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RESEARCH ARTICLE

ACTINOMYCETES FROM RICE FIELD SOIL AND THEIR ANTAGONISTIC ACTIVITY AGAINST RICE FUNGAL PHYTOPATHOGEN.

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Abstract

A total of 125 actinomycetes isolates were obtained from 25 rice field soil samples. All the isolates were screened for antifungal activity against *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani* using in vitro dual culture bioassay. A total of 30 isolates (24%) were displaying antagonistic activity against one or the other tested plant pathogenic fungi. Seventeen actinomycetes isolates showed antagonistic activity against *Fusarium moniliforme*, fourteen against *Rhizoctonia solani* and fourteen against *Helminthosporium oryzae*. Two isolates R5 and Rf81 were displaying antifungal activity against all the three tested fungi. Among the antagonist isolates, seventeen were found to produce volatile compounds and fifteen showed diffusible antimetabolite production. Out of these 30 isolates, 22 belonged to *Streptomyces spp*., 4 each to *Micromonospora* and *Microbispora*. Four isolates (R5, R37, Rf2 and Rf81) were selected on the basis of their antagonistic ability for field experiment . Highest inhibition of foot rot disease was showed by R5 treatment in nursery (5.12%) and field (8.49%) over control whereas the results of the nursery bed experiment with consortium of R5, R37, Rf2 and Rf81 inoculation to rice seeds showed disease incidence of 6.41%. Therefore, these results suggested that actinomycete isolate R5 may be used as a potential biocontrol agent against foot rot disease in Pusa basmati 1121.

Introduction:-

In Asia, more than 80% of the people live on rice, and their primary food security is entirely dependent on the volume of rice produced in this part of the world. The total global rice is declining gradually even with the extensive use of the modern varieties such as high yielding and hybrid varieties. Moreover, the problems associated with the green revolution technological packages, compounded by the problems of soils, water, pest and diseases have further complicated the efforts to maintain farmer's existing yields. The currently used fungicides are either less effective or of a great environmental concern. Hence, it is necessary to develop environmentally safe and potent fungicides of natural origin.

Potential use of microbes based biocontrol-agents as replacement or supplements for agrochemicals has been addressed in many recent reports (Shimizu et al, 2000). With the increased concern about conserving natural resources as air, soil and water, natural or biological control of plant diseases has received increased emphasis. Biological control of plant diseases is slow, gives few quick profits, but can be long lasting, inexpensive and harmless to life (Dhingra and Sinclair, 1995).

Actinomycetes, a Gram-positive bacteria in order Actinomycetales, are part of the indigenous soil microflora involved in the turnover of recalcitrant plant organic matter and produce a balance in the ecosystem (Miyadoh,

1997).Actinomycetes are known to produce bioactive substances,especially antibiotics that are effective against phytopathogenic fungi (Crawford et al, 1993; Bressan, 2003). Biocontrol with beneficial bacteria is one promising alternative to fungicides (Li et al, 2008). Hydrolases such as chitinase contribute to degradation of fungal cell walls (Korsten et al, 1994) Paulitz and Belanger, 2001; Helisto et al, 2001). Chitin is the second most abundant polysaccharide in nature and a major component of fungal walls, insect exoskeletons and crustacean shells. Chitinase secreted by a BCA is likely to be effective against pathogenic fungi, the cell walls of which are mainly chitin. The present study aims to isolate and screen efficient soil actinomycetes and to evaluate their antagonism against selected fungal pathogens.

Material and methods:-

Procurement of standard fungal cultures:-

Cultures of *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani* were obtained from Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The fungal cultures were maintained on Glucose Yeast Extract Agar and stored at 4ºc.

Soil sampling:-

Twenty soil samples fields of rice were collected from Punjab Agricultural University, Ludhiana. The samples were collected in polythene bags and transported to laboratory for immediate processing.

