CHARACTERIZATION OF ENDOPHYTIC AND RHIZOSPHERIC ACTINOMYCETES FROM *GLADIOLUS SP.*

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
MICROBIOLOGY
(Minor Subject: Biochemistry)

By

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CERTIFICATE I

This is to certify that the thesis entitled, "**Characterization of endophytic and rhizospheric actinomycetes from *Gladiolus sp.***" submitted for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Microbiology** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Jagpreet Kaur (L-2011-BS-09-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that the thesis report entitled, "**Characterization of endophytic and rhizospheric actinomycetes from *Gladiolus sp.***" submitted by **Jagpreet Kaur** (Admn. No. **L-2011-BS-09-IM**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)** in the subject of **Microbiology** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

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Abstract

The present study was undertaken to isolate endophytic and rhizospheric actinomycetes from *Gladilous* and to evaluate their plant growth promoting traits. Out of 90 isolates, 40 were from rhizospheric soil and 50 from roots of *Gladiolus* plants. Sixteen isolates from rhizospheric and 14 from endophytic were observed to produce IAA ranged from 4.3-79.9 µg/ml. Eighteen (20%) isolates were observed to solubilize phosphate in the range of 6.1-64.5mg/100ml. Fifteen (16.6%) isolates displayed siderophore production. Twelve isolates were producing hydroxamate in the range of 5.5 to 39.4µg/ml and nine isolates produced catechol ranging between 12.1-35.3µg/ml. Fifty out of 90 isolates were observed to produce the gibberellic acid ranging from 12.5-50.6 µg/ml. Ten isolates were found to produce HCN and 5 isolates were able to show ACC deaminase activity. The antagonistic activity was displayed by twelve isolates against *Fusarium oxysporum*. On the basis of PGP traits and antifungal activity, isolate Sc9 was selected for green house study which was presumptively identified as *Streptomyces* sp.. This actinomycete isolate could be used as potential plant growth promoting and biocontrol agent.

Keywords: Endophytic and rhizospheric actinomycetes, plant growth promotion, antagonistic properties, *Gladiolus*

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ਮੌਜੂਦਾ ਅਧਿਐਨ ਗਲੈਡੀਓਲਸ ਵਿੱਚੋਂ ਐਡੋਫਿਟਿਕ ਅਤੇ ਰਹਾਇਜ਼ੋਸਫੈਰਿਕ ਐਕਟਿਨੋਮਾਈਸੀਟਸ ਦਾ ਨਿਖੇਖਣ ਕੀਤਾ ਗਿਆ ਸੀ ਅਤੇ ਪੌਦਾ ਵਿਕਾਸ ਦਰ ਤਰੱਕੀ ਲਈ ਪੜਤਾਲ ਕੀਤਾ ਗਿਆ ਸੀ । ਨੱਬੇ ਆਇਸੋਲੇਟ ਵਿੱਚੋਂ 40 ਰਹਾਜ਼ੋਸਫੈਰਿਕ ਮਿੱਟੀ ਵਿੱਚੋਂ ਅਤੇ 50 ਗਲੈਡੀਓਲਸ ਦੀਆਂ ਜੜ੍ਹਾਂ ਵਿੱਚੋਂ ਆਈਸੋਲੇਟ ਕੀਤਾ ਗਿਆ ਸੀ। ਸੋਲਾਂ ਰਹਾਇਜ਼ੋਸਫੈਰਿਕ ਅਤੇ 14 ਐਂਡੋਫਿਟਿਕ ਐਕਟਿਨੋਮਾਈਸੀਟਸ ਨੇ ਆਈ ਏ ਏ 4.3-79.9 ਮਾ. ਗ੍ਰਾ/ਮਿ.ਲੀ. ਪੈਦਾ ਕੀਤਾ ਸੀ। ਅਠਾਰਾਂ (20%) ਫਿਸਦੀ ਆਈਸੋਲੇਟਸ ਨੇ 6.1-64.5 ਮਿ. ਗ੍ਰਾ./ਮਿ. ਲੀ. ਫਾਸਫੇਟ ਸੋਲੂਬਲਾਇਜ਼ ਕੀਤਾ ਸੀ। ਪੰਦਰਾ (16.6%) ਆਈਸੋਲੇਟ ਨੇ ਸਾਈਡਰੋਫੋਰ ਪੈਦਾ ਕੀਤਾ ਸੀ। ਬਾਰਹਾ ਆਈਸੋਲੇਟਸ ਨੇ ਹਾਈਡਰੋਕਸਾਮੇਟ ਕਿਸਮ ਦਾ ਉਤਪਾਦਨ 5.5 ਤੋਂ 39.4 ਮਾ. ਗ੍ਰਾ/ਮਿ. ਲੀ. ਅਤੇ ਨੌਂ ਆਈਸੋਲੇਟ ਨੇ ਕੈਟਾਕੋਲ ਕਿਸਮ ਦਾ ਉਤਪਾਦਨ 12.1 ਤੋਂ 35.3 ਮਾ. ਗ੍ਰਾ./ਮਿ. ਲੀ. ਤੱਕ ਪੈਦਾ ਕੀਤਾ ਸੀ । ਨੱਬੇ ਵਿੱਚੋਂ ਪੰਦਰਾਂ ਆਈਸੋਲੇਟਸ ਨੇ 6 ਜ਼ਿਬਰੇਲਿਕ ਐਸਿਡ 12.5-50.6 ਮਾ. ਗ੍ਰਾ/ਮਿ. ਲੀ. ਪੈਦਾ ਕੀਤਾ ਸੀ। ਦਸ ਆਈਸੋਲੇਟਸ ਨੇ ਐੱਚ.ਸੀ.ਐੱਨ. ਅਤੇ ਪੰਜ ਆਈਸੋਲੇਟਸ ਨੇ ਏ.ਸੀ.ਸੀ. ਡੀਐਮੀਨੇਜ਼ ਸਰਗਰਮੀ ਦਿਖਾਈ ਸੀ। ਐਨਟਾਗੋਨਿਸਟਿਕ ਸਰਗਰਮੀ 12 ਆਈਸੋਲੇਟਸ ਨੇ *Fusarium oxysporum* ਦੇ ਖਿਲਾਫ਼ ਦਿਖਾਈ ਸੀ ।ਪੌਦਾ ਵਿਕਾਸ ਦਰ ਤਰੱਕੀ ਅਤੇ ਐਂਟਾਗੋਨੀਸਟਿਕ ਸਰਗਰਮੀ ਦੇ ਆਧਾਰ ਤੇ ਆਈਸੋਲੇਟ SC9 ਨੂੰ ਗਰੀਨ ਹਾਊਸ ਦੇ ਅਧਿਐਨ ਲਈ ਚੁਣਿਆ ਗਿਆ, ਜਿਸ ਦੀ ਪਹਿਚਾਣ *Streptomyces aureus* ਕੀਤੀ ਗਈ ਸੀ । ਇਸ ਐਕਟੀਨੋਮਾਈਸੀਟਸ ਨੂੰ ਪੌਦਾ ਵਿਕਾਸ ਦਰ ਅਤੇ ਬਾਇਓਕੰਟਰੋਲ ਅਜੰਟਾ ਦੀ ਤਰ੍ਹਾਂ ਪ੍ਰਯੋਗ ਕੀਤਾ ਜਾ ਸਕਦਾ ਹੈ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF PLATES

CHAPTER I

INTRODUCTION

Flowers relate to us a beautiful story of reproduction and bringing something sweet and tender into this world. *Gladiolus* (*Gladiolus hortulanus* L.H. Bailey) is an important cut flower in many countries including India mainly because of its many different colorful spikes which can be used in different floral arrangements at the hotel discussion, meeting, function, festival, celebration, religious ceremony, wedding, expression of love, etc. *Gladiolus* belonging to the family Iridaceae (Sinha and Roy 2002). *Gladiolus* is mainly grown from corms. It is also propagated by seeds but takes four seasons for blooming and seed set limited to genotypes and climate. Once corms are planted, leaves and flowering spikes arise from the buds on the corms.

Gladiolus is a seasonal and long-duration cut flower crop that develops a stable microclimate for a range of diseases. Corm rot and vascular wilt of *Gladiolus*, caused by *Fusarium oxysporum* f. sp. *gladioli*, cause significant yield loss to the crop leading to decline in quality and quantity of the spikes and planting materials (Rana *et al* 2004). *Alternaria* sp. and *Sclerotium* sp. are responsible for serious yield losses (Chaiharn *et al* 2009). Fusarium wilt is one of the most serious diseases of *Gladiolus* which affect plants in the field and corms in storage (Riaz *et al* 2009, 2010). This disease causes considerable crop losses each year. In addition to direct economic losses, quality of the economic product also decreases with even moderate levels of disease infestation. Resistance and susceptibility are the two ends of the disease spectrum. Vascular corm rot is also called "yellows" on infected plants in the field which is caused by *Fusarium oxysporum* f. sp. *gladioli*, which deteriorates its quality and market value (Chandel and Bhardwaj 2000). The infected corms show brownish to black dry rot symptoms which results in yellowing of leaves, stunted plant growth, discoloured flowers and even destruction of the corms (Ram *et al* 2004).

Actinomycetes can be characterized as differentiating prokaryotes which exhibit strain specific types of morphological differentiation. Actinomycetes are a specific group as bacteria. Morphologically they resemble fungi because of their elongated cells that branch into filaments or hyphae which are belong to phylum *actinomycetales*, included taxa of Gram-positive microorganisms. Endophytes are microorganisms that live within plants as a part of their life without causing visible disease. Endophytes have a wide range of antimicrobial strains comprising important sources of a variety of bioactive metabolites, including anticancer, antidiabetic and antimicrobial compounds (Strobel and Daisy, 2003).

Actinomycetes occur in plant rhizospheric soil and produce active compounds which can protect roots by inhibiting the growth of potential fungal pathogens. This may be achieved through the production of enzymes which degrade the fungal cell wall or antifungal

compounds (Getha *et al* 2005; Errakhi *et al* 2007). Rhizodeposition of various exudates provide an important substrate for the soil microbial population (Marschner and Baumann 2003).

The environmental friendly microorganisms are gaining attention to be used as biological control of plant infection. These microorganisms have proved to be helpful in PGP and play an significant role in nutrient cycling (Bhattacharyya and Jha 2012) and infection control. Although many attempt to control this disease, the problem is still widespread due to development of fungicidal resistance in disease causing pathogen and phytotoxic effects in plant.

Plant growth promoting traits (PGP) have proven to be a promising agricultural approach that plays a significant role in plant protection from diseases and enhances growth of plant (Fernando *et al* 2006; Fatima *et al* 2009). It involves the application of variety of microbes alone or in combination with other antagonistic agents (Spadaro and Gullino 2005). Antagonism is an important PGP mechanisms shown by different microbes against other phytopathogenic microbes due to the production of different metabolites like siderophores, IAA, ACC and antibiotics (Lugtenberg and Kamilova, 2009).

Keeping in view the extensive utility of actinomycetes, the present investigation has been undertaken to fulfill following objectives:

1. To isolate actinomycetes from roots and rhizospheric soil of *Gladiolus* sp.
2. To study plant growth promoting potential of actinomycete isolates from *Gladiolus* sp.
3. Evaluation of effectiveness of screened actinomycete isolates as potential growth promoting and antagonistic against fungal pathogens of *Gladiolus* in green house.

