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RESEARCH ARTICLE

Endophytic actinomycetes from *Azadirachta indica* A. Juss.: Characterization and anti-microbial activity

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Abstract

A total of 35 isolates of endophytic actinomycetes were obtained from 20 trees of *Azadirachta indica* A. Juss. These were then screened and evaluated for their biocontrol potential against an array of pathogenic fungi and bacteria. The dominant genus identified was *Streptomyces* sp. (23) and rest was related to other genera, like *Micromonospora* sp. (5), *Microbiospora* sp. (5) and *Nocardia* sp. (2). The distribution of endophytic actinomycetes was 42.9, 37.1 and 20 % in roots, stems and leaves respectively. Eight of the isolates were showing inhibitory activity against different plant pathogenic fungi and also fifteen isolates were displaying antagonism against human pathogenic bacteria. These results not only further our understanding of plant–microbe interactions but also indicate that there is an untapped resource of endophytic microorganisms that could be exploited in the biotechnological, medicinal and agricultural industries.

Introduction:-

Actinomycetes represent a large proportion of the soil microbial biomass which represents a diverse group of filamentous Gram-positive bacteria characterized by high G+C content in their genomes. Actinomycetes are also found as endophytes that colonize the plant tissues. Endophytes enter plant tissues primarily through the root zone; however aerial portion of plants such as flowers, stems and cotyledons may also be used for entry. The first endophytic actinomycete to be identified and studied was the *Frankia* species. *Frankia* are nitrogen fixing actinomycetes that form actinorrhizae with eight families and over 200 species of angiosperms (Strobel and Daisy, 2003). Since the isolation of *Frankia*, a number of other biologically active endophytes and root-colonizing microorganisms belonging to actinomycetal group have been isolated or detected. The most common endophytic actinomycete isolated from surface-sterilized plant tissues is *Streptomyces* sp. (Coombs et al., 2004).

The association of actinomycetes with plants is found to confer many advantages to host plants such as the production of phytohormones and siderophores, the nitrogen fixation and the protection against plant pathogens by producing antibiotics or extracellular enzymes (Ezra et al., 2004). Endophyte infected plants often grow faster than the non-infected ones. This effect is due to the production of the phytohormone Indole-3-acetic acid (IAA) (Coombs, 2002), Pteridic acid A and B, which has auxin like activity (Misk and Franco, 2011), phosphate solublizing activity (Provorov et al., 2002), production of siderophores (Coombs and Franco, 2003), cytokinin and other plant growth promoting substances by the infecting endophytes. They play a crucial role in the recycling of refractory biomaterials by decomposing complex mixtures of polymers in dead plant, animal and fungal materials producing several volatile sesquiterpene substances namely, geosmins, responsible for the characteristic ―wet earthy odor.

Endophytes may produce a plethora of substances e.g. novel antibiotics, antimycotics, immunosuppressants, and anticancer compounds of potential use to modern medicine, agriculture, and industry (Bailey et al., 2006). The introduction of endophytic actinomycetes into plants with the ability to colonize the internal tissue would further

enhance the stability and increase their potential effectiveness as biocontrol agents (Lee et al., 2004). An endophytic actinomycete isolated from creeping vine (*Monstera* sp.) was also reported as *Streptomyces* sp. producing coronamycin, a complex substance of novel peptide antibiotics with the activity against pythiaceous fungus and human fungal pathogen, *Cryptococcus neoformans* (Igarashi et al., 2002). Some endophytic *Streptomyces* sp. can enhance crop yield through protection of their host against pathogens as in case of 'take all disease' of wheat (Wakelin et al., 2004). Because of the production of broad-spectrum antimicrobial compounds by actinomycetes, they may have antagonistic effects on the growth and performance of nitrogen-fixing mutualistic bacteria (Costa and Loper, 2012).

Each part of *Azadirachta indica* is used in medicines for centuries now and thus commercially exploitable. It is also considered to be a natural source for medicines and industrial products. The bark, seeds, leaves, fruit, extracts and oils of the *A. indica* tree contain pharmacological constituents which offer some impressive therapeutic qualities, like antimicrobial, anti-pyretic and anti-inflammatory, anti-tumour, anti-helminthic activities (Shenpagam et al., 2012). The aim of the work reported in this paper was to screen the endophytic actinomycetes isolated from parts of the *A. indica* tree, for their potential as biocontrol agents of a range of antagonistic bacteria and fungi and to find effective plant growth promoting agents for enhanced crop yield.