Isolation of actinomycetes:-

Actinomycetes population from the soil of rice fields were determined by serial dilution and spread plating method on Starch Casein Agar (Küster and William, 1964). Ten grams of soil sample was taken and transferred to an Erlenmeyer flask containing 90 ml of sterile distilled water. The soil sample was serially diluted upto 10^{-5} levels. Aliquot (1ml) from serial logarithmic dilution of each suspension was pipetted onto the surface of duplicate Petri plates containing Starch Casein Agar (SCA). The inoculum was spread evenly over the surface using glass spreader. Petri plates were incubated for 7-10 days at 28ºc. After the incubation period, the mean colony count was determined and recorded as colony forming unit (CFU/g) of each sample. The suspected colonies were picked up and purified on SCA medium and incubated at 28^0C for 5-7 days. Isolated colonies were streaked on SCA after purification and then subcultured on slants and stored at 4°C.

Antagonistic effect against phytopathogenic fungi:-

The actinomycete isolates were evaluated for their antagonistic activity against *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani* by dual-culture in-vitro assay (Khamna et al, 2009). Fungal discs (8mm in diameter), 5 days old on GYE at 28ºc, were placed at the center of GYE plates. Actinomycete discs (8mm) 5 days old, grown on starch casein agar, incubated at 28ºc were placed on opposite sides of the plates, 3 cm away from fungal disc. Plates without the actinomycete disc served as controls. All the plates were incubated at 28ºc for 14 days and colony growth inhibition (%) was calculated by using the formula:

Inhibition (%) = [(Fungal growth radius of control - Fungal growth radius in the direction of actinomycete) / Fungal growth radius of control] × 100

Production of volatile antifungal compounds:-

The production of volatile antifungal compounds released by isolates was assayed by sealed plate method as demostrated by Fiddaman and Rossal (1993). Two hundred microlitre cultures from five day old broth of actinomycete isolates were spread on Petriplates containing SCA medium. After incubation at 28°C for 4-5 days, a second Petri plate containing PDA was inoculated with a 6mm of the test fungi and placed over the actinomycete culture. The two plates were sealed together with parafilm and further incubated at 28°C. A Petriplate containing agar medium without actinomycete was placed over the PDA medium inoculated with the fungal pathogen served as control. A radial growth of the test fungus was measured after 5 days.

Production of diffusible antimetabolites:-

Production of diffusible metabolites by actinomycete isolates against *H. Oryzae*, *F. Moniliforme* and *R. Solani* was assayed by the method of Montealegre et al. (2003). Glucose yeast extract plates covered with a cellophane membrane were overlayed with SCA medium and inoculated with 100µl of bioantagonistic actinomycete isolate suspension. After incubation for 5days at 28°C, the membrane along with the grown isolate was removed and the plate was inoculated in the middle with 10mm disc of pure fungal culture. Plates were incubated at 28°C for 5 days

and the growth of pathogen was measured.

Morphological and biochemical characterization of the isolates:-
From thirty actinomycete isolates which displayed high antagonistic activity and their colonies were characterized morphologically and physiologically to the genus level (Shirling and Gottlieb, 1966).

In vivo biocontrol assay for testing effectiveness of potential actinomycete isolates as antagonists against Fusarium moniliforme:-
Field experiment:-
Seed Treatment:-
Seeds of basmati rice cultivar Pusa Basmati 1121 were treated with inoculum of *F. Moniliforme* for 2 hrs and then inoculated seeds were dipped in suspension of four actinomycete isolates showing antagonism (R5, R37, Rf2 and Rf81) against *F. Moniliforme* again for 2 hrs. Untreated seeds were taken as control. Seeds were sown in five nursery beds (four beds for treated seeds and one for control) in soil (1 x 1 meter plot size) in 15 rows having 30 seeds per row.

Seed Treatment with consortium of actinomycetes isolates:-
Seeds of basmati rice Pusa Basmati 1121 treated with inoculum of *F. Moniliforme* for 2 hrs and then inoculated seeds were dipped in consortium suspension of actinomycetes isolates (R5, R37, Rf2 and Rf81) showing antagonistic activity towards *F. Moniliforme* again for 2hrs. Untreated seeds were taken as control. Seeds were sown in two nursery beds (one bed for treated seeds and one for control) in soil (1 x 1 meter plot size) in 13 rows having 20 seeds per row.