CHAPTER II

REVIEW OF LITERATURE

The Gladioli (*Gladiolus* L.) is one of the most widely cultivated flowers in the world, together with other cut flowers such as roses (*Rosa*), chrysanthemums (*Dendranthema* L.) and carnation (*Dianthus caryophyllus* L.) (Horn 2002). Cut flowers not only offer aesthetical beauties but also have become a commercial object contributing national economics by providing millions of dollars through exporting overseas (Akpinar and Bulut 2011). *Gladiolus* hybrids are among the preferred cut flowers due to their different sizes, shades, and excellent vase life (Bose *et al* 2003). A large corm is capable of producing 25-200 cormels depending as cultivars and propagation method (Sinha and Roy 2002).

Gladiolus stands fourth in the international cut flower trade after carnation, rose and chrysanthemum. The propagation by corm may transmit several bacterial, fungal and viral diseases such as *Fusarium* corm rot, *Botrytis* blight, bacterial leaf rot, etc. which cause crop damage and commercial loss (Aftab *et al* 2008). *Gladiolus* (*Gladiolus* f. sp. *gladioli*), "Queen of bulbous flower crops" infected by wilt disease which is caused by *Fusarium oxysporum* and Kulkarni (2006) identified it as *F. oxysporum* f. sp. *gladioli* on the basis of morphological and cultural characterization. The percentage of Disease incidence was observed in a range of 20.00 to 61.53 percent. The highest disease incidence % was observed in Dharwad district (42.81%), followed by Belgaum district (27.46%) and least in Bangalore district (22.41%).

Chemical pesticides are still a valuable and effective method to control the disease but with harmful effects on a variety of non-target microorganisms and environmental pollution. The use of biofertilizers as alternate for chemicals, has attracted interest in many recent reports (Patil *et al* 2011; Gronemeyer *et al* 2012). Although this method is a slow process but it can be long lasting, cheap and harmless to life.

Actinomycetes are among the broadly distributed group of microorganisms in nature (Oskay *et al* 2004). These are Gram-positive bacteria belonging to order Actinomycetales and having high G+C (55-78 percent). They are part of the indigenous soil microflora involved in the turnover of recalcitrant plant organic matter and produce a balance in the ecosystem. They are present in high proportion in the soil, rhizosphere and roots of plants (Kimura and Asakawa 2006). Some residents of the nodule is responsible for nitrogen fixation to plants (Okazaki 2003).Interactions between actinomycetes and plant such as fixing nitrogen, plant growth hormone production or protecting plant against fungal infection have been known. Another important role of actinomycetes is their ability to produce bioactive compounds, including antibiotics, anti-viral and anti-cancer agents.

There are many reports on the agricultural implications of actinomycete in biological control of plant pathogens (Ghorbani *et al* 2007) and with following inoculation of these

organisms causes triggering of signal transduction in host plants which leads to initiate defense responses to deal with the stresses at cell, tissue and organ level (Hasegawa *et al* 2006). Furthermore, many actinomycetes produce antibiotics, some of which helpful in controlling soil-borne fungal pathogens (El-Mehalawy *et al* 2004; Jain and Jain 2007). Some actinomycete isolates can also improve plant growth (El-Tarabily *et al* 2009; Palaniyandi *et al* 2011) by producing promoters such as siderophores to improve nutrient uptake (Tokala *et al* 2002) or IAA to help growth of roots (El-Tarabily 2008).

Among, many species of actinomycetes, *Streptomyces* produces and secrete a wide array of biologically active compounds including antibiotics, hydrolytic enzymes and enzyme inhibitors (Compant *et al* 2005; Shantikumar *et al* 2006) and also recognized to enhance soil fertility and proved to possess antagonistic activity against plant pathogens (Aghighi *et al* 2004). *Streptomyces* sp. has been described as rhizospheric bacteria (Miller *et al* 1990), antifungal biocontrol agents useful in controlling fungal root diseases. Plant root exudates stimulate rhizospheric growth of actinomycetes that are strongly antagonistic to fungal pathogens, while the actinomycetes utilize root exudates for growth and synthesis of antimicrobial substances (Yuan and Crawford 1995). Actinomycetes living inside flowering plant roots and soils are poorly studied. Previous studies on *Catharanthus roseus* and *Withania somnifera* plants showed that the rhizospheric actinomycetes can induce the resistance of *Catharanthus roseus* to fungal pathogens (Kamara and Gangwar 2015).

2.1 Distribution of actinomycetes

The majority of actinomycetes are free living and found widely distribute in many natural environments including various soil, freshwater habitat, marine habitat, organic matter habitat and colonize plants. Actinomycetes have proven to be a rich source of important natural products especially anibioitics. Approximately 10,000 antibiotics have been found, and almost half of them are produced by *streptomyces* that originated in soil (Lazzarini *et al* 2000). Recently, the rate of discovery of new compounds from existing genera obtained from common soil has decreased therefore it is critical that novel actinomyetes from unexplored habitats such as marine, hot spring be pursued as sources of novel antibiotics and others bioactive compounds.

2.1.1 Rhizospheric actinomycetes

Plant-associated actinomycetes may indirectly benefit the plants by preventing the growth of plant pathogens through different mechanisms and through stimulation of plant defense mechanisms (Weyens *et al* 2009). Rhizospheric actinomycetes produce active compounds which can protect roots by inhibiting the growth of fungal pathogens which may be achieved through the production of enzymes which degrade the fungal cell wall. (Errakhi *et al* 2007). Actinomycetes enhance plant growth by producing biologically active substances such as indole-3-acetic acid (IAA) to help growth of roots and produce siderophores to

improve nutrient uptake (Suzuki *et al* 2004). Actinomycetes also produce various antibiotics (chloramphenicol, neomycin and streptomycin), biologically active material like B vitamins, auxins and others.

2.1.2 Endophytic actinomycetes

Actinomycetes are 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. Endophytes may produce a plethora of substances e.g. novel antibiotics, antimycotics, immune suppressants, and anticancer compounds of potential use to modern medicine, agriculture, and industry (Strobel and Daisy 2003). 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 (Coombs *et al* 2004).

Endophytic actinomycetes are also called as the microbial chemical factories within plants (Igarashi *et al* 2004). They are found inside the tissues of nearly all healthy plants and are the free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Kafur and Khan (2011) isolated 38 endophytic actinomycetes from leaves of *Catharanthes roseus* (L.) G. Don of family *Apocynaceae*. Among the 38 isolates 20 morphologically different isolates were screened for antagonistic activity against fungi *Candida albicans, Botrytis cinerea, Curvulari alunata, Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani.* The selective isolation of endophytic actinomycetes indicated the richness of microbial diversity in *Catharanthus roseus* and screening for antimicrobial activity should be investigated for a comprehensive identification and potential use as source of bioactive agents.

Many endophytic actinomycetes have showed PGP traits including IAA activity, ACC deaminase activity, ammonia production, catalase activity, siderophore production, phosphate solubilization and chitin hydrolysis (Singh and Padmavathy 2014). Endophytic actinomycetes are attractive because their secondary metabolites might be promising sources of novel antibiotics and growth regulators of other organisms, as suggested by Matsukuma *et al* (1994) and Okazaki *et al* (1995). Endophytic actinomycetes are particularly considered as potential sources of bioactive compounds and various novel compounds. Most actinomycetes in soil belong to the genus *Streptomyces* (Goodfellow and Simpson 1987) and 75% of the biologically active compounds are produced by this genus. Actinomycetes occur in the plant rhizosphere soil and produce active compounds (Suzuki *et al* 2000). Matsumoto *et al* (1998) isolated actinomycetes from fallen leaves and genus *Microbispora* was frequently found. Actinomycetes are the main source of antibiotics and endophytic actinomycetes isolated from medicinal plants have considerable development potential (Mini P 2012) and have ability to produce a variety of bioactive metabolites including antibiotics, plant growth promoters, plant

growth inhibitors and cell wall-degrading enzymes such as cellulases, hemicellulases and chitinases which can be applied to agricultural packages (Hasegawa *et al* 2006; Sharma 2014).

2.2 Mechanisms of mode of action of actinomycetes

2.2.1 Plant growth promoting actinomycetes (PGPA)

Nowdays, increased public concern about environmental problems causing directly or indirectly by the use of fertilizers, pesticides, herbicides and fungicides, has prompted researchers to consider alternatives and chemical strategies for facilitating plant growth in agriculture, horticulture and silviculture (Glick *et al* 2007). Root colonizing actinomycetes commonly referred to as actinorhiza exert beneficial impact on plant development directly or indirectly. The plant growth promoting endophytic actinomyctes possess similar mechanism for following functions: production of biological control agents, production of plant growth promoting compounds such as auxin, cytokinins and gibberellins, siderophore production to bind Fe^{3+} from the environment or suppression of stress ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Sun *et al* 2009; Nimnoi *et al* 2010).

Thirty actinomycete strains were isolated by Franco-Correa *et al* (2010) from the rhizosphere of field-grown plants (*Trifolium repens* L.) and identified by microscopic characteristics, biochemical tests and molecular techniques. These *Streptomyces* isolates were tested for their capabilities of solubilizing/mineralizing sparingly phosphate sources, N2-fixation and/or siderophore production, typical traits of Plant Growth Promoting Rhizobacteria (PGPR). The three actinomycete strains improved AM mycelial growth in absence of the test plant, and MCR9 and MCR26 also stimulate AM fungal spore germination. Inoculation of clover plants with either of the selected actinomycetes enhanced plant growth and N acquisition. Co-inoculation of actinomycetes and *Glomus mosseae* produced synergic benefits on plant growth and MCR9 and MCR24 also on P acquisition. The three selected actinomycetes improve AM formation by clover plants and *Glomus mosseae* (Quan *et al* 2010).

Biocontrol by use of plant growth promoting traits (PGP) represents a potentially attractive alternative disease management approach since PGPR are known for growth promotion and disease reduction in crops (Jetiyanon and Kloepper 2002). PGP traits seem to promote growth through suppression of plant disease-causing organisms (Zehnder *et al* 2001; Ji *et al* 2006; Veerubommu and Kanoujia 2011), competition for space, nutrients and ecological niches, production of antimicrobial substances, or through production of phytohormones and peptides acting as bio stimulants without negative effects on the user, consumer or the environment (Jimenez-Delgadillo 2004).

2.2.2 Indole Acetic Acid (IAA)

Actinomycetes have the ability to promote plant growth by producing phytohormones such as auxin or gibberellins. The auxins are a group of indole ring compounds that have the ability to improve plant growth by stimulating seed germination, root initiation, cell elongation and seedling growth (EI-Tarabily 2008). Indole-3-acetic acid is a common natural auxin and is a product of L-tryptophan metabolism in microorganisms (Sapak *et al* 2008).

