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Evaluation of *Streptomyces* spp. for growth promotion in *Phaseolus vulgaris* and their application for induced resistance against *Fusarium solani*

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Three *Streptomyces* isolates (ARHS/ PO15, ARHS/PO26 and ARHS/PO27) obtained from the rhizosphere soil of *Solanum tuberosum* were identified as *Streptomyces tricolor* (NCBI KX894280)*, S. flavogriseus* (NCBI KX894281) *and S. griseus* (NCBI KX894282) by 16S rDNA technology. Experiments were carried out to find out the growth promoting activity of these isolates on *Phaseolus vulgaris* and biocontrol activity against root rot pathogen (*Fusarium solani*). *Streptomyces tricolor* showed maximum growth promotion activity by enhancing plant growth in terms of shoot length, root length, number of leaves and leaf area. Accumulation of key defense enzymes like chitinase, β-1,3 glucanase, phenyl alanine ammonia lyase and peroxidase also increased in *Streptomyces* treated plants compared to untreated or fungal infected plants. Indirect immunofluorescence technique also confirmed the increase level of chitinase in the treated plant tissues. The results indicated that *Streptomyces* can be used as potential plant growth enhancer as well as biocontrol agent against *Fusarium solani,* root rot pathogen of *Phaseolus vulgaris*.

Key words: *Phaseolus vulgaris, Fusarium solani*, root rot, *Streptomyces tricolor, Streptomyces flavogriseus, Streptomyces griseus*

INTRODUCTION

Phaseolus vulgaris Linn. is commonly known as French bean is one of the important cash crop of India which is cultivated extensively in north east (Dochhil *et al* ., 2013) as well as other parts of India. Being one economically important crop its cultivation is very extensive in many places. But there are many factors that affect the yield of this vegetable like plant growth, plant health, disease etc. Root diseases of bean limit yields wherever the crop is grown and constitute a major constrain to bean production worldwide. Several organisms cause Root rot, among which *Fusarium solani, Rhizoctonia solani* and *Fusarium oxisporum* are main. Root rot caused by *Fusarium solani* results in yield loss of upto 84% in United States. *Fusarium solani* root rot on bean is widespread and occurs in most bean fields throughout the world. Cultural and chemical disease control methods are of limited value against root rot as stated by Hagerty *et al*. (2015). However, biocontrol by microorganisms can be an alternative. Plant growth promoting potential of *Streptomyces* on bean is already reported. Biocontrol of soil borne fungal pathogens has been studied by Gopalakrishnan *et al*.(2013).

The present study aims at revealing the role of three identified isolates of Streptomycetes, *Streptomyces tricolor* (KX894280)*, S. flavogriseus* (KX894281) *and S. griseus* (KX894282) for *in vivo* evaluation of the growth promoting activity on *Phaseolus vulgaris* and biocontrol efficacy against fungal root rot pathogen, *Fusarium solani*.

MATERIAL AND METHODS

Selection of Streptomyces isolates

Three Streptomycetes strains *Streptomyces tricolor* (NCBI KX894280)*, S. flavogriseus* (NCBI KX894281) *and S. griseus* (NCBI KX894282) were selected for *in vivo* evaluation of the growth promoting activity on *Phaseolus vulgaris.* Identification of the isolates were already done by their 16s rDNA sequences (Ray *et al*., 2016a).

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Growth promotion activity of Phaseolus vulgaris by Streptomyces isolates

Two cultivar of *Phaseolus vulgaris* were selected for field trial, Jwala(CV2) and Kholar (CV3). Three *Streptomyces* species - *Streptomyces tricolor* (NCBI KX894280), *S. flavogriseus* (NCBI KX894281) *and S. griseus* (NCBI KX894282) were selected for *in vivo* evaluation of the growth promoting activity on *Phaseolus vulgaris*. Application of *Streptomyces* isolates were done by seed coating , soil drench and foliar spray. For seed coating the method as described by Errakhi *et al*., (2007) was followed with some modifications. For soil drenching the method of Karimi *et al* (2012) was followed. For foliar spray the cell pellet suspended in sterile distilled water was sprayed on the leaves of plants after a few drop of Tween-20 was added to it. Growth improvement in the treated seedlings was observed 15 days after treatment and dry biomass were measured after three months of inoculation. For growth promotion average root length, shoot length, leaf number, and dry weight were measured against control.