Seedling Treatment:-
Seedlings raised from infected seeds were treated with potential actinomycete isolates inoculum (R5, R37, Rf2 and Rf81) for 2hrs before transplanting and these seedling were transplanted in field ($1^{1/2}$ x $1^{1/2}$ meter plot size) in duplicate manner accordingly as treatment given in nursery beds in 8 rows having 10 plants per row with plant to plant spacing 15 cm and row to row spacing 20 cm.

Field experiment
Incidence of disease (% Disease incidence)
The plants were counted with foot rot disease symptoms and then disease incidence was measured as follows:

$$\text{Incidence of disease (\%)} = \frac{\text{Total number of dead seeds}}{\text{Total number of seeds sown}} \times 100$$

Results and discussion:-

Isolation of actinomycetes from rhizospheric soil:-
A total of 125 isolates of actinomycete were obtained from rhizospheric soil of rice field from different locations of Punjab Agricultural University Ludhiana. Our results are in conformity with the work of other researches. Priya and Kalaichelvan (2012) reported 57 isolates from rhizospheric soil samples collected from paddy fields of different parts of Tamil Nadu. Elamvazhuthi and Subramanian (2013) obtained 15 isolates of actinomycetes from upland paddy field soils. Harikrishnan and Shanmugaiah (2013) obtained 102 isolates of actinomycetes from 18 rice rhizospheric soil samples. Muangham, Pathomaree and Duangmal (2014) isolated 210 melanogenic actinomycetes from 75 rice rhizospheric soil samples.

The data on occurrence and enumeration of actinomycetes from rhizopheric soils showed that mean colony count ranged from 3.1×10^4 to 4.7×10^4 cfu/g of soil .Our results are supported by Punngram et al (2008) who reported total actinomycete counts from rice rhizospheric soil sample were from 3.2×10^3 - 6.5×10^5 cfu /g . Merckx et al (1987) suggested that the rhizosphere represents a unique biological niche that supports abundant and diverse saprophytic microorganisms due to a high input of organic materials derived from the plant roots and root exudates. Hata et al (2015) reported the actinomycete population in the rhizosphere ranging from 93.09 x 10^3 CFU/g to 359.60 x 10^3 CFU/g in rice field soil.

In vitro screening of actinomycetes isolates for antagonistic potential against phytopathogenic fungi:-
Out of 125 isolates obtained from rhizospheric soil of rice 30 (24%) were displaying antagonistic activity against one or the other tested plant pathogenic fungi. Out of 30 isolates 12 were from nursery and 18 from field soil (Table 1). A total of 17 actinomycete isolates were displaying antagonistic activity against *F. moliniforme* two isolates R37 and Rf81 exhibited maximum antagonistic activity with percent inhibition of 26.67% and 28.33% respectively. Fourteen actinomycete isolates showed antagonistic activity against *H. oryzae*. Two isolates Rf35 and Rf81 were displaying maximum antifungal activity with percent inhibition of 50%, as evident from Table 1. Fourteen actinomycetes isolates exhibited antagonistic activity against *R. solani* with highest percent inhibition by R37 and Rf65 (29.41% each). Isolate Rf37 was showing antifungal activity against *R. solani* and *F. moniliforme* whereas isolates Rf81 and Rf5 was showing antagonism against *H. oryzae*, *R. solani* and *F. moniliforme*. Plant root exudates stimulate growth of rhizospheric actinomycetes that are strongly antagonistic to fungal pathogens, while the actinomycetes utilize root exudates for growth and synthesis of antimicrobial substances (Crawford et al, 1993; Yuan and Crawford, 1995). Our results are in accordance with several workers. Ningthoujam et al (2009) obtained 35 actinomycete isolates from various habitats in Tamil Nadu and screened for activity against some major rice fungal pathogens such as Curvularia oryzae MTCC 2605, Pyricularia oryzae MTCC147, H. oryzae, F. oxysporum MTCC287 and R. solani and found that four isolates showed potent antagonistic activities in dual culture assay.