IAA production is a common trait among endophytic microorganisms, that posses the ability to produce IAA, belong to a vast range of bacterial phyla/classes have been isolated from multiple plants, including poplar, soybean, epiphytic and terrestrial orchids, cactus, potato and strawberry. IAA production by endophytic bacteria has been associated with the promotion of plant root growth, enhanced production of lateral roots and increases in root volume and biomass (Taghavi *et al* 2009).

IAA producing actinomycetes are commonly isolated from both the rhizo- as well as the endosphere. Several Streptomyces sp., such as *S. olivaceoviridis*, *S. rimosos* and *S. viridis*, have the ability to produce IAA and improve plant (Sapak *et al* 2008). A total of 445 actinomycetes were isolated by Khamna *et al* (2009) from rhizospheric soils of 16 medicinal plants. Morphological and chemotaxonomic studies indicated that 89% of the isolates elonged to the genus *Streptomyces*, 11% were non-streptomycetes viz. *Actinomadura* sp., *Microbispora* sp., *Micromonospora* sp., *Nocardia* sp, *Nonomurea* sp. and three isolates were unclassified. The highest number and diversity of actinomycetes were isolated from *Curcuma mangga* rhizosphere soil. Twenty-three *Streptomyces* isolates showed activity against at least one of the five phytopathogenic fungi: *Alternaria brassicicola*, *Collectotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium digitatum* and *Sclerotium rolfsii*. Thirty-six actinomycete isolates showed abilities to produce indole-3-acetic acid (IAA) and 75 isolates produced siderophores on chrome azurol S (CAS) agar. *Streptomyces* CMU-PA101 and *Streptomyces* CMU-SK126 had high ability to produced antifungal compounds, IAA and siderophores.

The total of 26 *Streptomyces* sp. isolated from rhizosphere by Frediansyah and Sudhiana (2013) were tested for ability to produce indole-3-acetic acid (IAA) in yeast malt extract (YM) medium containing 2 mg/mL tryptophan calorimetric estimation of IAA was carried out by adding Salkowski reagent in culture supernatent and was measured at λ 530 nm. To ensure the IAA production in *Streptomyces* isolates, gene involved in IAA biosynthesis was detected by amplifying Tryptophan Monooxigenase (iaaM) gene. The study of the effect of tryptophan on the production of IAA was measured at different concentrations of tryptophan (0, 1, 2, 3, 4, 5 mg/mL) in the culture medium. The result showed that there were two *Streptomyces* sp. isolates which could produce IAA, namely *Streptomyces* sp. MS1

(125.48 µg/mL) and *Streptomyces* sp. BR27 (104.13 µg/mL). This result indicated the IAM pathway was predicted involved in the biosynthesis of IAA in the selected isolates. Both of the isolates were able to produce IAA after 24h incubation and the highest production was at 120h incubation at tryptophan concentration of 2 mg/mL and 1 mg/mL, respectively. Therefore, it is concluded that *Streptomyces* sp. isolates were able to produce IAA and potentially to be utilized as biostimulant agent.

Jamil *et al* (2015) conducted an experiment at the Horticultural research field to investigate the effect of plant growth regulators on flower and bulb production of *Hippeastrum*. There are ten treatments comprising of three concentrations of three growth regulators viz., IAA (20, 60 and 100 ppm), ethrel (100, 300 and 500 ppm) and GA3 (100, 300 and 500 ppm) along with control (soaked in water). Flower and bulb characteristics of *Hippeastrum* influenced significantly by different levels of growth regulators. Application of IAA at 60 and 100 ppm and GA3 at 100, 300 or 500 ppm twice as foliar spray at an interval of 30 days promoted the number of bulblets on the treated plants. The highest number of bulblets per plot (40.00), bulbs weight per plot (4056 g) along with bulb yield (40.56 t/ha) were also obtained in GA3 at 500 ppm.

2.2.3 ACC deaminase activity of actinomycetes

Many PGP actinomycetes have found to enhance growth of the plant by the activity of enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. This enzyme hydrolysis ACC which is the immediate biosynthetic precursors of ethylene in plant tissues to ammonia and α ketobutyrate (Glick *et al* 2007). Minute quantity of the ACC exuded from plant is transformed by ACC deaminase enzyme of microbes resulting in lesser quantity of ACC level in plant (Penrose and Glick 2001).

Treatment of plants with PGPR producing ACC deaminase can considerably be more resistant to the harmful effects of ethylene due to stressful conditions (Glick *et al* 2007) and result in inhibition in level of root elongation (Glick and Penrose 2004) and promotion of plant growth (Glick *et al* 2007). Small quantity of ethylene improved initiation of root and growth while maximum production of ethylene produced via plant roots can inhibit root elongation. Although ACC deaminase production had reported by a variety of microbes such as bacteria (Glick and Penrose 2004), yeasts and fungi but mostly bacterial strains have been studied in detail to evaluate their PGP characteristic (Glick *et al* 2007).

Endophytes possessing ACC deaminase activity can reduce the level of stress ethylene and assist their host plant cope with various biotic and abiotic stresses. ACC deaminase activity was evaluated in the biocontrol and plant growth-promoting fungus. The potential of actinomycetes to promote the plant growth through the ACC deaminase production under gnotobiotics and green house conditions has been studied. Sixty four isolates of *Streptomyces* spp. isolated from a tomato rhizosphere in the United Arab Emirates

were originally selected for their ability to produce ACC deaminase and IAA production. Two isolate and *S. atrovirens* no. 26 produced ACC deaminase and, whilst *S. filipinensis* no. 15 also produced IAA (El-Tarabily 2008) and resulted in the reduction of the endogenous levels of ACC in both roots and shoots and increased plant growth, under green house conditions.

2.2.4 Phosphate-solubilizing microorganisms

Phosphorus (P) is the second most important nutrient of plant. It has a definite role in plant metabolism such as cell division, development and transport of photosynthesis nutrient and regulation of metabolic pathways. Depending on soil pH, phosphate is present in combination with other minerals, organic matter and variety of microorganisms. It is found in the form of inorganic phosphate in the soil which is produced by weathering of parent rock while organic phosphate derived from decayed plants, animals or microorganisms.

The use of phosphate solubilizing actinoycetes as biofertilizers has concurrently increased phosphorous uptake in plants and improved yields in several crop species. A laboratory study was conducted to isolate, identify and characterize the phosphate solubilizing microbes from different crop soils such as Okra, Chilli, tomato, Cotton and Egg plant. The population dynamic of phosphate solubilizing actinoycetes were higher in the rhizosphere soil of tomato followed by brinjal. Based on the solubilization zone production in the solid medium, two isolates from each crop plant were selected and used for studies. The selected strains differed in utilization of different carbon, nitrogen, amino acid and vitamin sources (Baliah *et al* 2016).

Actinomycetes isolated from the rhizosphere were capable of increasing availability of phosphorus to plants either by mineralization of organic phosphate or by solubilisation of rock phosphate by production of acids (Hinsinger *et al* 2003). Among the six selected actinomycetes; *Streptomyces griseus, Streptomyces cavourensis* and *Micromonospora aurantiaca* strains show significantly improved wheat plant growth in test tubes as well as in rock phosphate soil. The strains showing the best phosphate release abilities were also having most important stimulatory effect on shoot and root plant growth was at least partially linked to phosphate supply (Hamdali *et al* 2008). Gangwar *et al* (2012) reported that twenty isolates of endophytic actinomycetes from rice plants and seventeen from wheat plants were able to solubilize phosphate. It is suggested that these actinomycetes could be used as plant growth promoters. The amount of phosphate released from rock phosphate was higher in the presence of actinomycete strains than in the presence of plant alone, indicating the important contribution of rock phosphate solubilizing actinomycetes in the improvement of plant growth (Welch *et al* 2002).

2.2.5 Siderophore Producing microorganisms

The siderophore-mediated competition for iron is one among the mechanisms

responsible for the antagonistic activity of *Streptomyces* sp. (Tokala *et al* 2002). The secreted iron-chelating compounds bind the ferric ions (Fe^{3+}), and are taken up by microbial cells through specific recognition by membrane proteins (Srivastava *et al* 2008). The presence of iron-chelating compounds makes the microbes better competitors for iron, preventing this way the growth of the pathogen microorganisms. Siderophores produced by biocontrol actinoycetes have a higher affinity for iron than those produced by some fungal pathogens, allowing the former microbes to scavenge most of the available iron, preventing the proliferation of fungal pathogens (Hillel 2005). *Streptomyces* have been reported to produce hydroxamate type siderophore which inhibit phytopathogen growth by competing for iron in rhizospheric soils (Khamna *et al* 2009). Siderophore exhibited considerable structural variability and affinity for iron, which determine the growth of microbe under competitive conditions when iron availability is a limiting factor. Competition for iron is considered as the possible method for phytopathogen control (Cao *et al* 2005).

Gangwar *et al* (2014) recovered a total of 60 endophytic actinomycetes from root, shoot and leaf tissues of *Musa acuminata*. Thirty four out of 60 isolates (56.66%) from banana leaves and root tissues produced siderophores which was noticeable by orange zones around the colonies on the CAS agar plates. The diameter of halo formed varied from 2 to 4.5 cm on the CAS agar plates and the most of the isolates belonged to *Streptomyces* sp., maximum diameter was produced by *Streptomyces viridis* (BL43). Thirty isolates were producing hydroxamate type of siderophore in the range of 4.1±0.5 to 128.6±1.1µg/ml and 16 isolates produced the amount of catechol type of siderophore ranging between 9.2±0.7 and 147.3±0.5 µg/ml. Maximum hydroxamate type of siderophore was produced by *S. Viridis* BL43 and maximum catechol type prodtion by *S. cinereus* BR28. In a parallel study the authors (Gangwar *et al* 2014), reported that nine endophytic isolates from three Indian medicinal plants, produced the amount of hydroxamate-type of siderophores ranging between 5.9-64.9 µg/ml. Only four isolates were able to produce catechol-type of siderophores in the range of 11.2-23.1 µg/ml, in this case.

2.3 Plant fungal pathogens

Plant diseases caused by plant pathogenic fungi continuously threaten the sustainability of global crop production (Elizabeth and Handelsman 1998). The fungus *Sclerotinia sclerotiorum* causes Sclerotinia blight, also known as whitemold or white rot (Agrios 1988). This fungus attacks not only crucifers but also a wide variety of other crop plants in field and storage. *Fusarium oxysporum* is an anamorphic species that includes both pathogenic and nonpathogenic strains (Smith *et al* 1988). Plant pathogenic forms cause a wilt disease and are grouped into 'formae speciales' (f.sp) based on their host range; some are further subdivided into pathogenic races. Fungal diseases, which have been controlled using the microorganisms and their products include diseases caused by organisms in the genera

Alternaria, Colletotrichum, Fusarium, Helminthosporium, Macrophomina, Phoma, Phytophthora, Pythium, Rhizoctonia, Sclerotium, Thelaviopsis and Verticillium (Chet 1990).

Riaz *et al* (2007) reported 100% disease incidence and 20% plant mortality, with reductions in shoot and root biomass of 63 and 100%, respectively, when *Gladiolus grandiflorus* corms grown in a pot culture system were inoculated with *F. oxysporum f. sp. gladioli*. The causative organism of wilt, *Fusarium oxysporum f. sp. ciceris* is widespread in chickpea growing areas resulting in considerable economic losses (Arunodhayam *et al* 2014). *Fusarium* root rot (*Fusarium* sp.) is one of the most important seedling diseases of coneflower (Echinacea sp.) in Alberta greenhouses. Wang *et al* (2005) suggested that *fludioxonil* and *Trichoderma* could be integrated into a disease management program for *Fusarium* root rot in coneflower.