Fungal pathogen

The root rot pathogen *Fusarium solani* (NFCCI 606) was obtained from NFCCI, Pune and maintained in the Immuno Phytopathology Laboratory, Department of Botany, North Bengal University.

In vitro antagonistic effect on Fusarium solani by Streptomyces isolates

Streptomyces isolates were tested for antagonistic effect against *Fusarium solani* by dual culture method (Skidmore and Dickinson, 1976). Inhibitions of the radial growth of fungal pathogen by the test organisms confirmed their antagonistic activity.

Artificial inoculation of the plant by fungal pathogen

Fifteen days old *Phaseolus vulgaris* plants were inoculated with *Fusarium solani* grown in sand maize meal media. *Fusarium solani* was mass multiplied in the sand maize meal media following the method of Biswas and Sen (2000). Fungal inoculum was added carefully to the rhizosphere of the plants. To determine the effect of *Streptomyces* on disease reduction four different set up were taken in each case: untreated control plant, inoculated with *F.solani*, treated with *Streptomyces* and inoculated with both *Sreptomyces* and *F. solani*. Disease intensity was calculated using 6 point scale following Mathew and Gupta(1996). Disease incidence was calculated following the method of Xue *et al*,(2013).

Assay of enzymes activities

Phenylalanine ammonia lyase (PAL activity of plants of four different set up was performed by the method as described by Chakraborty *et al*, (1993). Peroxidase activity was estimated by the method described by Chakraborty *et al*, (1993). Chitinase activity was estimated by the method of Bollar and Mauch, (1988). â- 1,3 Glucanase activity was measured by the method as described by Pan *et al*, (1991).

Total phenol content

Assay of total Phenol content of Leaves and root were done by the method of Mahadeven and Sridar, (1982).

Fluorescence antibody staining and microscopy

Indirect fluorescence staining of cross section of root and leaf for study of immunolocalization of Chitinase enzymes was done using FITC labeled goat anti rabbit IgG following the method of Chakraborty and Saha, (1994).

RESULTS AND DISCUSSION

Growth promoting activity

Three isolates selected for the study, *Streptomyces griseus*(KX894282), *S. tricolor*(KX894280) and *S. flavogriseus* (KX894281) showed positive effect in growth promotion of *Phaseolus vulgaris. Streptomyces* spp were applied in field in form of seed coating, Soil drench and foliar spray and measurement was taken in 7days, 15 days and 30 days interval. The results are summarised in the Table 1 and Table 2. Growth parameters taken into consideration are shoot length, root length, number of leaves and leaf area. *Streptomyces tricolor* (KX894280) was found to be the most effective one for plant growth promotion followed by *S. flavogriseus* (KX894281) and *S. griseus* (KX894282). Results are summarized in the Table 1 and Table 2. Increase in the total dry mass of the

plants after 30 days of inoculation (Fig 1) correspond to the result obtained for growth promotion activity of *Streptomyces* spp. El-Tarabily(2008) has already reported the ability of *Streptomyces* spp. in growth improvement of plants. Effect of *Streptomyces* formulation on growth of *Vigna radiata* was evaluated by Ray *et al*.,(2016b). The use of *Streptomyces griseus* and *Streptomyces tricolor* for the seed treatment of barley, oat, wheat and carrot to increase their growth has already been reported by Jog *et al* (2014).

***Biocontrol activity of Streptomyces* spp.**

To assess the role of *Streptomyces* spp. on biocontrol of root rot disease caused by *Fusarium solani, in vitro* antagonistic test was performed which confirmed positive effect of *Streptomyces* in controlling *F. Solani* (Table 3 and Fig 2). Biocontrol activity of *Streptomyces griseus* against *Penicillium, Botrytis, Fusarium, Rhizoctonia* and *Alternaria* have already been reported by Danaei et al., (2014), Anitha and Rebeeth(2010). In pot condition the disease severity in *Phaseolus vulgaris* inoculated with the pathogen *F. Solani* increased with time but the maximum disease severity was reduced in the pots where the soil was pretreated with *Streptomyses* spp. Among the three isolates, *S. flavogriseus* was the most effective in inhibiting the root rot disease followed by *S. tricolor* and *S. griseus*. CV2=Jwala, were more susceptable to the disease than the plants of CV3=Kholar (Fig 3). Simillarly El-Mohamedy *et al*., (2013) controlled root rot of *Phaseolus vulgaris* caused by *Fusarium solani* by *Trichoderma harzianum.*