Effect of volatile compound production on growth inhibition of pathogenic fungi:-
Fourteen isolates of actinomycetes displayed antifungal activity against *H. oryzae*. Out of these 14 isolates only six were able to produce volatile compounds (Table 2). The growth inhibition was in the range of 19.66-35.29%. Seven out of 15 antagonistic isolates were found to produce volatile compounds against *R. solani* and showed growth inhibition in the range of 19.57-52.94%. Six out of seventeen were produced volatile compounds against *F. moniliforme* in the range of 21.54-35.33%. Maximum inhibition was observed in plates R5 and Rf81 35.53 and 35.29 respectivly**.** Volatile organic compounds produced by *Streptomyces* spp. and other species of actinomycetes were reported to cause growth abnormalities in different fungi, including *F.oxysporum*. Priya et al (2012) reported that volatile organic compounds from the actinomycetes significantly inhibited the fungal growth and completely prevented the pigment production. The pigments of pathogenic fungi, such as melanin were reported to be interrelated with fungal pathogenicity and could endow fungi some special recovery function, such as antiradiation, antioxidation and scavenging free radical. Therefore, it seems that the volatiles produced by *Streptomyces sp.5* and *Streptomyce ssp.7* would be possible to play a significant role in reducing the pathogenic fungal infection ability. Cordovez et al (2015) tested the antifungal activity of volatile organic compounds (VOCs) produced by the *Streptomyces* isolates from disease suppressive soil, hyphal growth of *R. solani* was measured during exposure to VOCs from each of the isolates. In the control, fungal hyphae reached the edge of the agar plates after 2 days of incubation.

All *Streptomyces* strains were able to significantly retard the growth of *R. solani*. Streptomyces strains W47 and W214 were the most inhibitory. When exposed for 2 days to the VOCs produced by these isolates, radial hyphal growth was reduced by 57 and 41%, respectively. *Streptomyces* also produces VOCs which reduce the incidence and/or the severity of several plant diseases caused by fungi and cause morphological abnormalities in different fungi (Moore and Stotzky, 1973; Wan et al, 2008; Boukaew et al, 2013; Wang et al, 2013; Wu et al, 2015).

Effect of diffusible antimetabolites production on growth inhibition of pathogenic fungi:-
Reduction in radial growth of all the tested pathogenic fungi was observed after incubation due to production of diffusible antifungal metabolites growth inhibition varied from 12.76 to 21.76%. Seven out of seventeen isolate were producing volatile compounds against *F. moniliforme* in the range of 16.76-21.76% (Table 3). Fourteen isolates of actinomycetes were displaying antifungal activity against *H. oryzae*. Out of these only six were able to produce antimetabolites. The growth inhibition was shown by these isolates in the range of 15.76-21.66%. Six out of 14 isolates were found to produce volatile compounds against *R. solani* in the range of 12.76-16.54%. Diffusible antimetabolites produced by *Streptomyces spp*. and other species of actinomycetes were reported to cause growth abnormalities in different fungi, including *F. oxysporum*. Priya (2012) revealed that *F. oxysporum*, *Botrytis cinerea*, *Rhytisma acerinum* and *Macrophomina phaseolina* were highly sensitive to diffused metabolites on agar and on cellophane followed by volatiles produced by isolate GACMPT- 57.