Materials and Methods:-

Sample collection:-

Twenty healthy trees of *A. indica* (Indian lilac) were randomly selected from different locations in Ludhiana district of northern India and samples of leaves, stems and roots were taken as per standard practices.

Isolation of endophytic actinomycetes:-

The stem, root and leaf segments were washed in running tap water to remove adhered epiphytes and soil debris and subjected to surface sterilization as described by previous researchers (Taechowisan and Lumyong, 2003). After the treatment, the plant samples were soaked in 10% $NaHCO_3$ solution for 10 min to inhibit fungal growth. Small fragments of each sample were mashed using sterile pestle and mortar and an aliquot of 1ml of suspension was poured on to Petri plates containing starch-casein agar (SCA) and spread with the help of glass spreader. Petri plates were incubated at 28^0C for 7-10 days. To verify the surface sterilization procedure, aliquots of rinsed water were spread on SCA medium and incubated at 30°C for 3-5days.

Characterization of actinomycete isolates:-

Actinomycete isolates were tentatively identified according to traditional morphological criteria (Ruan et al., 1990; Yan, 1992; Tan et al., 2006).

Dual Culture Antagonistic Bioassay:-

The actinomycete isolates were evaluated for their antagonistic activity against five phyto pathogenic fungi: *Alternaria brassicicola*, *Fusarium oxysporum*, *Phytophthora dresclea*, *Rhizoctonia solani* and *Helminthosporium oryzae* by dual-culture in vitro assay on PDA plates. Spread plate method was used for detecting antagonism against 6 pathogenic bacteria: *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Klebsiella sp.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and one pathogenic yeast *Candida albicans* on Sabouraud's dextrose agar was also evaluated. A lawn of these pathogenic cultures was prepared on nutrient agar plates by spreading 0.1 ml of these pathogenic cultures. A well was made in the center of each nutrient agar plate. Culture filtrate (50µl) of the actinomycete isolates was put in that well and incubated at 37ºC. After 4-7 days of incubation at 28^0C, the width of inhibition zones was measured.

Optical microscopy:-

Optical research microscopy was performed with fungal culture of *Fusarium oxysporum* to observe the changes in cell wall of the fungus due to the effect of secondary metabolites produced by endophytic actinomycetes isolates (*Streptomyces cinereus* AR16). A drop of lactophenol cotton blue (LBCB) stain was placed in the centre of a clean glass slide. A small portion of colony was scratched and placed in the drop of stain with the help of inoculation loop. Then it was teased into small bits with the help of teasing needle. A cover slip was placed on top and gentle pressure was applied to avoid any inserted air bubble. It was then observed under 10X, 20X and 40X objectives of Leica Optical Research microscope (Model LM 5000B).

Scanning electron microscopy (SEM):-
SEM was employed to evaluate the effect of *Streptomyces albosporus* (AZS96) on the fungal cell wall of *Fusarium oxysporum* culture using chemical fixation and liquid osmium fixation technique (Bozzola and Russell, 1996). The sample was then placed in the vacuum dessicator overnight, stubbed and sputter coated with gold in E-1010 Ion sputter coater machine to be viewed under secondary electron imaging mode in Hitachi S-3400N scanning electron microscope.

Identification of one of the Microbial Culture using 16S rDNA based Molecular Technique:-
DNA was isolated from the culture. Its quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel (Gel Image-1). The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of **1326bp** 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

Results and Discussion:-

Endophytic fungi are reported ubiquitously from each and every higher plant, which has been investigated for their endophytic microbial complement (Gond et al., 2007). These microbes have emerged as strong candidates to fulfill the division between the discovered and undiscovered genera/species of fungi (Hawksworth, 1991). These microbes are of greater interest for the huge reservoir of natural bioactive compounds that they produce (Kongsaree et al., 2003). Endophytic actinomycetes are now considered as exciting novel sources for obtaining new bioactive compounds and have been reported from several hosts such as tomato, banana, wheat, and maize with promising anti-microbial activity against pathogenic strains (Verma et al., 2009).