Accumulation of Defense enzymes

Level of four major defense enzymes were quantified following application of *Streptomyces* spp and after challenge inoculation of *Phaseolus vulgaris* with root rot pathogen *Fusarium solani* in pot condition. Application of *Streptomyces* spp. in form of seed coating , soil drench and foliar spray were found to positively affect the biochemical properties of *Phaseolus vulgaris*. As disease establishment also changes the level of plant defense enzymes so the biochemical response of root tissue of plants following application of *Streptomyces* spp. and challange inoculated with the pathogen *F. Solani* was determined. Level of the defence enzymes Phenylalanine ammonia lyase (PAL), Peroxidase (POX), Chitinase(CHT) and â- 1,3 Glucanase(GLU) were found to increase significantly in the plants treated with the *Streptomyces* spp. in comparison to the untreated plants (Figs 4-8). Among the four different set up: untreated control plant, inoculated with *F.solani*, treated with *Streptomyces* and inoculated with both *Sreptomyces* and *F. solani,* it was found that the level of the enzymes were highest in the plants treated with both pathogen and *Streptomyces* spp. and lowest in the untreated plants. Among the two cultivars it was found that enzyme activity was higher in the cultivar Kholar than cultivar Jwala. Total phenol content of the plants was also estimated and it revealed increase in level of phenolics in the treated plants in comparison to the untreated plants. The result corresponds to the findings of earlier experiments where it was found that level of the defense enzymes increase in plants after infection by pathogenic organisms (Nandi *et al*.,2013, Parihar *et al* 2012) As was found in the present study that the level of defense enzyme was higher in the plants infected with the pathogens and treated with the *Streptomyces* spp., Chakraborty *et al* (2016) also studied changes in levels of different defense related enzymes, Phenylalanine ammonia lyase (PAL), Peroxidase (POX), Chitinase (CHT) and â-1,3Glucanse (GLU) in som plants following treatment with bioinoculants and infection with *Colletotrichum gloeosporioides*. Allay and Chakraborty, (2010) also reported enhanced activities of defence enzymes chitinase, glucanase and peroxidase in mandarin plants during disease suppression against fusarial root rot.

Induction of resistance and immuno-localization of chitinase in plant tissue

Localization of the plant defense enzyme chitinase was observed by FITC labelling as well as by conventional biochemical estimation process.Strong reactin with FITC in plant tissue indicate the induction of chitinase enzyme. In 2009, Chakraborty and co-workers studied the expression of chitinase in leaves of treated tea plants following induction with salicylic acid using immunofluorescent techniques.Following this technique root and leaf samples were collected from healthy plants without any treatment, plants treated with *Streptomyces* and inoculated with the pathogen. As level of chitinase was higher in *Streptomyces flavogriseus* treated plants so samples were collected from plant treated with *S.*

Table1: Effect of *Streptomyces* spp. on shoot and root growth in *Phaseolus vulgaris*

Treatment		Shoot length(cm)			Root length(cm)		
		7 days	15 days	30days	7 days	15 days	30days
CV Jwala	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
CV Kholar	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 50 plants. ± denote standard error. CV2=Cultivar 2(Jwala), CV3=Cultivar3 (Kholar). Treatment with *Streptomyces griseus, Streptomyces tricolor*, *Streptomyces flavogriseus*

Table 2: Effect of *Streptomyces* spp. on leaf growth in *Phaseolus vulgaris*

Treatment		Leaf number			Leaf area(cm^2)		
		7 days	15days	30days	7 days	15 days	30days
CV2	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
CV3	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 50 plants. ± denote standard errorCV2=Cultivar 2(Jwala), CV3=Cultivar 3(Kholar), Treatment with *Streptomyces griseus, Streptomyces tricolor*, *Streptomyces flavogriseus*

Table 3: *In vitro* antagonistic activity of *Streptomyces* spp. against *Fusarium solani*