2.4 Biological methods

Biological control includes total or partial destruction of a pathogen population by other microorganisms. *Fusarium oxysporum* thrives well and causes several diseases in some soils known as conducive soils. It develops much less and causes much milder disease in other soils such as Lateritic clay soil, known as suppressive soil. Mohamed and Gomaa (2000) studied the effect of bioagents and agricultural chemicals on Fusarium wilt incidence and growth characters of gladiolus plants. For efficacy, two biological control agents (BCA) i.e. *Trichoderma harzianum* and *Bacillus subtilis* and two agricultural chemicals (sulfur and lime-calcium hydroxide) were compared with recommended fungicides Vitavax (captan) 75wP and Rizolex-T50 (Tolelofos- methyl + thiram), as soil or corm-soaking treatments in controlling Fusarium disease on gladiolus 'Peter Pears'. The effects of these agents, chemicals and fungicides on several plant growth parameters were also studied. It was observed that most of the treatments decreased the diseased corm number, as well as increased the survival of the plant growth and corm formation. Growth inhibition of pathogen by *Trichoderma harzianum*, *T. viride* and *T. virens* has been reported by Sharma and Chandel (2003). Mishra and Mukhopadhyay (2005) reported integrated and biological control of gladiolus corm rot and wilt caused by *Fusarium oxysporum* f. sp. *gladioli*. The suppression of disease is due to the presence of microbiota especially *Streptomyces* spp. in Lateritic clay soil that suppress infection (Chandel and Deepika 2010). Parveen *et al* (2016) reviewed on the use of microbial antagonists (fungi and bacteria), botanicals and compost extracts as biocontrol agents against different pathogenic fungi causing postharvest fungal rots in rosaceous fruits which shows that they can play an important role in the bio-management of fungi causing rot diseases.

Coombs *et al* (2004) examined biocontrol efficacy of endophytic actinomycetes against *G. graminis* var. *tritici* of wheat. Six strains which showed significant biocontrol effects in naturally infested soil tests had varied degrees of antifungal activities. Endophytic

actinomycetes with tomato were obtained by Inderiati and Franco (2008) noted that 13 of 15 strains having varying levels of antagonistic activity showed the suppressive effects on damping-off caused by *Rhizoctonia solani* significantly. *Streptomyces thermocarboxydus* showed strong *in vitro* antagonism but resulted in the lowest disease suppression *in vivo*. In case of soybean, inoculation of selected endophytic actinomycetes, *Streptomyces sp.* isolated from sweet pea which showed antagonistic ability against fungal plant diseases could infect and improve nitrogen uptake of the soybean plantto about 83% compared to uninoculation control treatment and such endophyte could be compatible well with *Bradyrhizobium* (Thapanapongworakul 2003).

2.5 Antifungal activity of rhizospheric actinomycetes

Over the past one hundred years, research has repeatedly demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens (Cook 2000). Soil microorganisms have the potential for the management of crop diseases. A variety of soil microorganisms have demonstrated activity in the control of various soil borne crop pathogens, including *Fusarium* wilt pathogens (Bloemberg and Lugtenberg 2001).A total of 137 actinomycetes cultures, isolated by Gopalakrishnan *et al* (2010) from twenty five different vermicomposts, were screened for their antagonistic activity against *Fusarium oxysporum f. sp. ciceri* (FOC) by dual-culture assay. From which, five most promising FOC antagonistic isolates i.e. CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90, were screened for the production of indole acetic acid (IAA), protease, cellulase, hydrocyanic acid (HCN), siderophore and antagonism activity against *Rhizoctonia bataticola*, causing dry root rot in chickpea and sorghum. All of the five produced siderophore and HCN, KAI-32 and KAI-90 produced cellulose, CAI-24 and CAI-127 produced protease and four of them (except KAI-90) produced IAA,. In the dual-culture assay, three of the isolates, CAI-24, KAI-32 and KAI-90, also inhibited all three strains of *R. bataticola* in chickpea, while two of them (KAI-32 and KAI-90) inhibited the tested strain in sorghum.

2.6 Antifungal activity of endophytic actinomycetes

Endophytic actinomycetes have gaining attention of investigators as biological control agents of plant pathogens due to their antifungal activities against plant pathogens i.e. *Rhizoctonia solani*, *Verticillium dahliae*, *Plectosporium tabacinum*, *Gaeumannomyces graminis* var. *tritici, F. oxysporum*, *Pythiumaphanidermatum* and *Colletotrichum orbiculare* (Cao *et al* 2005; El-Tarabily *et al* 2009 and Shimizu *et al* 2009). *Streptomyces roseosporus* W9 obtained from wheat plants showed highest antagonistic activity against ten different pathogenic fungi tested (Gangwar *et al* 2012).

Although antifungal agents are produced by several different groups of microorganisms, it is endophytic actinomycetes which may offer the greatest promise from chemotherapeutic point of view as well as novel source of antifungal agents. As the

endophytic actinomycetes are producing a number of antifungal antibiotics, they may be the treasure troves for pharmaceutical agents and agrochemical compounds. Taechowisan *et al* (2003) isolated 59 endophytic actinoycetes from the roots of *Zingiber officinales* and *Alpinia galangal* and tested against *Candida albicans* and phytopathogenic fungi, *Fusarium oxysporum*.

The in vitro antifungal activity of endophytic *Streptomyces* sp. has been investigated by Gandotra *et al* (2012) against *Candida albicans* and *Candida parapsilosis*. About 9 Genera of endophytic actinomycetes (*Streptomyces*) were isolated from various parts on different media. *Streptomyces* showed significant antifungal activity against *Candida albicans* when performed by fermented broth filterate method. Also, GS broth supported the greatest antifungal activity. Augustine *et al* (2014) were isolate 312 actinomycetes from soil samples on chitin agar which purified and screened for their antifungal activity against pathogenic fungi. Out of these, 22% of the isolates exhibited activity against fungi.

Endophytic microbes existing in plants were isolated and screened for inhibition of several pathogenic fungi and for plant growth promtion. Of 369 isolates from 13 medicinal plants, 94 isolates had antifungal activity against the phytopathogenic fungus *Dothiorella gregaria*. Antifungal activity against *Sclerotinia sclerotiorum* and *Botryosphaeria dothidea*, the presence of genes coding for type I polyketide synthases (PKSs) and PKSs for a polyene component, chitinase activity, and production of siderophores and indole-3-acetic acid were also evaluated. Four selected representative antagonistic endophytes (SLS23, SSD41, SSD49 and SSD60) and their biocontrol and plant-growth-promoting activities were studied. These isolates significantly improved the growth of soybean and tomato and varied in their control efficacy against plant diseases caused by *S. sclerotiorum* and *B. dothidea* (Liu *et al* 2016).

2.7 Advantages of biological control

One of the goals for the use of biocontrol in agriculture is to avoid the pitfalls associated with chemical control such as development of resistance in the target pathogen and environmental degradation (Benbrook *et al* 1996). The advantage of biocontrol strategies is the slow development of resistance by the pathogens and pests to the antagonistic metabolites produced by biocontrol agents (Handelsman and Stabb 1996). This is because most biocontrol agents produce more than one antagonistic compound and resistance to such multiple compounds should occur only at a very low frequency (Fiddman *et al* 2000). The use of biological control strategy also minimizes the negative consequences for human health and the environment (Elizabeth and Handelsman 1998). Also, biological control strategy does not pose serious environmental concerns.

Microbial biological control agents have been commercialized and marketed as biological control products (Helbig and Bochow 2001). Targets of those antagonists already being commercialized are mainly soil-borne and post harvest plant pathogens. *Streptomyces*

lydicus WYEC108 has been formulated in the commercially available product Actinovate ® and it may be able to control fungal plant pathogens effectively for fresh market tomatoes. Prestop® is a biological fungicide for control of damping-off and root diseases (*Pythium, Fusarium, Phytophthora* and *Rhizoctonia*) as well as for the control of *Botrytis* (grey mould) and *Didymella* (*Mycosphaerella*) gummy stem blight cucumber. It is based on the *Gliocladium* fungal strain J1446 which colonizes effectively roots and foliar parts of plants preventing the attack of plant diseases. *Streptomycetes* are known to include several antagonistic species that may inhibit growth of plant pathogenic microorganisms.

CHAPTER III

MATERIAL AND METHODS

3.1 Procurement of standard fungal cultures

Fusarium oxysporum was obtained from Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The fungal cultures were maintained on Glucose Yeast Extract Agar (GYE) and stored at 4ºC.

3.2 Sample collection

Twenty soil and sixteen root samples of *Gladiolus* were collected from various locations of Punjab Agricultural University, Ludhiana and carried to laboratory for immediate processing.

3.3 Isolation of actinomycetes from rhizospheric soil

One gram of soil sample was transferred to 9ml of sterile water blank and homogenized for 2 minutes on a votex mixture. Aliquots (0.1ml) from serial logarithmic dilutions of each suspension were pipetted onto the surface of duplicate petriplates containing Yeast Malt extract agar (YMEA) supplemented with nalidixic acid (50µg/ml). The inoculum was spread evenlr.y over the surface using the sterile glass spreader. The 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 YMEA medium and incubated at 28^0C for 5-7 days. Subculturing was done after every one month and stored at 4°C.

3.4 Isolation of endophytic actinomycetes

Root samples were washed in running water to remove adhered soil particles and sterilized by dipping in 70% ethanol for 5 minutes and sodium hypochlorite solution (0.9% available chlorine) for 20 minutes. To remove surface sterilization agents, samples were washed in sterile water 3 times and divided into small fragments. These fragments were macerated completely. One ml of suspension was transferred on to Petri plates containing Starch Casein Agar (SCA) media and was spread uniformly with glass spreader. Plates were incubated for 7-19 days at 28^0C. Isolated colonies were purified and then subcultured on slants and stored at 4^0C.

3.5 Maintenance of actinomycete isolates

Actinomycete isolates were maintained at 4ºC on SCA and YMEA slants.

3.6 Characterization of actinomycete isolates for functionality traits

3.6.1 Indole acetic acid (IAA) Production

Production of IAA was done by method of Gordon and Weber (1951).

3.6.1.1 Reagents

(a) Salkowski reagent: 1ml of 0.4 M $FeCl_3$ in 50 ml of 35% perchloric acid

(b) IAA stock solution: 100 µg ml^{-1} of 50 % ethanol

Actinomycete isolates were grown in Yeast malt extract broth in 100 ml Erlenmeyer flasks for 10 days. Ten milliliter of grown culture was taken in eppendroff tubes and centrifuged at 10,000 rpm for 20 minutes. Two drops of O-phosphoric acid were added to the supernatant if alkaline. Then 4 ml of reagent (a) was added and incubated for 25 minutes at room temperature for development of pink color and absorbance was recorded at 530 nm. Statistical analysis had been done by calculating standard error.