Isolation and characterization of endophytic actinomycete diversity:-
A total of 35 isolates of endophytic actinomycetes were obtained from tissues of roots, leaves and stems, revealing that healthy living tissues of A. indica trees harbour a variety of the endophytic actinomycetes. It is interesting to record the predominance of *Streptomyces* sp. from this host, as it was also dominant in another report whereby 87% of the *Streptomyces* sp., out of 2,174 actinobacterial strains were isolated from nearly 90 medicinal plant of tropical rain forests of China (Qin et al., 2009). Similarly, Gangwar et al (2014) obtained a total of 40 isolates of endophytic actinomycetes from the root, stem and leaf tissues of *A. vera, M. arvensis* and *O. sanctum* out of which most of the actinomycetes were identified as *Streptomyces* sp.

Besides *Streptomyces* sp. other genera like *Micromonospora* sp. *Microbispora* sp. and *Nocardia* sp. were also reported. Our results are in corroboration with the findings of Coombs and Franco (2003) who isolated *Streptomyces* sp., *Micromonospora* sp., *Nocardiodes albus, Streptosporangium/ Microbispora* from the surface sterilized tissues of healthy wheat roots. *Streptomyces* sp., *Nocardia* sp., *Pseudonocardia* sp. and *Promicromonospora* sp. were isolated from *Achillea fragrantissima* sp. (El-Shatoury et al., 2009).

Out of 35 isolates of A. indica, the majority were recorded from roots (n=15) followed by stems (n=13) and leaves (n=7) respectively (**Table 1**). In a study by Conti et al (2012), the isolation frequency was higher in the base leaves (12.5%), when compared to the top leaves (3.05%) of *Spermacoce verticillata* and in total 12 actinobacteria were obtained with *Microbispora* (41.66%) being present in majority followed by *Streptomyces* (25%), *Nocardia* (8.34%), and unidentified bacteria (25%). The results also revealed that the surface treatment was adequate for the isolation of endophytic actinomycetes, as surface sterilized imprinted Petri plate (control) did not produce any growth. Thus, all the actinomycetes recorded in this experiment must have been endophytic and not the epiphytic.

Table 1:- Distribution of endophytic actinomycetes isolates from *A. indica* trees.

Types	No. of isolates		
	Root	Stem	Leaf
Streptomyces albosporus	4	3	4
S. griseofuscus	2	5	-
S. grieseorubroviolaceus	2	-	-
S. aureus	1	-	-
S. flavus	-	-	2
Micromonospora sp.	4	1	-
Microbispora sp.	2	3	-
Nocardia sp.	-	1	1
Total	15	13	7

Values represent mean of three replicates

Based on colony and cultural characteristics, various subgroups were identified and among *Streptomyces* sp., the subgroups *S. albosporus* (n=11) was more frequently isolated followed by *S. grieseofuscus* (n=7) *S. griseorubroviolaceus* (n=2), *S. flavus* (n=2) and *S. aureus* (n=1), respectively (**Table 2**). Among *Streptomyces* sp., *S. albosporus* was the preponderant endophytic actinomycetes followed by *S. grieseofuscus*. Differences in the distribution of endophytic actinomycetes in the roots and in the leaves were observed. *Streptomyces albosporus* was obtained from all the plant parts, like, roots, stems and leaves of A. indica. The studies revealed that actinomycetes such as *Streptomyces* sp., *Micromonospora* sp., *Microbispora* sp. and *Nocardia* sp. were able to colonize internal tissues of a wide range of host plants.

Table 2:- Morphological and biochemical characterization of endophytic Streptomyces sp.

Property	***Streptomyces albosporus***	***S. griseofuscus***	***S. griseorubroviolaceus***	***S. cinereus***	***S. aureus***	***S. flavus***
Colony color	white	grey	purplish-pink	grey	grey	yellow
Pigmentation	-	grey	brown	-	yellow	yellow
Spore arrangement	chain	chain	chain	chain	chain	chain
Hydrolysis of Casein	+	-	-	+	+	-
Starch	+	+	+	+	+	+
Decomposition of Esculin	-	+	+	-	+	+
Tween-80	+	-	+	+	-	+
Xanthine	+	+	+	+	+	+
Hypoxanthine	+	-	+	+	+	-
Tyrosine	+	+	+	+	+	+
Effect on growth in the presence of Sodium azide	+	+	-	+	-	+
NaCl (5%)	+	+	+	+	+	+
NaCl (10%)	-	+	+	+	+	-