Description of species isolated:-

Thirty antagonistic isolates were further identified presumptively on the basis of morphological and biochemical characteristics. Twenty two isolates out of 30 were identified as *Streptomyces spp.* Based on colony and cultural characteristics. *Streptomyces* isolates were further characterized into various sub groups. Out of 22 isolates, 14 belonged to *Streptomyces cinereus*, and 8 to *S. albosporus*. Besides *Streptomyces sp*., other genra like *Micromonospora sp.* (n=4), and *Microbispora sp.* (n=4) were also reported. The isolates which are seleceted for field evaluation R5 belong to *Streptomyces albosporus*, R37 to *Streptomyces cinereus*, Rf2 to *Streptomyces cinereus* and Rf81 to *Streptomyces* albosporus. Results are in accordance with observations of Coombs and Franco (2003) and Okazaki (2003). Streptomyces sp. had 100% distribution in the rhizosphere of Seena occidentalis and 75% in the rhizosphere of Musa sapientum var parasidiaca. Intrameron et al (2011) reported that 26 actinomycetes out of 30 strains tested for antifungal activity belonged to genus *Streptomyces* and contained between 95-100% DNA homology in the 16S rRNA gene. Khalil et al (2014) obtained fifty isolates of *Streptomyces* spp; from Egyptian rice field soil. *Streptomycetes* have been determined largely because of morphological and biochemical criteria, resulting in the arrangement of strains into cluster groups. On the basis of the aerial and substrate mycelia, development of spiral spore chains and smooth spore surface of the strain, they are placed under the genus Streptomyces (Williams et al 1983).

Biocontrol assay for testing effectiveness of potential actinomycete isolates as antagonists towards Fusarium moniliforme:-

Field experiment on the basis of in-vitro antagonistic activity:-

Four isolates (R5, R37, Rf2 and Rf81) were used in treatment of seeds and seedlings in cultivar Pusa basmati 1121 against foot rot disease in field condition.

Nursery bed experiment:-

In the nursery bed experiment, treatment of R5 and R37 inocula to seeds showed least disease incidence 5.12% and 5.30% followed by Rf2 (6.11%) and Rf81 (8.89%). On the other hand, untreated seeds taken as control showed disease incidence 14.13% (Table 4 & 5).

Results are in accordance with Zarandi et al (2009) tested the potential of rhizospheric/ endophytic *Streptomyces sindeneusis* isolate 263 for the biological control of *Magnaporthe oryzae* the causal agent of rice blast. Treatment of plants with only pathogen resulted in typical blast symptoms and percent of diseased leaf area evaluated 8%. In the treatments with both pathogen and *Streptomyces* percent of diseased leaf area was evaluated to be 0.5% according to the method developed by IRRI. That is indicative of significant reduction in the number of lesions in pots which received the antagonist.

The fungicide control (phytopathogen + metalaxyl 3g a.i./L) and the negative control (treatment without the phytopathogen) did not show any damping off incidence. Besides, the positive control (treatment with the phytopathogen) demonstrated 98% of damping off. The actinobacterium strain 16R3B was more effective biocontrol agent against this phytopathogen (Francisco et al 2013).

Sitti and Christopher (2008) obtained 15 endophytic actinomycetes from seven week-old healthy tomato plants. In planta tests on *Rhizoctonia solani* infected tomato seeds revealed that 14 out of 15 tested isolates were able to significantly reduce the percentage of infected plants. Three of the isolates, TF2, TF21 and TF30 (all belonging to *Streptomyces* group) decreased disease incidence of the pathogen by at least 60%. Thirteen isolates were found to inhibit damping off significantly in vivo. Surprisingly, one isolate was inactive in vitro but had relatively high (40%) disease suppression in vivo, while *Streptomyces thermocarboxydus* (TF23) showed strong in vitro antagonism but resulted in the lowest disease suppression in planta.

Field experiment:-

Data of field experiment exhibited disease incidences of 8.49% (R5), 10.19%, (Rf81), 11.05% (Rf2) and 13.43 (R37) as shown in Table 4. Untreated infected seeds taken as control showed disease incidence of 18.62%. Interpretation after pair wise comparison of treatments showed that treatments R5-Rf81, R37-Rf2 and Rf2-Rf81 does not differ significantly in controlling the foot rot disease in Pusa basmati 1121. Rest of the treatments differed significantly in controlling the disease.