3.6.1.2 Standard curve of IAA

Standard curve was prepared by taking standard IAA solution (b) in 0-100 µg/ml concentration in different test tubes. One ml volume was made with distilled water and then 2 ml of reagent (a) was added to make the total volume to 3 ml and incubated for 25 minutes at room temperature. Standard curve was plotted with the different readings obtained by taking absorbance at 530 nm (Appendix II).

3.6.2 Phosphate solubilization

Characterization of isolates for phosphate solubilization was done by qualitative and quantitative method (Jackson 1973).

3.6.2.1 Qualitative method for Phosphate-solubilization

Screening of phosphate solubilizing isolates was done using National Botanical Research Institute-Bromo Phenol B (NBRI-BPB) medium. Actinomycete cultures were inoculate on petriplates and incubated for 7 days at 28°C. The plates were observed for formation of clear halos around colony.

3.6.2.2 Quantitative method for Phosphate-solubilization

Ammonium molybdate-Ammonium vandate reagent: Ammonium molybdate (22.5g) was dissolved in 400 ml distilled water. Then 1.25g of ammonium vandate solution in 250 ml of boiling water was added to ammonium molybdate solution and allowed to cool at room temperature. Concentrated nitric acid (250 ml) was then added to it and the volume was made to one litre.

Culture broth was digested with 20ml of triacid mixture and 50 ml volume was made with distilled water; specific aliquots were used to estimate the phosphorous by reacting with 5ml of ammonium molybdate reagent in nitric acid. The volume was made up to 50ml and yellow color was estimated at 470 nm using spectronic-20. From the standard curve using different concentrations the total phosphorus was estimated (Appendix II). Statistical analysis had been done by calculating standard error.

3.6.3 Siderophore production

Siderophore production was determined done both qualitatively and quantitatively.

3.6.3.1 Qualitative method

Siderophore production by actinomycete isolates was done with blue coloured

petriplates having dye chrome azurol S (CAS) (Schwyn and Neilands 1987). The growth medium used was Yeast malt extract agar (YMEA) for actinomycetes isolates. In 50 ml deionised water, 60.5 mg of CAS was dissolved and mixed with 10ml of a Fe^{3+} solution (1mmol/l $FeCl_3.6H_2O$ in 10mmol/l HCl). Slowly this was mixed with 72.9 mg of hexadecyltrimethyl ammonium bromide (HDTMA) earlier dissolved in 40 ml water. The resulting dye solution was autoclaved, cooled to 50-60ºC and mixed with 900 ml YMEA. The medium was allowed to solidify on Petri-plates. Formation of an orange halo around the colony is indicative of positive culture.

3.6.3.2 Quantitative method

Catechol-type siderophores were assessed by the method of Arnow (1937). To study the siderophore production, the isolates were allowed to grow in yeast malt extract broth and incubated for 10 days at 28ºC. The culture supernatant was separated by centrifuging the cultures at 10,000 rpm for 15 minutes. 20 ml of supernatant at pH 2 twice was used to prepare ethyl acetate extracts with an equal volume of solvent. One ml of 0.1 M of ferric chloride in 1 ml of 0.1 N HCl was added to prepare Hathway's reagent in 100 ml of distilled water. To this 1ml of 0.1 M potassium ferricyanide was added. To one volume of Hathway's reagent, one volume of sample was added. Absorbance was recorded at 560 nm with sodium salicylate as standard. The quantity of siderophore present in the sample was calculated from standard curve prepared using different concentrations of sodium salicylate (Appendix II).

Hydroxamate type of siderophores was evaluated by the method of Csaky (1948). 0.5 ml of 6 M H_2SO_4 was added to 0.5 ml of culture supernatant. Mixture was autoclaved and allowed to cool. 1.0 ml of 1% (wt/vol) sulfanilic acid in 30% (vol/vol) acetic acid and 0.5 ml of 1.3% (wt/vol) iodine in 30% (vol/vol) acetic acid were added. Excess I_2 was eliminated by the addition of 1.0 ml of 2% (wt/vol) Na_3AsO_2 solution after 5 min. A solution of α-naphthylamine (0.3% [wt/vol] in 30% acetic acid [vol/vol]; 1 ml) was added, and the total volume was increased to 10 ml with distilled water. Absorbance at 526 nm was recorded after 30 min at room temperature. Hydroxylamine hydrochloride was used as a standard for the estimation of hydroxamate siderophores (Appendix II). Statistical analysis had been done by calculating standard error.

3.6.4 Production of Gibberellic acid

Gibberellic acid production was done by method of Barrow *et al* (1995).

3.6.4.1 Reagents

(a) Zinc acetate solution: To 80ml of distilled water, 21.9g of zinc acetate was added and mixed with 10 ml of glacial acetic acid. Volume was made to 100ml.

(b) Potassium ferrocyanide: 10.6g of potassium ferrocyanide in 100ml of distilled water.

Cultures inoculated in their respective broth were incubated at 37ºC for seven days

and then centrifuge at 8000rpm for 10 min. To the test tubes, 15ml of the supernatant was taken and zinc acetate solution (2ml) was added. Two ml of potassium ferrocynide solution was added after two minutes and centrifuged at 8000 rpm for 10 min. Five ml of supernatant was added to 5 ml of 30% hyperchloric acid. Absorbance measured at 254nm in a UV-VIS spectrophotometer. The amount of gibberellic acid was calculated from standard curve (Appendix II). Statistical analysis had been done by calculating standard error.

3.6.5 Production of HCN

Cyanide production was detected by method as described by Lorck (1948). To the Petri plates amended with 10% Trypticase soya agar (TSA) medium and 4.4 g of glycine per litre, actinomycete isolates was inoculated. Piece of paper impregnated with 0.5% picric acid and 2% sodium carbonate was placed in the lid of each Petri plate and incubated for 3 to 5 days at 28^0C. Cyanide production will be indicated by change in color of filter paper from yellow to orange-brown.

3.6.6 ACC deaminase activity

The qualitative estimation was done by the method described by Govindasamy *et al* (2008). Actinomycete isolates were grown on Petri plates containing DF (Dworkin and Foster) salt minimal medium supplemented with 3 mM ACC. Growth of isolates on plates was compared to positive ($(NH_4)_2SO_4$ as N-source) and negative controls (DF minimal medium without ACC) after 3-4 days incubation at 28°C.

3.7 Screening of actinomycete isolates for antagonistic activity

The actinomycete isolates were screnned for their antagonistic activity against *Fusarium oxysporum*, by dual-culture *in vitro* assay. Fungal discs (8mm in diameter) were placed at the center of GYE plates. The actinomycetes isolates were spotted on opposite sides of the plates and incubated at 28ºC for 7-8 days. Plates without the actinomycetes served as control. Statistical analysis had been done by calculating standard error.Colony growth inhibition (%) was measured using the formula provided by (Yuan and Crawford 1995):

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

3.8 To determine the effect of fungicide carbendazim on the growth of actinomycete isolate Sc9 *in vitro*.

The Fungicide was supplemented in Nutrient agar (NA) medium before autoclaving in the range of 10ppm, 25ppm and 50ppm in three 250ml conical flasks respectively. Medium was autoclaved and poured into the sterile petriplates. Actinomycete isolate Sc9 was streaked on petriplates amended with fungicide and incubated for seven days at 28° C. Petri plates containing NA medium without fungicide served as a control. Growth of actinomycete isolate on different concentrations of fungicide amended petriplates was observed.

3.9 Scanning electron microscopic (SEM) studies of the antagonistic effect of potential actinomycete isolate Sc9 on fungal cell wall

Scanning electron microscopy (SEM) of one actinomycete isolate obtained from rhizospheric soil of *Gladiolus* (Sc9) treated with *Fusarium oxysporum* culture was performed (Bozzola and Russel 1999). The samples were processed, freeze dried and stubbed on aluminium stub using double sided carbon sticky tape. The stubbed samples were 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.

3.10 Morphological and biochemical characterization of the isolates

3.10.1 Morphological description of actinomycete isolates was done on the colony shape, mass, color, spore color, spore arrangements, pigment production and Gram staining. To study the colony morphology, the isolates were cultured on starch casein agar medium at 28ºC. The reverse colony color, color of diffusible pigment and spore chain morphology were noted (Ruan *et al* 1990, Yan 1992, Cao *et al* 2004, Tian *et al* 2006). The microscopic morphology was studied by examining Gram stained smears.

3.10.2 Biochemical characteristics

Actinomycete isolates was identified biochemically using Casein, starch, esculin and Tween-80 hydrolysis. Decomposition of tyrosine, xanthine and hypoxanthine was also studied.

3.10.2.1 Hydrolysis of Casein

The test for casein hydrolysis was performed by the procedure described by Gordon and Smith (1955). A 10% suspension of skimmed milk powder in water and an equal volume of 4% water agar autoclaved separately and cooled. They were mixed and poured in to Petri plates. The plates were inoculated in the center and incubated at 28^0C for 48 hrs. Colonies were observed for zone of clearance beneath and around the growth.

3.10.2.2 Hydrolysis of Starch

Ninety ml of nutrient agar medium was prepared. To this 1 gram of potato starch suspension in 10 ml of cold distilled water was added and autoclaved. The medium was poured into sterile Petri plates and inoculated with actinomycete isolates. After incubation period, Grams iodine solution was flooded into plates. Hydrolysis of starch was indicated by clear zone surrounding actinomycete colony.

3.10.2.3 Hydrolysis of Esculin

To the Nutrient agar medium, 0.1% w/v Esculin and 0.05% ferric citrate were added and autoclaved. The plates were inoculated and incubated for 10 days. Hydrolysis of esculin was showed by blackening of medium around the colony.

3.10.2.4 Hydrolysis of Tween- 80

1% of Tween 80 and 0.01% of $CaCl_2$ were added to nutrient agar, autoclaved and poured into sterile petriplates. The hydrolysis of tween 80 was indicated by an opaque zone formed due to precipitation of calcium salt around the colony (Jones *et al* 1979).

3.10.2.5 Decomposition of Hypoxanthine, Tyrosine and Xanthine

A concentration of 0.5% (w/v) was used for testing hypoxanthine and tyrosine decomposition, whereas 0.4% concentration for xanthine decomposition. The media were sterilized by autoclaving at 121^0C for 15 minutes and poured into petriplates in aliquots of 20 ml. In the center of plate test isolate was inoculated and incubated. A zone of clearance appearing around and beneath the colony was taken as evidence of decomposition.

3.11 Evaluation of effectiveness of actinomycete isolates as plant growth promoting and potential antagonists against *Fusarium oxysporum* under green house conditions

3.11.1 Inoculum preparation of potential antagonists

The potential isolate (Sc9) was grown in nutrient broth medium for 5 days. Healthy corms of *Gladiolus* were surface sterilized with 0.1% $HgCl_2$ for 3 min followed by treatment with 95% ethanol for 5 minutes and then successive washing with sterilized distilled water. The surface sterilized corms were immersed overnight in the antagonist suspension containing 10^8cfu/ml.