(+) = Growth detected
(-) = Growth not detected

Dual Culture Antagonistic Bioassay:-

Eighteen (51.4%) out of 35 isolates were showing promising activity against different plant pathogenic fungi as shown in **Table 3**. Most of the isolates displaying antagonistic activity belonged to the *Streptomyces* sp. (n=13) and rest belonged to *Micromonospora* sp. (n=3), *Microbispora* sp. (n=1) and *Nocardia* sp. (n=1). In the present study, *Streptomyces albosporus* was found to have antifungal activity against *F. oxysporum, A. brassicicola, P. dresclea* and *R. solani* while *Streptomyces griseofuscus* was effective against *F. oxysporum, P. dresclea, A. niger* and *R. solani* (Table 3). The isolates of *Streptomyces griseorubroviolaceus* were observed to have antifungal activity against *F.*

oxysporum, P. dresclea and *A. niger* and *Streptomyces aureus* showed antifungal activity against *F. oxysporum* and *P. dresclea*. This may be attributed to the hydroxamate type siderophores production ability of *Streptomyces* sp., which inhibit phytopathogen growth by competing for iron in rhizosphere soils (Khamna et al., 2009). One isolate each of *Micromonospora* sp. AZS10, *Microbispora* sp. AZR3, three cultures of *S. albosporus* AZL3, AZR9 and AZR14 was active only against *P. dresclea* showing 27.2, 27.0, 35.7, 28.2, 37.2 inhibition percentage respectively. Three of the *S. albosporus* isolates AZS4, AZL5 and AZL6 were displaying 56.2, 62.5 and 63.7% inhibitory activity against *F. oxysporum.* One isolate of *Nocardia* sp. AZL2 was observed to have antifungal activity against only *P. dresclea* (50%). *S. griseorubroviolaceus* AZR7 was the only isolate inhibitory against *A. niger* (75%). The results are in accordance with Verma et al (2009) who reported that thirty two out of the fifty five (58%) endophytic actinomycetes isolates from *A. indica* were found to have broad spectrum significant antimicrobial activity. In the study by Gangwar et al (2014), it was found that 8 endophytic actinomycetes were having strong inhibitory activity against *A. brassicicola* and *F. oxysporum* rather than *Aspergillus niger, Aspergillus flavus, Botrytis cinerea, Penicillium digitatum, Penicillium pinophilum, Phytophthora drechsleri* and *Colletotrichum falcatum*. *Saccharopolyspora* O-9 from *O. sanctum* strongly inhibited all the pathogenic fungi, and maximum percent inhibition was observed against *A. brassicola* (71.4%). Many isolates of endophytic *Streptomyces* exihibited antagonism against either one or more than one tested phytopathogens. *Streptomyces albosporus* O-11 and *S. albosporus* A-4 antagonized all the tested fungi except *A. flavus* and *P. pinophilum*.

Table 3:- Antifungal activity (% inhibition) of the isolates

.**Genus**	**Isolate**	**A.niger**	**A.brassicicola**	**F.oxysporum**	**P.dresclea**	**R.solani**
S. albosporus	AZS96	-	42.25±0.8	63.75±0.8	26.95±0.7	-
S. albosporus	AZS13	-	23.50±0.8	42.50±0.8	-	20.25±0.6
S. griseofuscus	AZR1	-	-	60.00±0.3	-	33.25±0.9
S. griseofuscus	AZS11	-	-	-	46.50±0.9	29.25±0.9
S. griseorubroviolaceus	AZR13	-	-	68.75±0.6	30.25±0.5	-
S. aureus	AZR10	-	-	62.50±0.1	8.25±0.4	-
Micromonospora sp	AZR12	-	50.00±0.7	75.00±0.2	-	-

Values represent mean of three replicates

Moreover, about 4% isolates recovered showed a broad antimicrobial spectrum against both pathogenic fungi and bacteria. Actinomycetes-fungus antagonism has been demonstrated for a variety of plant pathogens such as, *Alternaria, Rhizoctonia, Verticillium, Fusarium, Phytophthora* and *Phytium* sp. (Aghighi et al., 2004). The ability of isolates to inhibit the growth of fungal pathogens is due to the secretion of the secondary metabolites by actinomycetes and this shows that endophytic actinomycetes can be developed as potential biocontrol agents. The antagonistic test revealed that mostly the isolates from roots showed antagonistic activity against phytopathogenic fungi and lesser from stems and leaves. Non-*Streptomyces* isolates such as *Amycolatopsis*, *Micromonospora*, *Nonomuraea* and *Nocardia* also demonstrated promising activities against pathogens (Huang et al., 2012).