Isolates	Inhibition of *Fusarium solani* (%)
Streptomyces griseus (ARHS/PO/15)	62.2
Streptomyces tricolor (ARHS/PO/26)	66.7
Streptomyces flavogriseus (ARHS/PO/27)	88.5

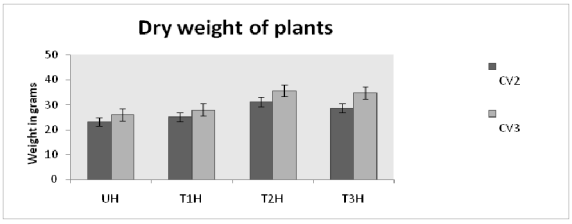

Fig. 1 Dry weight of *Phaseolus vulgaris* treated with *Streptomyces* formulation. UH= Untreated healthy, T1H= Treated with *S. griseus*, T2H= treated with *S. tricolor*, T3H=Treated with *S. flavogriseus*. [CV2 =Jwala, CV3=Kholar]

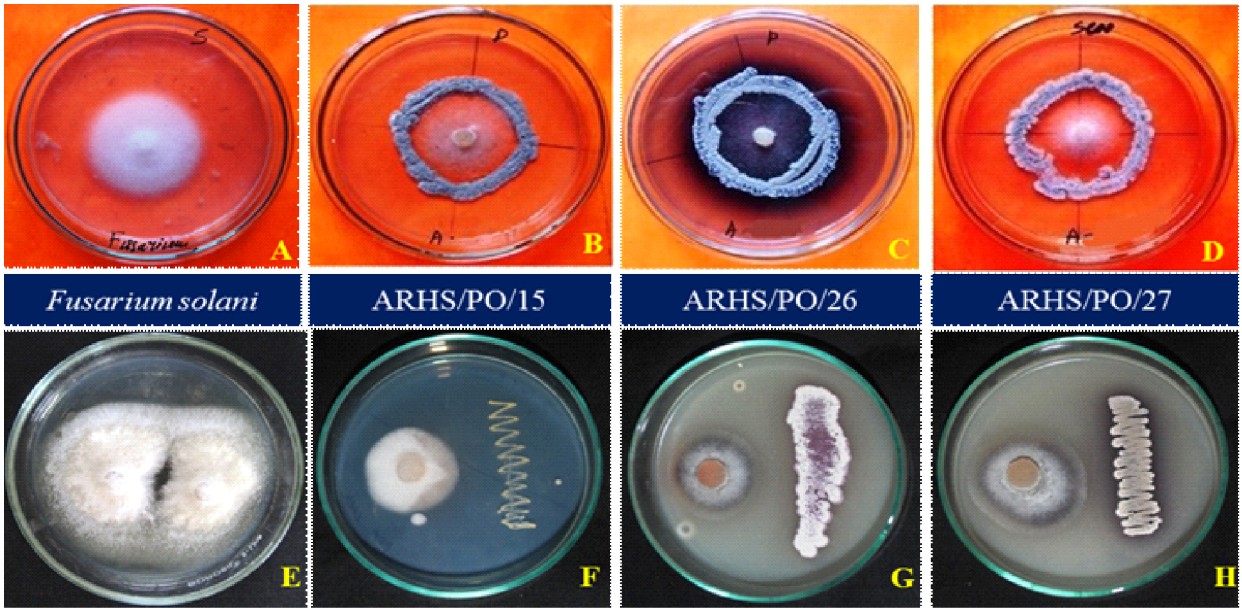

Fig.2 (A-H) *In vitro* assay of *Streptomyces griseus* -ARHS/PO/15 (B,F), *Streptomyces tricolor* - ARHS/PO/26(C,G), *Streptomyces flavogriseus* -ARHS/PO/27 (D,H) showing antagonistic activity against *Fusarium solani.* Control (A,E), Radial growth (B,C.D), Linear growth (F,G,H)