The results of our study are in accordance with Yating et al (2014), who tested rhizospheric/ endophytic actinomycete strains F22-29 against Rape clubroot. Their studies in pots showed that the occurrence of disease in

both the seed coating and root irrigation with actinomycete fermentation liquid F22-29 (28.57 and 34.29%) was lower than the infected control (60%). Same isolates when tested for inhibition of Chinese cabbage clubroot resulted in disease incidence of 56.67%, which was 26.09% lesser as compared to its control. In another trial, the disease incidences for seed coating and root irrigation with F22-29 against Brassica napus clubroot were 35.00% and 60.00% respectively. The inoculation reduced disease incidence by 50.00% and 14.29% as compared to control. Additionally, they reported control efficiency of seed coating with F22-29 to be significantly better than the root irrigation with it.

Consortium treatment:-

The results in table 6 revealed that in the nursery bed experiment combined treatment of R5, R37, Rf2 and Rf81 inocula to rice seeds showed disease incidence of 6.41%, which was comparatively lower than untreated control (16.90%). This indicates that the consortium of these treatments may prove to be a potential biocontrol antagonist against foot rot disease in Pusa Basmati 1121.

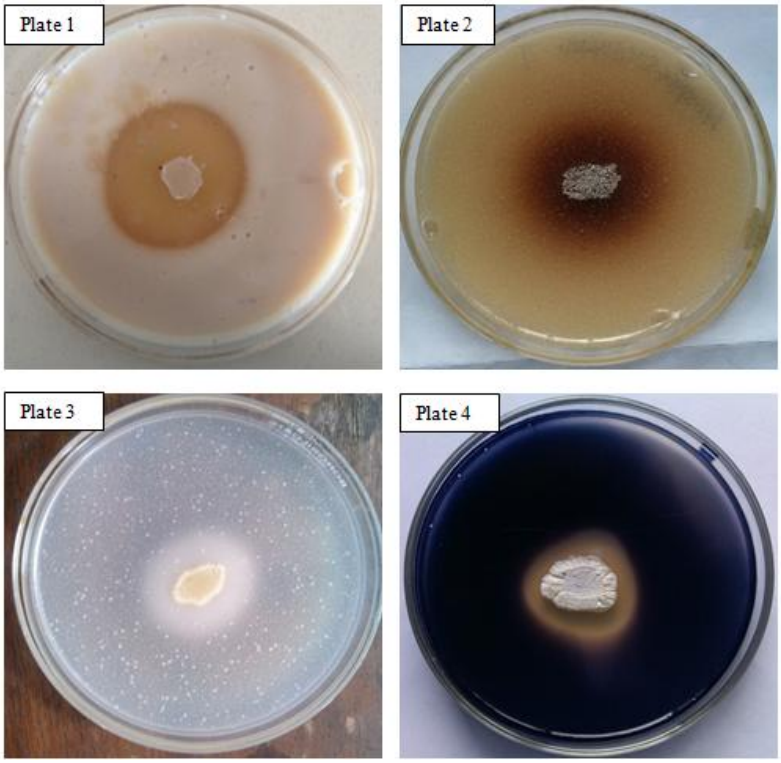

Fig. 1: Biochemical characterization of actinomycete isolates obtained from rhizosphere of rice plants.
Plate 1: Hydrolysis of Casein by *Streptomyces albosporus*, R8
Plate 2: Degradation of Tyrosine by *Streptomyces cinereus*, R24
Plate 3: Hydrolysis of Tween-20 by *Micromonospora spp.*, R35
Plate 4: Hydrolysis of Starch by *Microbiospora spp.*, Rf26

Table 1:-Antifungal activity (%inhibition) of actinomycete isolates obtained from rhizospheric soil of rice plants from nursery and field soil.