Streptomyces sp. was more active against pathogenic bacteria (**Table 4**). *Streptomyces griseofuscus* were more active against pathogens, and these were displaying antagonistic activity against *Yersinia enterocolitica, Klebsiella* sp., *Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa*. Isolates of *Streptomyces albosporus* were observed to have antibacterial activity only against *E. coli*, but did not form any clear zone and had only retarded the growth of bacteria. The isolates of *Streptomyces griseorubroviolaceus* were observed to have antibacterial activity against *E. coli* and *Klebsiella* sp. and *Streptomyces flavus* was active against *Klebsiella* sp. Isolates of *Micromonospora* sp. were displaying maximum antibacterial activity against *E. coli, Klebsiella* sp. and *Staphylococcus aureus* and *Microbispora* sp. were observed to have inhibitory activity against *Pseudomonas aeruginosa* and *E. coli*. The isolates of *Nocardia* sp. were active against *E. coli* and *Pseudomonas aeruginosa*. None of the isolates were able to inhibit the activity of *Candida albicans* and *Aeromonas hydrophila*.

Table 4: Antibacterial activity of isolates against human pathogens.

Genus	Isolate	Yersinia	E. coli	S.aureus	Klebsiella	P. aeruginosa
S. griseofuscus	AZR1	++	±	±	±	±
S. griseofuscus	AZR11	±	±	±	±	±
S. griseofuscus	AZS2	-	±	±	+	+
S. griseofuscus	AZS9	±	±	±	+	+
S. griseorubroviolaceus	AZR7	-	±	-	±	-
S.griseorubroviolaceus	AZR13	-	±	-	±	-
Microbispora sp.	AZS1	-	±	-	-	±
Microbispora sp.	AZS5	-	±	-	-	±
Microbispora sp.	AZS12	-	±	-	-	±
Microbispora sp.	AZR3	-	±	-	-	±
Nocardia sp.	AZL2	-	±	-	-	±
Nocardia sp.	AZS3	-	+	-	-	+
Micromonospora sp.	AZR5	-	±	±	±	-
Micromonospora sp.	AZR12	-	+	+	+	-
Micromonospora sp.	AZS10	-	±	+	±	-

*(+) zone of inhibition ranged between 4 and 7 mm, (++) zone of inhibition ranged between 8 and 15mm,
(±) variable inhibition, (-) no inhibition

The results were supported by another researcher (Verma et al., 2009) who reported that 60% of the isolates recovered showed a broad antimicrobial spectrum against both bacteria and fungi. These are also supported by Nanjwade et al. (2010), who reported that six out of nine isolates of actinomycetes obtained from the soil samples screened for antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* and showed significant antimicrobial activity against both Gram-positive and Gram-negative organisms. Also, the study by Shempagm et al. (2012) revealed that, among endophytic actinomycetes obtained from *Azadiracta indica*, *Ocimum sanctum* and *Phyllanthus amarus*, three isolates from *Azadiracta indica* isolate had a promising effect antibacterial activity against the various pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antimicrobial screening against gram positive bacteria *Staphylococus aureus* (ATCC-6538) and *Bacillus subtilis* (UFPEDA-16) evaluated by Conti et al [30] using agar plug assay and showed that 28.57% of the isolates (fungi and actinobacteria).

Optical microscopy:-

The intact hyphae and numerous spores of *Fusarium oxysporum* were observed in the control fungus using optical microscopy (Fig. 1:**A**) as compared to the *Fusarium oxysporum* inoculated with AZS96 (Fig. 1:**B**). The result indicated that potent antagonists inhibited the growth of the test fungi by releasing extracellular diffusible metabolites that inhibited the hyphal growth of *Fusarium oxysporum* and spore number, spore size was also reduced and bursting of spores was also observed along with thinning of hypha. Our results are in accordance with Taechowisan et al. (2005) who reported that fungal mycelium along the edges of the colonies facing CMUAc130 appeared thickened, with bulbous-like formations along the ends under a dissecting microscope. On the control plate fungal mycelium was showing regular thin hypha with normal sized spores.