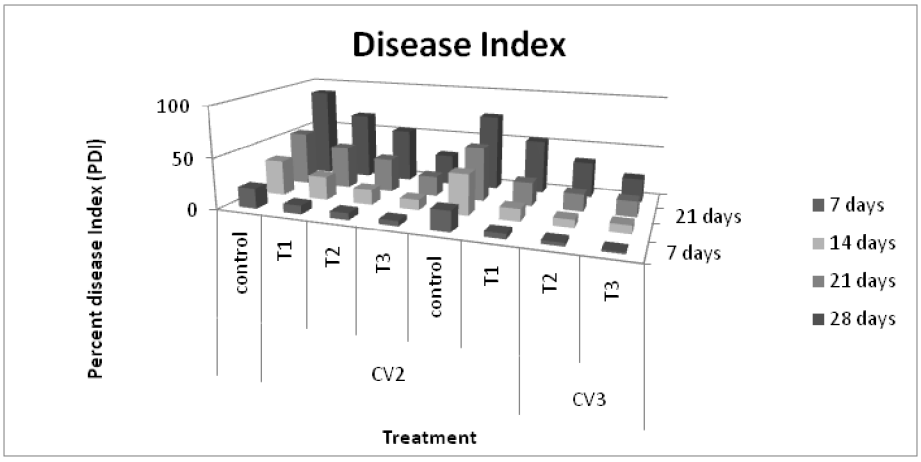

Fig.3 Disease index after challenge inculcation with *Fusarium solani* and host response upon respective isolate treatments in two cultivars of *Phaseolus vulgaris.* T1= Treated with *Streptomyces griseus,*T2=Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus* [CV2 =Jwala, CV3=Kholar].

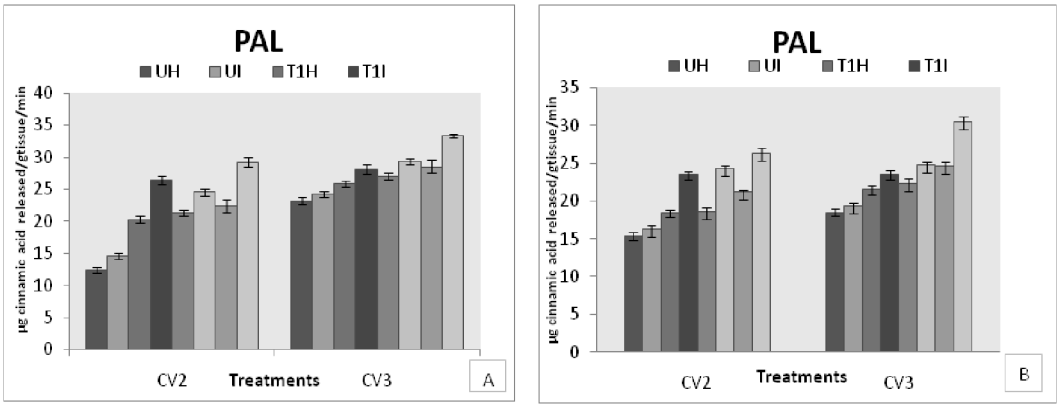

Fig. 4 PAL activity in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani;* UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus*.[CV2=Jwala, CV3=Kholar]

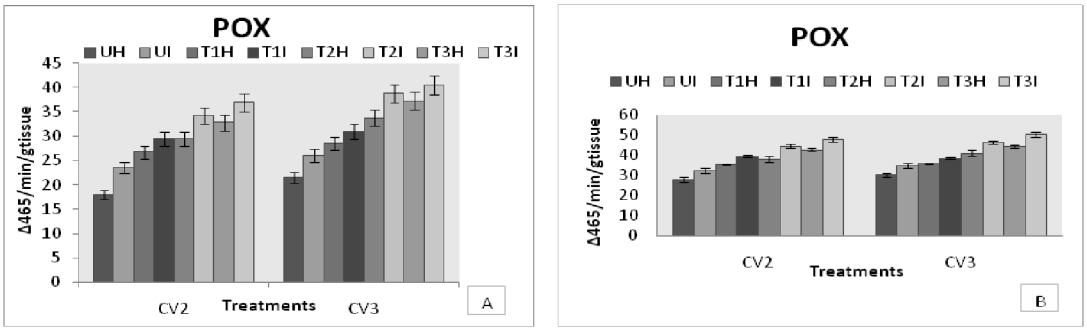

Fig. 5 Peroxidase activity in (A) leaf & (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus*. [CV2=Jwala, CV3=Kholar].

