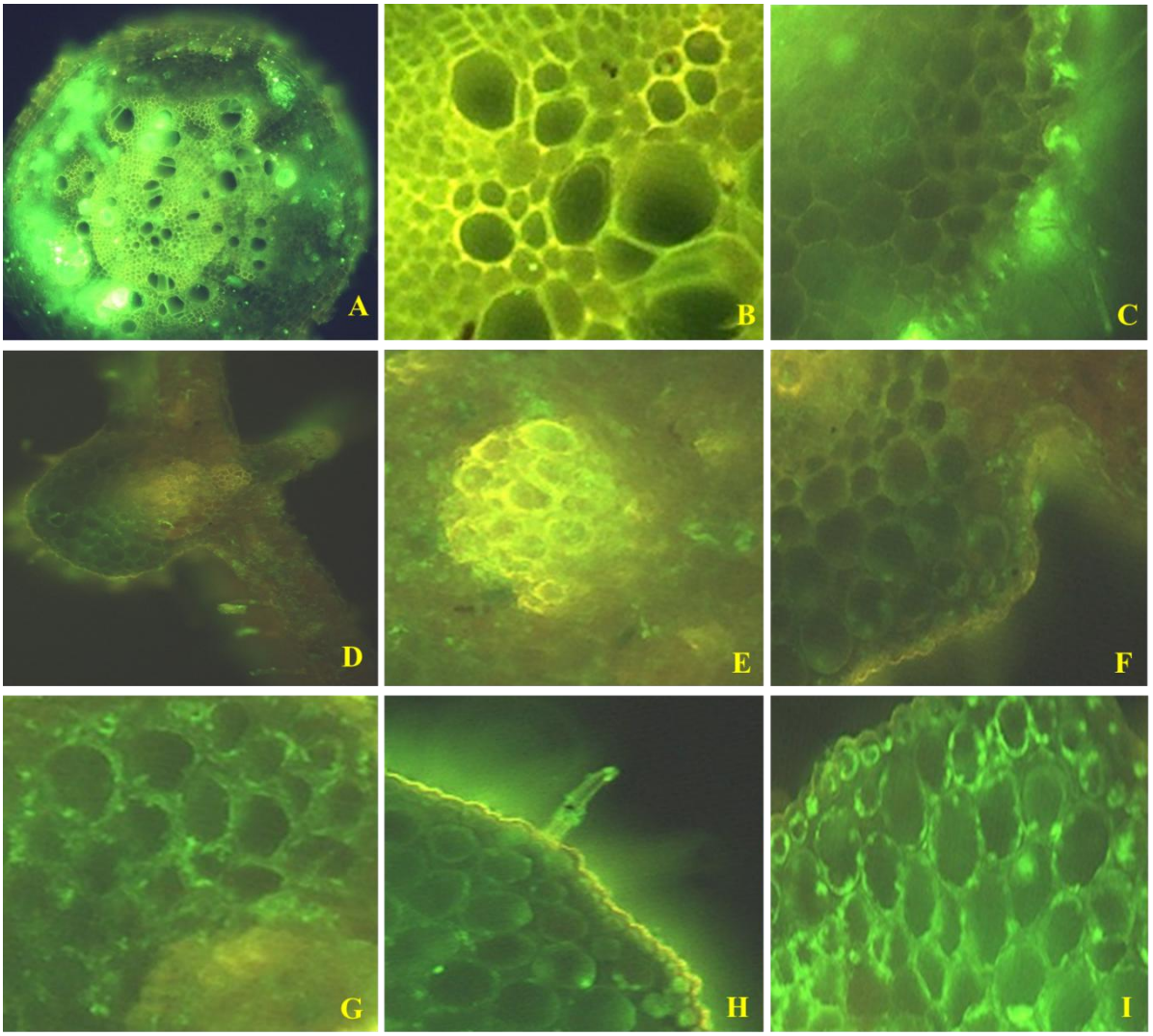


Figure 54: FITC labeling of leaf and root tissue of *Phaseolus vulgaris* with Pab of glucanase enzyme after treatment with *S.flavogriseus* (KX894281) and pathogen challenge . (A-C) TS of root tissue ; (D-F) TS of leaf tissue ; (G&F) Localization of glucanase enzyme, in cortical tissue of root (G), root hair and epidermal layer in root(H) and vascular tissue in leaf midrib area (I).