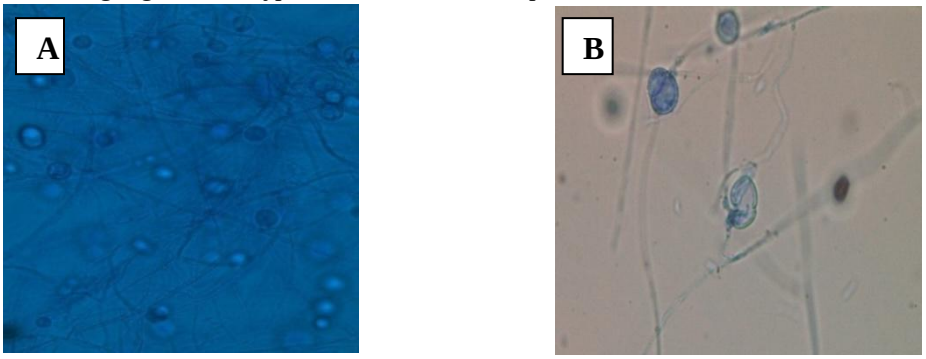

Fig 1. Control (A) and *Fusarium oxysporum* infected with strain AZS96 shows reduction in the spore size, number of spores and thinning of hyphae and bursting of spore in (B).

Scanning electron microscopy:-

Scanning electron microscopy was done with the co-culture containing *F. oxysporum* and endophytic actinomycete isolate AZS96. SEM showed degradation of cell walls of *Fusarium oxysporum* mycelia growing towards AZS96 as compared to control. The single culture plate of *Fusarium oxysporum* served as control, which showed the regular vegetative cells (Fig. 2: **A**), whereas fungal colony inoculated with isolate AZS96 (Fig. 2: **B**) showed that hypha got disrupted and reduced in size, whereas in control intact cells had a smooth surface with overall intact morphology. The spores were also partially deformed and reduced in size. Many cells were enlarged and elongated, but having extremely low viability. Our results are in corroboration with Tang-um and Niamsup (2012) who reported that breakage of the cell walls of *Fusarium oxysporum* f. sp. lycopersici mycelia growing towards *Streptomyces* sp. P4 as compared to control *F. oxysporum*. It is suggested that the actinomycetes isolate produced extracellular secondary metabolites and/or hydrolytic enzymes including chitinase, which play a crucial role in fungal growth inhibition.

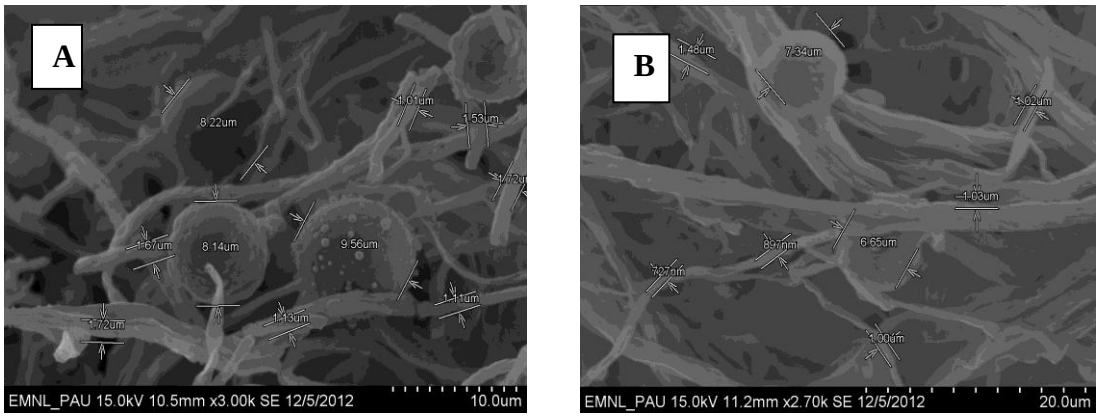

Fig 2. Scanning electron microscopic analysis of *Fusarium oxysporum* grown alone showed regular, radial growth (A) and co-cultured with AZS96 reduction in number and size of spores along with hyphal degradation in (B).