3.11.2 Fungal inoculum preparation

Fusarium oxysporum was grown on potato dextrose agar and discs of fungi were transferred to 250 ml Erlenmeyer flasks containing autoclaved wheat and sand. The flasks were incubated at 25ºC for 7 days at 200rpm. The rate of inoculum applied to the potting mixture was 10 gm of fungi per pot.

3.11.3 Soil infestation

Soil was taken from field and sterilized by autoclaving at 121^0C for 1 hr for 3 consecutive days. Corms were grown in pots, using completely randomized block design (CRD) with 6 treatments and 3 replications each. Five bulbs were sown per pot containing sterile soil on 15 September, 2016. The treatments were:

T1- Control with sterile soil only

T2- Corms inoculated with fungal spore suspension

T3- Corms inoculated with potential actinomycete isolate (Sc9)

T4- Corms inoculated with potential actinomycete isolate (Sc9) + fungal spore suspension

T5- Recommended NPK (40:40:160)

T6- NPK+ potential actinomycete isolate Sc9

3.11.4 Observations to be recorded

3.11.4.1 Percentage of corm germination

Total numbers of corm germinated were counted and then percent germination was

calculated as follows:

$$\text{Germination (\%)} = \frac{\text{Total number of corm germinated}}{\text{Total number of corm sown}} \times 100$$

3.11.4.2 Plant growth promoting attributes

3.11.4.2.1 Fresh weight of shoot and root

Plants were removed with root system intact and then measured for fresh weight of shoot and root after 30 and 60 Days of sowing (DAS).

3.11.4.2.2 Dry weight of shoot and root

Plants were randomly selected and uprooted plants from each pot were sun dried and then oven dried at 60^0C for 1 day.

3.11.4.2.3 Root and shoot length

Plants root and shoot lengths were measured separately using simple measuring scales in triplicates.

3.11.4.2.4 Incidence of disease (Wilt incidence %)

The plants were counted with wilting symptoms and then wilt incidence was measured as follows:

$$\text{Incidence of disease (\%)} = \frac{\text{Total number of wilted plants}}{\text{Total number of plants}} \times 100$$

3.11.4.2.5 Inhibition of disease development (%)

Inhibition of disease was calculated by comparing treatments with control.

$$\text{Inhibition of disease development (\%)} = \frac{\text{Wilt incidence}}{\text{Wilt incidence in control}} \times 100$$

CHAPTER IV

RESULTS AND DISCUSSION

Actinomycetes are filamentous, Gram-positive bacteria which produce antibiotics of agricultural and medicinal value. Actinomycetes participate in agriculture as biocontrol agents. Endophytic actinomycetes are also known to stimulate plant growth due to the production of phytohormones (Nimnoi *et al* 2010) and keep a check on the growth of phytopathogens through production of enzymes, antibiotics or siderophores (Quecine *et al* 2008).

The present investigation was undertaken to isolate endophytic and rhizospheric actinomycetes from *Gladiolus* plants and to evaluate their PGP and antifungal activity against *Fusarium oxysporum* in pot conditions.

4.1 Isolation of actinomycete diversity

A total of 90 isolates of actinomycete were obtained from the rhizospheric soil and roots of *Gladiolus* plant. Out of 90, forty were isolated from rhizospheric soil and 50 from roots of *Gladiolus* (Table 1). The data on occurrence and enumeration of actinomycetes from rhizopheric soil showed that *Gladiolus* has the population density of $2.5x10^5$c.f.u g^{-1} (Plate 1 and 2).

These results are in accordance with the reports of Karthikeyan *et al* (2007) who isolated and enumerated actinomycetes from rhizosphere soil of four medicinal plants *viz Aloe vera, Catharanthus roseus* (L.), *Coleus forskholii,* and *Ocimum sanctum.* The actinomycetes population was found to be 12.22 x 10^5 c.f.u g^{-1} in *O. sanctum*, 10.44 x 10^5 c.f.u g^{-1} in C. *roseus*, 8.44 x 10^5 c.f.u g^{-1} in *A. vera* and 6.22 x 10^5 c.f.u g^{-1} in *C. forskholii*. Similarly, Gil *et al* (2009) reported 1.91×10^4 c.f.u g^{-1} of actinomycetes from different soil samples. Qin *et al* (2009) obtained 2174 actinomycete strains from 90 selected medicinal plants of tropical rain forests in China. Roy *et al* (2011) also isolated 31 rhizospheric strains of actinomycetes from different niche habitats of Sheopur district, Madhya Pradesh, India. Gajendran *et al* (2012) isolated 135 rhizospheric actinomycetes from soil of five flowering plants i.e. *Chrysynthemum indicum, Crossandra infundibuliformis, Barlleria cristata, Polianthes tuberose and Cantharanthus roseus*. The actinomycete diversity was found to be highest in *Cantharanthus roseus.* Gangwar *et al* (2014) obtained 40 endophytic actinomycete isolates from the leaf, stem and root of *A. vera*, *M. arvensis* and *O. sanctum,* out of which most of the actinomycetes were recognized as *Streptomyces* sp. Kamara and Gangwar (2015) obtained 100 actinomycete isolates (50 from *Catharanthus roseus* and 50 from *Withania somnifera*) from 30 soil samples. The actinomycete population was found to be highest in *Withania somnifera* (3.9 $x10^5$ c.f.u g^{-1}) followed by *Catharanthus roseus* (3.2 $x10^5$ c.f.u g^{-1}). Therefore, this implies that nature of vegetation occurring at the sampling sites probably

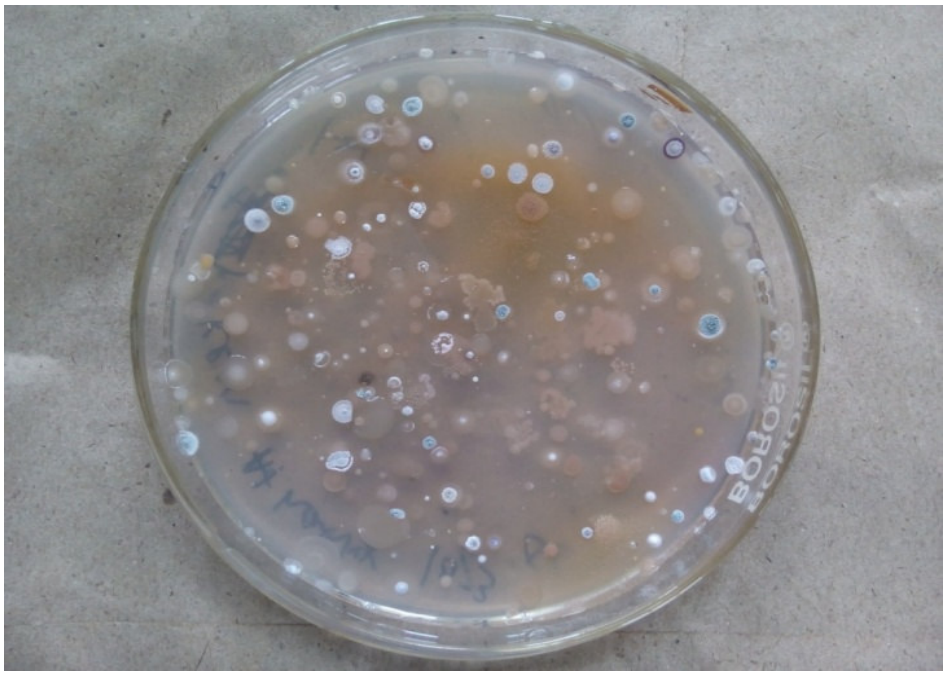


Plate 1: Rhizospheric colonies

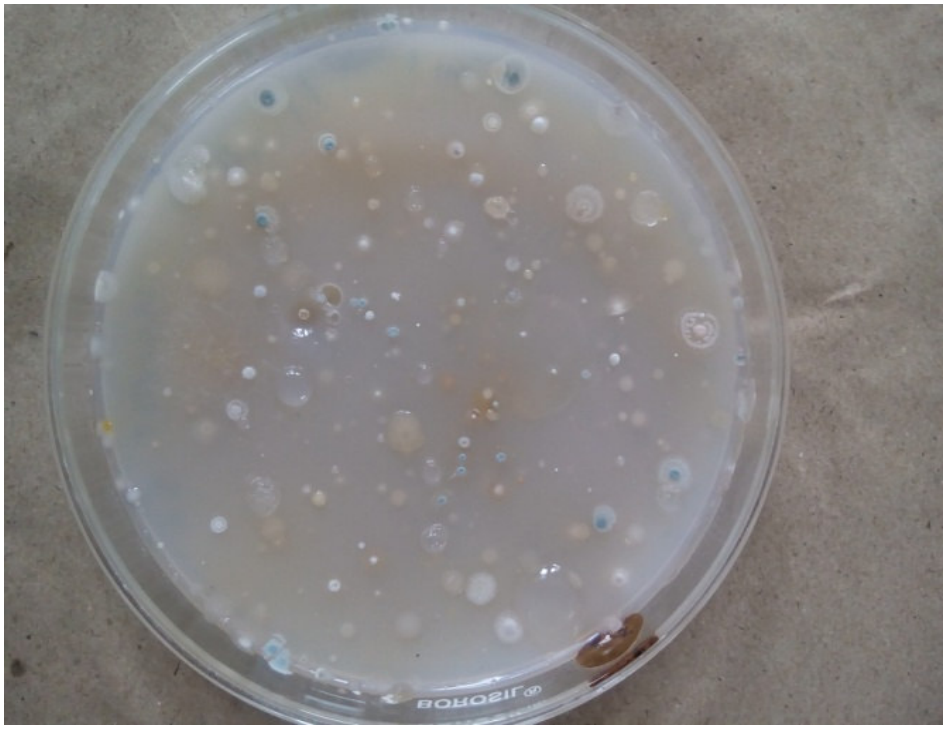


Plate 2: Endophytic colonies

influence the actinomycete populations through root exudates and sloughed off root material.

Table 1: Population density of actinomycete isolates from *Gladiolus* plant

Occurrence	No. of samples	Colony count	No. of isolates
Rhizospheric	20	2.5×10^5(c.f.u/g)	40
Endophytic	16	-	50

4.2 Production of Indole-3-acetic acid (IAA)

Out of 90 isolates, thirty (16 from soil and 14 from roots) were observed to produce the phytohormone IAA. Indole acetic acid production ranged from 4.3-27.4 µg/ml in the absence and 8.1-79.9 µg/ml in the presence of tryptophan. Maximum IAA production was reported in rhizospheric actinomycete isolate Sc9 (79.9 µg/ml), followed by Sb3 (68.9 µg/ml) and Sh2 (68.3 µg/ml) in the presence of tryptophan whereas Sc13 showed minimum IAA production (8.1µg/ml). Whereas IAA production without tryptophan was maximum in isolate Sb3 (27.4µg/ml), followed by Sc9 (19.6µg/ml) and Sh2 (17.5µg/ml). Here, isolate Sc13 showed minimum IAA production (4.3µg/ml), as evident from Table 2.