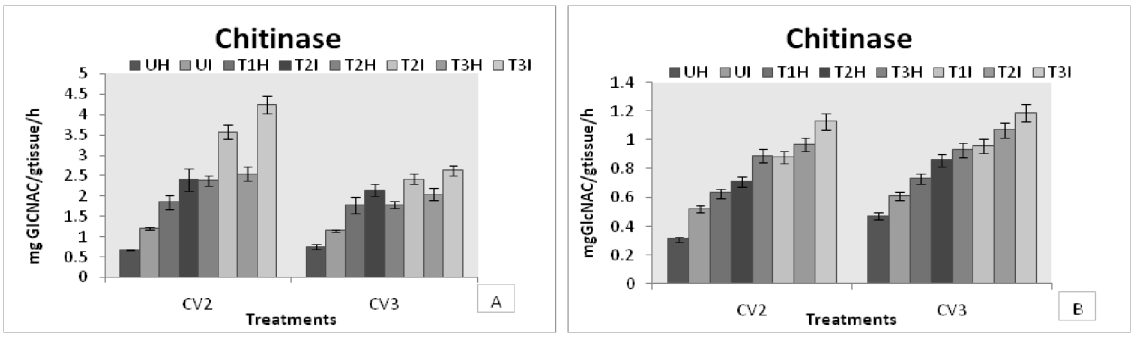

Fig. 6 Chitinase activity in (A) leaf and (B) roots of *P. vulgaris* treated with *Streptomyces* formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus*. [CV2=Jwala, CV3=Kholar].

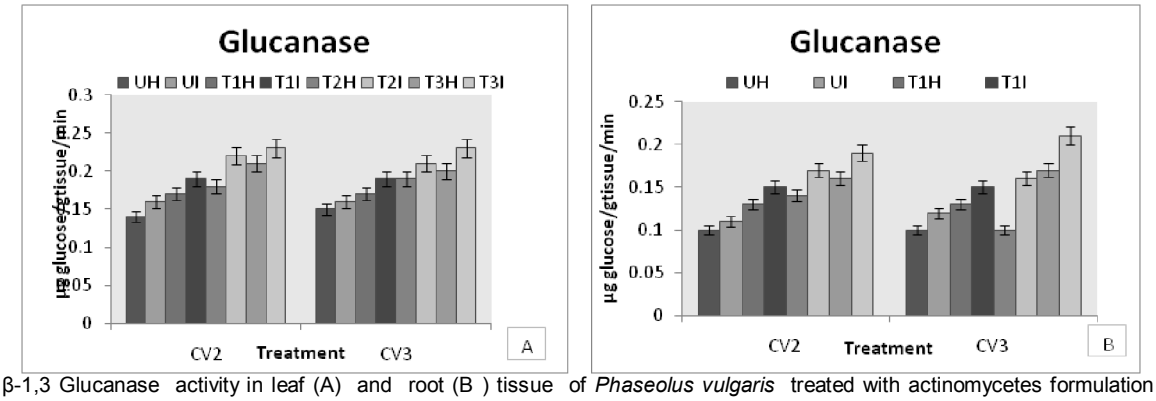

Fig. 7 β-1,3 Glucanase activity in leaf (A) and root (B) tissue of *Phaseolus vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*. UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus* [CV2=Jwala, CV3=Kholar]

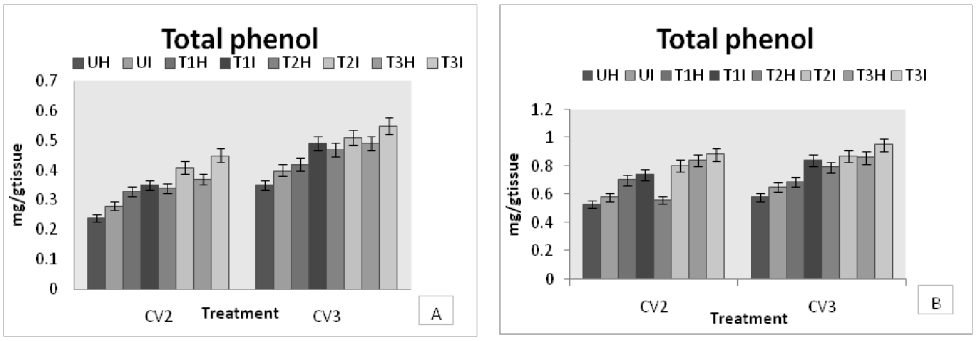

Fig.8 : Total phenol content in (A) leaf and (B) roots of varieties of *P. vulgaris* treated with actinomycetes formulation before and after challenge inoculation with *Fusarium solani*; UH= Untreated healthy, UI=Untreated Inoculated, T1= Treated with *Streptomyces griseus,* T2= Treated with *Streptomyces tricolor,* T3=Treated with *Streptomyces flavogriseus.* [CV2=Jwala, CV3=Kholar]