Identification of one of the Microbial Culture using 16S rDNA based Molecular Technique:-

The culture, which was labeled as AZS96was found to be Rhodococcus qingshengii strain BJC15-A38 (GenBank Accession Number: JX401475**.**1) based on nucleotide homology and phylogenetic analysis.

The isolate shared 88-95% identity to those of the closely related strains. Isolate is the most commonly related to JX401475**.**1 under a bootstrap supported value of 100% (Fig. 3).

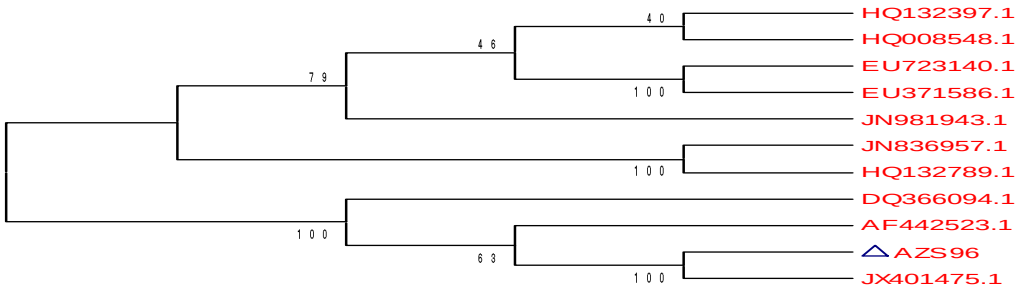

Fig 3. Evolutionary relationships of 11 taxa.

Conclusion:-

The study revealed that endophytic actinomycetes isolated from *A. indica* have broad-spectrum antimicrobial activity and are also the valuable reservoirs of novel bioactive compounds. Not only the *Streptomyces*, but also the other genera of actinomycetes were also act as the antagonistic agents displayed antifungal activity as well as antibacterial activity. Our survey also suggested that medicinal plants are a potent source of endophytic actinomycetes with wide plant growth promoting activities for the development of sustainable agriculture.

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References:-

1. Aghighi S, Bonjar G H S, Rawashdeh R, Batayneh S, Saadoun I (2004) First report of antifungal spectra of activity of Iranian actinomycete strains against *Alternaria solani, Alternata alternate, Fusarium solani, Phytophthora megasperma, Verticillium dahlia* and *Saccharomyces cerevisia*. Asian J Plant Sci **3:** 463-71.
2. Bailey B A, Bae H, Strem M D, Robert D P, Thomas S E, Crozier J, Samuels G J, Choi I k- Young, Holmes K A (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta **224:** 1449–64.
3. Bozzola J J, Russell D L (1996) Electron Microscopy, Second Edition. pp 202-39.
4. Conti R, Cunha I G B, Siqueira V M, Souza-Motta C M, Amorim E L C, Araújo J M (2012) Endophytic microorganisms from leaves of *Spermacoce verticillata* (L.): Diversity and antimicrobial activity. J Appl Pharm Sci **2 (12):**17-22.
5. Coombs J T (2002) Antibiotics for wheat. Science Now! http:// www.abc.net.au/science/news/stories/s351442.htm. The national science forum, 20–22 August, 2001.
6. Coombs J T, Franco C M M (2003) Isolation and identification of actinobacteria from surface sterilized wheat roots. Appl Environ Microbiol **69:** 5603-08.
7. Coombs J T, Michelson P P, Franco C M M (2004) Evaluation of endophytic bacteria as antagonists of *Gaeumannomyces graminis* var. tritici in wheat. Biol Control **29:** 359-66.
8. Costa J M, Loper J E (1994) Characterization of siderophore production by the biological- control agent *Enterobacter cloacae*. Mol Plant Microbe Interact **7:** 440-48.
9. El-Shatoury S, El-Kraly O, El-Kazzaz W, Dewedar A (2009) Antimicrobial activities of actinomycetes inhabiting *Achillea fragrantissima* (Family: Compositae). Egy J Nat Toxins **6:** 1-15.
10. Ezra D, Castillo U F, Strobel G A, Hess W M, Porter H, Jensen J B, Condron M A M, Teplow D B, Sears J, Maranta M, Hunter M, Weber B, Yaver D (2004) *Coronamycins*, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp. Microbiol **150:** 785-93.
11. Gangwar M, Dogra S, Gupta U P, Kharwar R N (2014) Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. Afr J Microbiol Res **92:** 182-91.
12. Gond S K, Verma V C, Kumar A, Kumar V, Kharwar R N (2007) Study of endophytic fungal community from different parts of *Aegle marmelos* Correae (Rutaceae) from Varanasi (India). World J Microbiol Biotechnol **23:** 1371-75.
13. Hawksworth D L (1991) The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol Res**95:** 641-55.
14. Huang X L, Zhuang L, Lin H P, Li J, Goodfellow M, Hong K (2012) Isolation and bioactivity of endophytic filamentous actinobacteria from tropical medicinal plants. Afr J Biotechnol **11:** 9855-64.
15. Igarashi Y, Iida T, Yoshida R, Furumai T (2002) Pteridic acids A and B, novel plant growth promoters with auxin-like activity from *Streptomyces hygroscopicus* TP-A0451. toibitnA J **55:** 764-67.
16. Khamna S, Yokota A, Lumyong S (2009) Actinomycetes isolated from medicinal plant rhizosphere soil: diversity and screening of antifungal compound, indole-3-acetic acid and siderophore production. World J Microbiol Biotechnol **25:** 649-55.
17. Kongsaeree P, Prabpai S, Sriubolmas N, Vongvein C, Wiyakrutta S (2003) Antimalarial Dihydroisocoumarins Produced by *Geotrichum* sp., an Endophytic fungus of *Crassocephalum crepidioides*. J Nat Prod **66:** 709-11.
18. Lee S, Flores-Encarnacion M, Contreras-Zentella M, Garcia-Flores L, Escamilla J E, Kennedy C (2004) Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in Cytocrome C biogenesis genes. J Bacteriol **186:** 5384-91.
19. Misk A, Franco C (2011) Biocontrol of chickpea root rot using endophytic actinobacteria. Biol Control **56:** 811-22.
20. Nanjwade B K, Chandrashekhara S, Shamarez A M, Goudanavar P S, Manvi F V (2010) Isolation and morphological characterization of antibiotic producing actinomycetes. Trop J Pharm Res **9:** 231-36.
21. Provorov N A, Borisov A Y, Tikhonovich I A (2002) Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscular mycorrhiza. J Theor Biol **214:** 215-32.