Table 2: Indole acetic acid (IAA) production by rhizospheric actinomycete isolates

Isolates	IAA production (µg/ml)	
	Without Tryptophan	With Tryptophan
Sc2	11.6 ± 0.1	23.9 ± 0.2
Sc13	4.3 ± 0.2	8.1 ± 0.1
Sc17	12.5 ± 0.1	23.6 ± 0.1
Sm4	16.2 ± 0.05	25.3 ± 0.05
Sb2	5.4 ± 0.1	33.6 ± 0.04
Sc12	8.6 ± 0.1	12.3 ± 0.08
Sh2	17.5 ± 0.2	63.6 ± 0.1
Sd1	15.7 ± 0.1	46.4 ± 0.1
Sc9	19.6 ± 0.2	79.9 ± 0.2
Sm9	5.9 ± 0.1	42.7 ± 0.1
Sd10	11.5 ± 0.03	54.7 ± 0.1
Sc20	5.9 ± 0.05	58.6 ± 0.05
Sc11	8.5 ± 0.1	34.1 ± 0.1
Sb3	27.4 ± 0.1	68.9 ± 0.2
Sc7	13.5 ± 0.05	52.6 ± 0.1
Sc16	18.3 ± 0.1	44.4 ± 0.1

Values indicate mean of three replicates**.**

Among endophytic actinomycete isolates, maximum IAA production was observed in isolate Rm9 (60.7µg/ml), followed by Rm4 (59.4µg/ml) and Rc1 (58.9µg/ml) in the presence of tryptophan. Whereas Rc2 isolate showed minimum IAA production (12.6µg/ml). Indole acetic acid production without tryptophan was maximum in isolate Rm4 (32.1µg/ml), followed by Rm9 (27.2µg/ml) and Rc1 (19.5µg/ml). Here, minimum IAA production showed by Rxy (6.7µg/ml), as evident from Table 3.

Table 3: Indole acetic acid (IAA) production by endophytic actinomycete isolates

Isolates	IAA production (µg/ml)	
	Without Tryptophan	With Tryptophan
Rd6	15.1 ± 0.06	19.3 ± 0.1
Rc7	13.3 ± 0.1	42.9 ± 0.2
Rb2	18.9 ± 0.1	54.7 ± 0.2
Rc1	19.5 ± 0.2	58.9 ± 0.1
Rb3	12.5 ± 0.1	19.5 ± 0.1
Rc2	8.3 ± 0.2	12.6 ± 0.1
Rd5	13.9 ± 0.1	52.7 ± 0.2
Rm3	15.2 ± 0.07	45.5 ± 0.1
Rm9	27.2 ± 0.05	60.7 ± 0.2
Rm5	13.9 ± 0.07	47.6 ± 0.1
Rm4	32.1 ± 0.1	59.2 ± 0.1
Rxy	6.7 ± 0.05	37.9 ±0.1
Rm10	16.3 ± 0.1	28.8 ± 0.2
Rm8	9.4 ± 0.1	23.9 ± 0.1

Values indicate mean of three replicates**.**

Our results are in aggrement with the Poonguzhali *et al* (2008) isolated rhizospheric actinomycetes from *Brassica campestris* which had shown to produce IAA in the range of 6.02–29.75 µg/ml. Pornthip (2010) isolated 83 actinomycetes from rhizospheric soil of twenty three plants. Among which 10 isolates were able to produce IAA in the range from 8.1–28.5 µg ml^{-1}. *Streptomyces* sp. CMUMH021 showed highest ability to produce IAA. Vasconcellos *et al* (2010) isolated 103 rhizospheric actinomycetes from Araucaria forests. Out of which, 37 isolates were able to produce indole-acetic acid ranging from 2-50µg/ml. Ruanpanun *et al* (2010) isolated 27 endophytic actinomycete from five tomato plants. All endophytic strains were screened for their ability to produce IAA. Eighteen strains were able to produce IAA ranging from 20-127µg/ml. Highest IAA production was recorded to be 127µg/ml by *Streptomyces* sp. PT2. Khamna *et al* (2010) isolated rhizospheric *Streptomyces* spp. from fourteen medicinal plants, out of which 11.2% produce indole-3-acetic acid in a yeast malt

extract medium supplemented with 2 mg/ml L-tryptophan. In the rhizosphere soils, root exudates are the natural source of tryptophan for rhizosphere micro-organisms, which may enhance auxin biosynthesis in the rhizosphere. Nimnoi *et al* (2010) isolated 10 endophytic actinomycetes producing IAA ranging between 9.85–15.4 µg/ml. Gajendran *et al* (2012) report that out of 135 rhizospheric actinomycete isolates from five flowering plants i.e. *Chrysynthemum indicum, Crossandra infundibuliformis, Barlleria cristata, Polianthes tuberose* and *Cantharanthus roseus*. Ten isolates were able to produce IAA in significant amount. Ameur and Ghoul (2012) isolated seven actinomycetes from rhizospheric soil in north Algeria of *Catharanthus*. All the actinomycete strains produced IAA in variable rates, from which isolate SF5 produced highest level of IAA as compared to other strains in the absence (55.58±0.7µg/ml) and presence (104.76±0.2µg/ml) of tryptophan.

4.3 Phosphate solubilization

4.3.1 Qualitative method of phosphate solubilization

Eighteen (20%) out of 90 isolates were observed to solubilize phosphate (Table 4 and Plate 3). These results are supported by Oliveira *et al* (2010) who reported that 16.2% out of seventy endophytic actinomycetes isolates obtained from tomato plants showed a positive reaction for phosphate solubility. Gangwar *et al* (2012) reported that twenty out of forty five endophytic actinomycetes isolates obtained from rice plants were able to solubilize phosphate. Gangwar *et al* (2014) reported that 30 out of 40 actinomycete isolates obtained from medicinal plants showed a positive reaction for phosphate solubility. This test indicated the ability of isolates to solubilize phosphate but not the amount of solubilized phosphate, therefore quantitative method was used.

4.3.2 Quantitative method of phosphate solubilization

All the isolates which were able to solubilize phosphate on NBRIP medium were further evaluated by quantitative method of phosphate solubilization. The amount of phosphate solubilized by actinomycete isolates varied in the range of 6.1-64.5mg/100ml, after 10 days of incubation (Table 4). The maximum amount of phosphate solubilized by Sc9 (64.5mg/100ml), followed by Sm9 (47.9mg/100ml) and Sh2 (43.7mg/100ml) and minimum by Sd1 (6.1mg/100ml). These results are in agreement with the observation of Hamdali *et al* (2008) who reported that *Streptomyces cavourensis* and *Streptomyces griseus* showed maximum phosphate solubilizing activity (0.83 and 0.58 µg/ml respectively). Gangwar and Kataria (2013) isolated fourty seven actinomycetes which were shown to solubilize phosphate ranging 0.02-0.68 mg/ml. *Streptomyces aureus* Mt-10 (0.68 mg/ml) was exhibiting maximum amount of phosphate solubilization. Passari *et al (*2015) isolated forty- two endophytic actinomycete isolate from roots of medicinal plants. Among them, 14 isolates (63.6%) were shown inorganic phosphate solubilization activity. The phosphate solubilization activity varied from 35 to 73% among the isolates, and *Streptomyces* sp. were identified as the highest

phosphate solubilization in 34 (73%) which was followed by *Leifsoniaxyli* ranging 24 (64%) and *Microbacterium* sp. ranging 21 (59%). Estimation of quantitative phosphate solubilization shown by endophytic actinomycetes in the range from 3.2 to 32.6 mg/100ml with the highest by *Streptomyces* sp. 34 (32.6 mg/ 100 ml), further followed by *Leifsoniaxyli* ranged 24 (31.5 mg/ 100 ml).

Table 4: Phosphate solubilization by actinomycete isolates after incubation of10 days

Isolates	Zone (cm)	Phosphate solubilization (mg/100ml)
Sm9	2.2	47.9 ± 0.1
Sd1	1.6	34.1 ± 0.1
Sc9	3.4	64.5 ± 0.2
Sd10	1.5	38.7 ± 0.1
Sh2	2.3	43.7 ± 0.05
Sc7	1.1	13.4 ± 0.1
Sc20	1.5	9.19 ± 0.1
Sd1	2.6	6.18 ± 0.1
Sc11	1.9	42.6 ± 0.2
Rm5	1.5	35.8 ± 0.06
Rm4	1.2	29.1 ± 0.06
Rm10	1.3	24.6 ± 0.1
Rxy	1.8	31.3 ± 0.2
Rm8	1.2	26.9 ± 0.1
Rb3	2.2	42.5 ± 0.1
Rm9	2.0	35.4 ± 0.1
Rc1	1.5	40.4 ± 0.1
Rd5	1.0	43.1 ± 0.1

Values indicate mean of three replicates**.**

4.4 Siderophore production

4.4.1 Qualitative estimation of siderophore

Among 90 isolates, fifteen isolates produced distinct orange halo on chrome-azurol S (CAS) plates indicating siderophore production (Table 5 and Plate 4). Diameter of halo varied from 1.0-4.2 cm on CAS agar plates, maximum being produced by isolate Sc9 (3.2cm) followed by Sh2 (2.7cm) and Rm9 (2.6 cm) and minimum being produced by Sc3 and Rd6 (1.2 cm). Our results are in accordance with Pornthip *et al* (2010) who