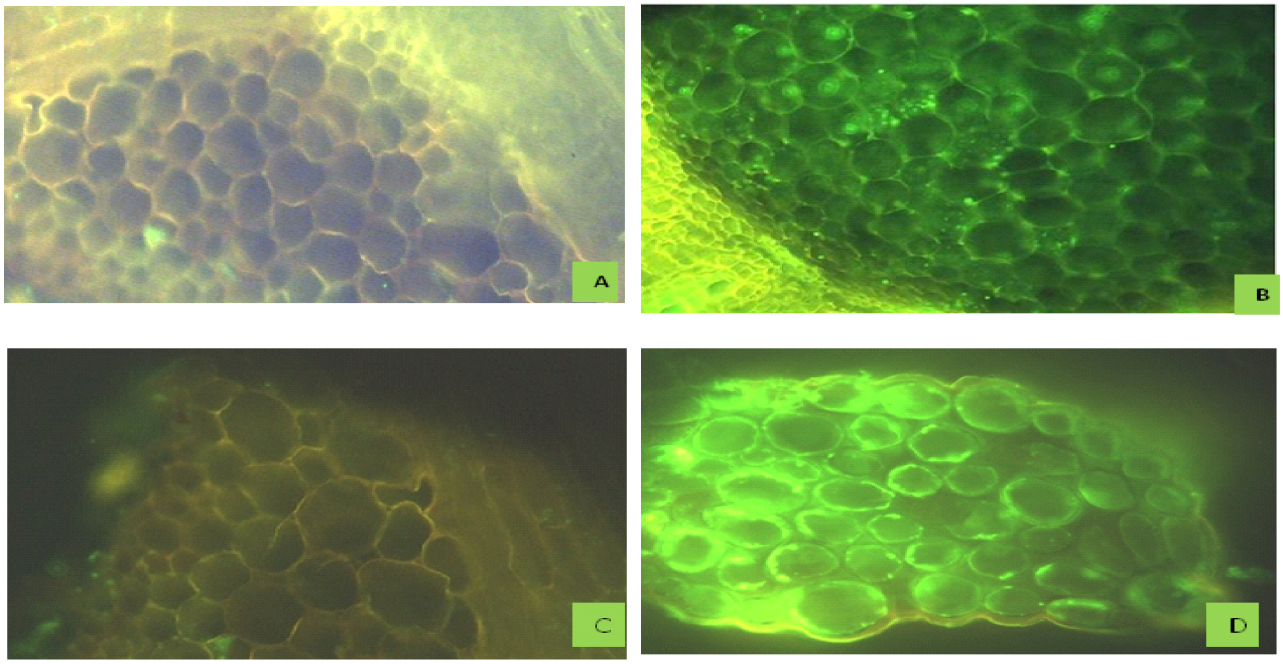

Fig. 9 (A-D) Transverse section of root tissue of *Phaseolus vulgaris* treated with *S.flavogriseus* (KX894281), inoculated with *F. solani* and reacted with PAb of chitinase following labelled with FITC conjugates. (A,C) Untreated inoculated control , (B,D) Treated with *Streptomyces* and inoculated with *F. solani* showing apple green fluorescence and localization of chitinase in cortical tissue. [A,B = Kholar (CV3) , C,D =Jwala (CV2)]

flavogriseus. The treated root and leaf tissue showed bright apple green fluorescent colour in the epidermal and cortical tissue of the plant samples. This observation indicate the localization of chitinase enzyme in the tissue (Fig 9). From the present study it can be concluded that *Streptomyces* spp. can be used for improvement of plant growth status as well as for management of root rot disease of *Phaseolus vulgaris* caused by *Fusarium solani.* Further study is needed to ascertain the effect of these *Streptomyces* spp. on other phytopathogenic fungi.

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