22. Qin S, Li J, Chen H H, Zhao G H, Zhu W Y, Jiang C L, Xu L H, Li W J (2009) Isolation, diversity and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol **75:** 6176-86.
23. Ruan J S, Liu Z H, Liang L N, Yang D C (1990) Study and application of actinomycetes. pp 1-18. Beijing: Bios-Scientific Publishers Limited ISBN 7-03-001499-5.
24. Shenpagam NH, Kanchana D, Sinduja G, Sandhya (2012) Isolation of endophytic actinomycetes from medicinal plants and its mutational effect in biocontrol activity. Int J Pharm Sci Res **3(11):** 4338-4344.
25. Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products microbial. Microbiol Mol Biol Rev **67**(4)**:** 491-502.
26. Taechowisan T, Lu C, Shen Y, Lumyong S (2005) Secondary metabolites from Streptomyces aureofaciens CMUAc130 and their antifungal activity. Microbiol **151:** 1691-95.
27. Taechowisan T, Lumyong S (2003) Activity of endophytic actinomycetes from roots of *Zingiber officinale* and *Alpinia galanga* against phytopathogenic fungi. Ann Microbiol **53:** 291-98.
28. Tan HM, Cao LX, He ZF, Su GJ, Lin B, Zhou SN (2006) Isolation of endophytic actinomycetes from different cultivars of tomato and their activities against *Ralstonia solanacearum* in vitro. World J Microbiol Biotechnol **22:** 1275-80.
29. Tang-um J, Niamsup H (2012) Chitinase production and antifungal potential of endophytic *Streptomyces* strain P4. Int J Sci Technol **6:** 95-104.
30. Verma VC, Gond SK, Kumar A, Mishra A, Kharwar RN, Gange AC (2009) Endophytic actinomycetes from *Azadirachta indica* A. Juss: Isolation, diversity and anti-microbial activity. Microb Ecol **57:** 749-56.
31. Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* sp. closely associated with wheat roots. Biol Fert Soils **40:** 36-43.
32. Yan XC (1992) Classification and identification of actinomycetes. pp 623-56. Beijing: Bios Scientific Publishers Limited ISBN 7-03-002022-7.