Isolates	Percent inhibition		
	Helminthosporium oryzae	***Rhizoctonia solani***	***Fusarium moniliforme***
R1	-	-	25.00
R3	-	-	8.33
R8	-	-	25.00
R5	**38.88**	**23.53**	**25.00**
R13	-	-	28.33
R17	27.78	17.65	-
R21	-	9.41	-
R24	-	-	13.33
R27	33.33	11.77	-
R28	27.78	17.65	-
R31	-	15.30	8.33
R37	-	**29.41**	**26.67**
Rf2	-	**23.53**	**21.21**
Rf4	38.88	-	-
Rf12	38.88	-	8.33
Rf17	-	11.77	-
Rf23	-	5.88	11.67
Rf26	27.78	-	21.67
Rf32	-	-	16.67
Rf35	50.00	-	-
Rf39	38.88	-	-
Rf43	33.33	-	-
Rf47	38.88	-	-
Rf52	33.33	-	-
Rf58	-	8.24	-
Rf61	33.33	-	11.67
Rf65	-	29.41	16.67
Rf72	-	23.53	-
Rf76	-	-	16.67
Rf81	**50.00**	**23.53**	**28.33**

Values indicate mean of three replicates.

Table 2:-Growth inhibition (%) of pathogenic fungi by rhizospheric actinomycete isolates due to volatile compounds production.

Isolates	Percent inhibition		
	Helminthosporium oryzae	***Rhizoctonia solani***	***Fusarium moniliforme***
R3	-	-	23.53
R5	**19.66**	-	**35.53**
R13	-	23.53	-
R21	-	23.53	-
R27	-	52.94	-
Rf2	-	-	**32.66**
Rf4	21.52	-	-
Rf12	-	29.41	-
Rf26	-	-	26.58
Rf39	23.53	-	-
Rf47	24.29	-	-
Rf52	-	23.53	-
Rf58	21.66	-	21.54
Rf65	-	19.57	-
Rf76	-	25.33	-
Rf81	**35.29**	-	**23.76**

Values indicate mean of three replicates.

Table 3:- Growth inhibition of pathogenic fungi by rhizospheric actinomycete isolates due to diffusible antimetabolites production.

Isolates	Percent inhibition		
	Helminthosporium oryzae	***Rhizoctonia solani***	***Fusarium moniliforme***
R3	-	-	17.33
R5	**16.53**	**-**	**19.66**
R21	-	13.33	-
R24	-	-	16.76
R27	19.53	-	**-**
R31	-	15.76	-
Rf2	**-**	**16.54**	**21.76**
Rf12	17.66	-	-
Rf23	-	13.33	-
Rf32	-	-	17.33
Rf39	15.76	-	-
Rf52	21.66	-	-
Rf58	-	12.76	19.54
Rf76	-	15.21	-
Rf81	**16.33**	**-**	**20.61**

Values indicate mean of three replicates.

Table 4:- Incidence of foot rot (% Disease incidence) in rice seeds implanted in nursery and seedlings in field.

Treatments	Percent disease incidence (%) in nursery	Percent disease incidence (%) in field after 45 days
R5	5.12	8.49
R37	5.30	13.43
Rf2	6.11	11.06
Rf81	8.89	10.19
Control	14.13	18.62

Table 6:-Effect of actinomycetes consortium on incidence of foot rot (Fusarium moniliforme) in nursery

Treatments	No. of seeds sown	No. of seeds germinated	Dead seedlings	Percent disease incidence(%) after 40 days
R5+R37+Rf2+Rf81	180	176	10	6.41
Control	180	169	24	16.90

Conclusion:-

The present study described the isolation and selection of actinomycetes from rice field soils as potential biocontrol agent to inhibit rice fungal diseases. Based on our data, isolates R5, R37, Rf2 and Rf81, which belonged to *Streptomyces spp*., showed the strongest broad spectrum antifungal antagonistic effect against three rice fungal pathogens. These *Streptomyces* isolates (R5, R37, Rf2 and Rf81) may prove to be potential biocontrol agents of rice plant pathogenic fungi and need to be tested in field.

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