4.15. Tissue and cellular location of Chitinase and Glucanase enzyme by FITC labelling in *Vigna radiata.*

Apart from the quantitative assessment of plant defence enzyme, localisation of chitinase enzyme in the plant tissue was also observed by FITC labelling Root and leaf samples were collected from treated and inoculated plants. Samples from healthy plants without any treatment were also collected for immunofluorescence study. Main objective of the study was to localize chitinase at the cellular level in the root and leaf tissue of *Vigna radiata*. Cross section of the root and leaf tissue were treated with normal antisera and PAb of Chitinase and labelled with FITC. The treated root and

leaves showed bright apple green fluorescence in the epidermal and cortical tissue which is indication of localization of Chitinase in this region. So, strong reaction with FITC in plant tissue indicated the induction of chitinase (PR 3) in *Vigna radiata* (Fig 55).

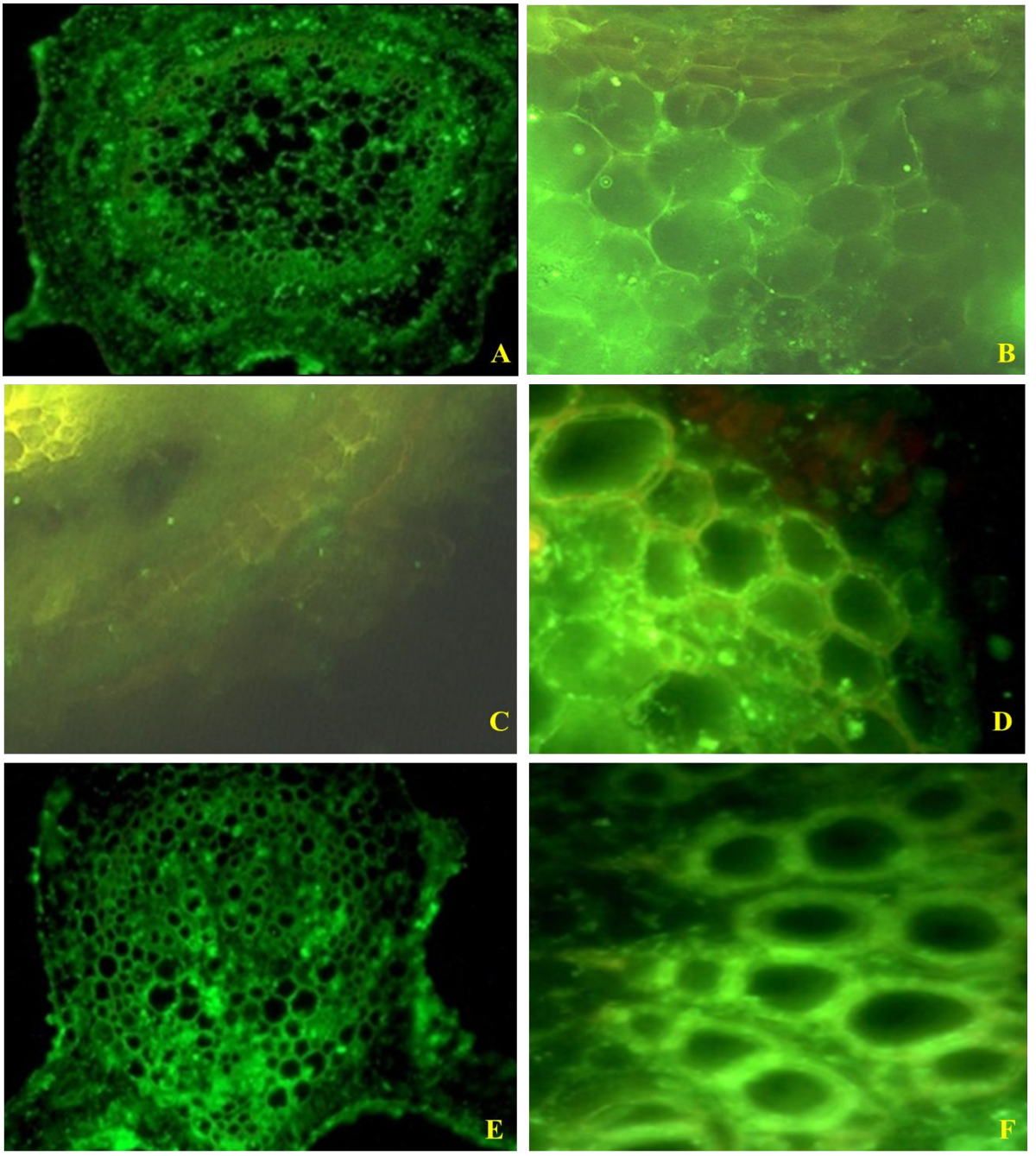


Figure 55: (A-F) FITC labeling of root & leaf tissue of *Vigna radiata* with Pab of glucanase enzyme & Pab of chitinase enzyme after treatment with *S. tricolor* (KX894280) and pathogen challenge . A-TS of root tissue of control ; (B-C & D)-TS of cortical & vascular tissue in roots ; (E & F) TS of leaf & localization of glucanase enzyme after pathogen challenge.