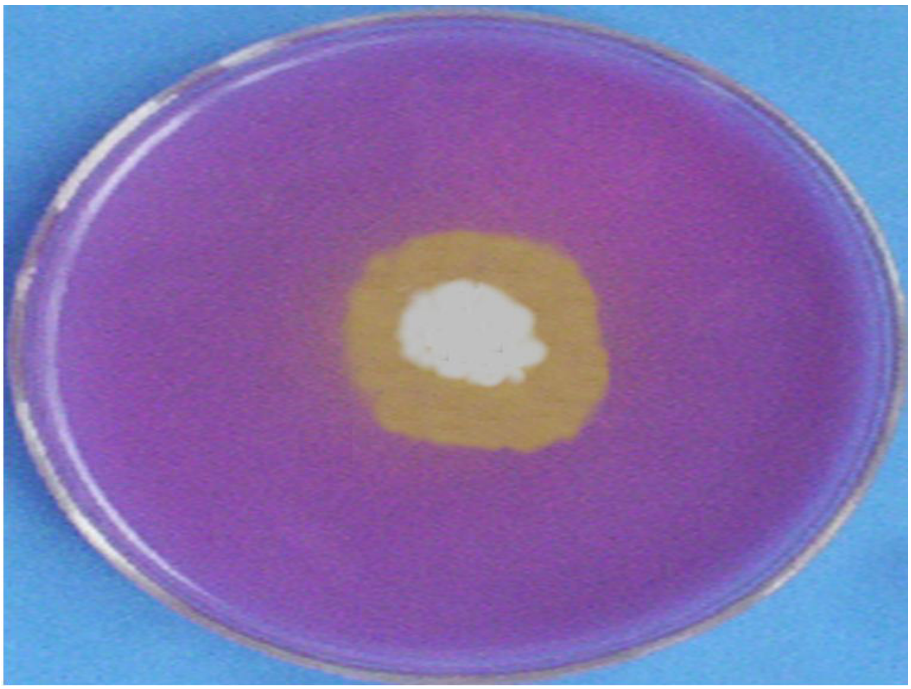


Plate 3: Phosphate solubilization by actinomycete isolate Sc9

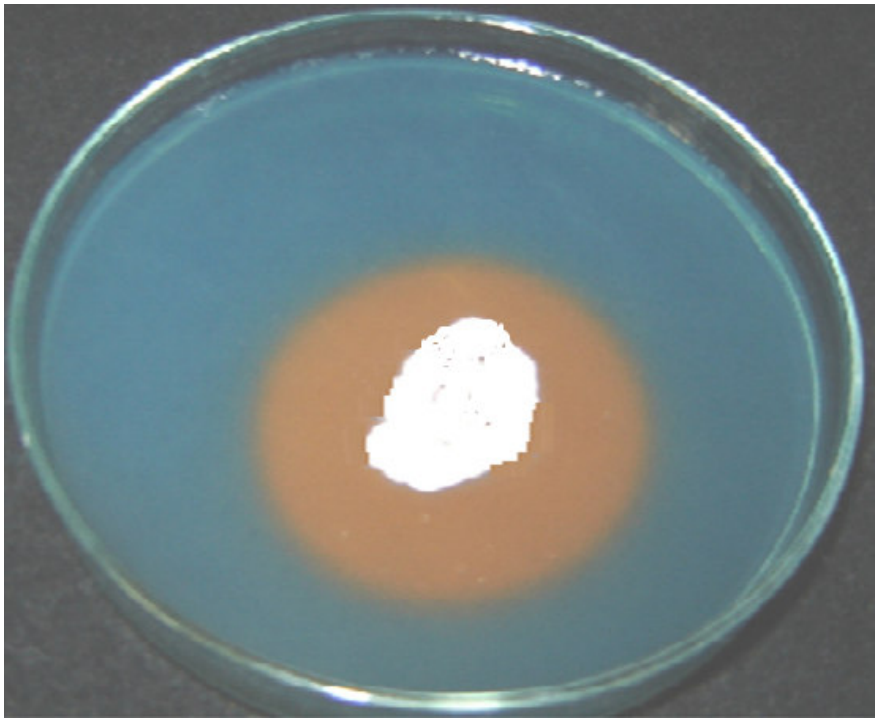


Plate 4: Siderophore production by actinomycete isolate Sc9

isolated 83 actinomycete isolates from soil of different plants. Thirty seven out of 83 isolates (44.5%) of rhizospheric actinomycete produced siderophore which was noticeable by orange zones around the colonies on the CAS agar plates.

4.4.2 Quantitative estimation of siderophore production

The siderophore producing isolates were further tested for detection of catechol and hydroxamate-type of siderophore. Twelve isolates were able to produce hydroxamate-type of siderophore ranging 5.5 to 39.4µg/ml and nine isolates produced catechol-type of siderophore ranging between 12.1-35.3µg/ml. Maximum hydroxamate-type of siderophore was produced by Sc9 (39.4µg/ml) followed by Sc16 (34.4µg/ml) and Sh2 (34.1µg/ml) and minimum was reported in Rc2 (5.5µg/ml). On the other hand, isolate Sc9 (35.3µg/ml) maximum catechol-type of siderophore production followed by Sc3 (34.8 µg/ml) and Sc12 (30.7µg/ml), while minimum yield was reported in Rb2 (9.5 µg/ml) (Table 5).

Table 5: Siderophore production by actinomycete isolates

Isolates	Zone(cm)	Hydroxamate (µg/ml)	Catechol (µg/ml)
Sc2	1.0	16.2 ± 0.1	-
Sc9	3.2	39.4 ± 0.1	35.3 ± 0.1
Sc12	1.9	-	30.7 ± 0.1
Sc3	1.2	18.9 ± 0.2	34.8 ± 0.1
Sb2	1.4	12.6 ± 0.2	-
Sh2	2.7	34.1 ± 0.1	13.9 ± 0.1
Sc20	2.1	-	18.1 ± 0.2
Sb3	1.9	16.2 ± 0.1	-
Sc16	1.5	34.4 ± 0.1	19.6 ± 0.1
Rm9	2.6	23.9 ± 0.05	-
Rb2	1.6	18.5 ± 0.1	12.1 ± 0.1
Rc2	1.3	5.5 ± 0.1	-
Rc7	2.4	11.6 ± 0.1	-
Rm3	1.4	-	16.4 ± 0.1
Rd6	1.2	19.6 ± 0.1	15.5 ± 0.2

Values indicate mean of three replicates**.**

Our results are in accordance with Nimnoi *et al* (2010) who observed that *Pseudonocardia halophobica* produced high amount for hydroxymate type of siderophore (39.30 µg/ ml). Kanchanadevi *et al* (2013) isolated six endophytic actinomycetes from agricultural crops and identified as *Propioni bacterium acnes, Nocardia sp* and

Micromonospora sp. All of these isolates produced siderophore in the range between 0.028µg/ml and 0.115µg/ml. Out of 40 endophytic actinomycetes from medicinal plants, 17 isolates produced catechol in the range from 2.68-51.6 µg/ml, while 22 isolates produced hydroxamate in the range from 8.71 to 144.21 µg/ml (Gangwar and Kataria, 2013). Gangwar *et al* (2014) isolated 60 endophytic actinomycetes from leaf, root and shoot of *Musa acuminata*. Thirty isolates were able to produce hydroxamate type of siderophore ranging 4.1±0.5 to 128.6±1.1µg/ml and 16 isolates were able to produce catechol type of siderophore ranging between 9.2±0.7 and 147.3±0.5 µg/ml.

4.5 Production of Gibberellic acid

Fifty out of 90 isolates were observed to produce the gibberellic acid. Out of which, 12 were from rhizosphere and 3 from roots of *Gladiolus.* Gibberellic acid production ranged from 12.5-50.6µg/ml. From these isolates, maximum gibberellic acid production was reported in Sc9 (50.6 µg/ml), followed by Sd10 (45.2µg/ml) and Sh2 (36.2µg/ml). Isolate Sc7 (8.1µg/ml) showed minimum gibberellic acid production as evident from Table 6. These results are supported by Solans (2011) who isolated 122 actinomycetes from *Ochetophila* plant. Among 122 isolates, maximum gibberellic acid production was reported in *Micromonospora sp.* (3.73µg/ml), followed by *Frankia sp.* (1.76µg/ml), *Actinoplanes sp.* (1.53µg/ml) and *Streptomyces sp.* (0.96µg/ml).

Table 6: Gibberellic acid production by actinomycete isolates

Isolates	**Gibberellic acid (µg/ml)**
Sb2	45.2 ± 0.1
Sc12	15.3 ± 0.1
Sd1	35.1 ± 0.05
Sh2	36.2 ± 0.05
Sd10	45.2 ± 0.06
Sm9	15.3 ± 0.05
Sc20	15.9 ± 0.2
Sc11	25.6 ± 0.2
Sc7	12.5 ± 0.1
Sb3	29.6 ± 0.1
Sc16	23.7 ± 0.1
Sc9	50.6 ± 0.1
Rm9	26.3 ± 0.2
Rm5	17.8 ± 0.1
Rm4	34.5 ± 0.05

Values indicate mean of three replicates**.**

4.6 Production of Hydrogen Cyanide and ACC deaminase activity

Production of HCN was assayed by change in color after incubation from yellow to light brown, moderate brown or reddish brown. Ten isolates was found to produce HCN (Table 7 and Plate 5). Out of which, Sc9, Sm6 and Rd9 were highest producers of HCN as evident from the reddish brown colour of filter paper. Five rhizospheric actinomycetes namely, Sc9, Sc16, Sb2, Sc20 and Sc7 showed ACC deaminase activity on DF medium (Table 7).

Table 7: HCN production and ACC deaminase activity of actinomycete isolates

Isolates	Zone of HCN	Growth on ACC
Sc9	+++	+++
Sh2	++	-
Sc10	+	-
Sb3	+	-
Sm6	+++	-
Sc16	++	+++
Sb2	+	+++
Sc7	-	+++
Sc20	-	+++
Rd9	+++	-
Rz1	++	-
Rc12	+	-

Values indicate mean of three replicates**.**

Our results are supported by Shete and Kapdnis (2012) who isolated 10 actinomycetes from soil. Out of these 10 isolates, only two isolates showed HCN production. Cruz and Paterno (2014) screened 59 actinomycete isolates for its growth promoting activities, fourteen out of which showed ACC deaminase activity.

4.7 Antagonistic activity of actinomycete isolates against *Fusarium oxysporum*

Out of 90 isolates, 12 (13.33%) displayed antagonistic activity against *Fusarium oxysporum*. Isolate Sc9 exhibited maximum percent inhibition of 81.2±0.2% against *F.oxysporum*, followed by Sh2 (72.7±0.1%) and Sc16 (69.4±0.1%). Rm9 showed minimum inhibitory activity of 50.9±0.1% (Table 8 and Plate 6).

Gangwar *et al* (2011) observed that 8 endophytic actinoycete isolates out 40, exhibited antagonistic cativity against nine phytopathogenic fungi i.e. *Alternaria brassicicola, Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Colletotrichum fulcatum, Fusarium oxysporum, Penicillium digitatum Penicillium pinophilum,* and *Phytophthora dresclea* in the range of 13.2 ± 0.2 to 71.4 ± 0.2.

Table 8: Antifungal activity (%inhibition) of actinomycete isolates against *Fusarium oxysporum*

Isolates	Percent inhibition
Sc9	81.2±0.2
Sh2	72.7±0.1
Sc20	63.5±0.2
Sc16	69.4 ±0.1
Sb2	53.5±0.5
Sc7	62.3±0.2
Sm4	51.2±0.2
Sc3	58.2±0.1
Rd5	63.5±0.4
Rm9	50.9±0.1
Rxy	65.8±0.3
Rb3	57.6±0.1

Average ± standard error from three replicates

Values indicate mean of three replicates**.**

Kamara and Gangwar (2015), out of the 39 rhizospheric isolates from *Catharanthus roseus* and *Withania somnifera*, 9 showed antifungal activity against *Alternaria alternata*, 19 against *Fusarium oxysporum*, 20 against *Helminthosporium oryzae*, 14 against *Macrophomina phaseolina*, 10 against *Penicillium* sp. and 16 against *Rhizoctonia solani.* These results concluded that actinomycetes from rhizospheric soil are the most promising candidates to be used as biocontrol agent against phytopathogenic fungi.

On the basis of plant growth promoting traits and antifungal activity isolate Sc9 was selected for green house study.

4.8 Effect of carbendazim on the growth of actinomycete Sc9

Carbendazim, a systemic benzimidazole fungicide, is applied repeatedly to control plant diseases including soil borne fungal diseases, over a growing season. The carbendazim conc. (50, 25, 10ppm) has no effect on the growth of actinomycete Sc9 (Plate 7). Yang *et al* (2010) observed the inhibition of carbendazim on growth of mycelium was stronger than on spore germination,but the inhibition of hymexazol on spore germination was stronger than on growth of mycelium. Hymexazol stimulated growth of melon,and had better effect on controlling melon wilt when applicated with carbendazim.The control effect of the mixture of carbendazim and hymexazol reached to 82.21%～86.67%,which was higher by 15.19% and 38.75% than single treatment with carbendazim and hymexazol,respectively.

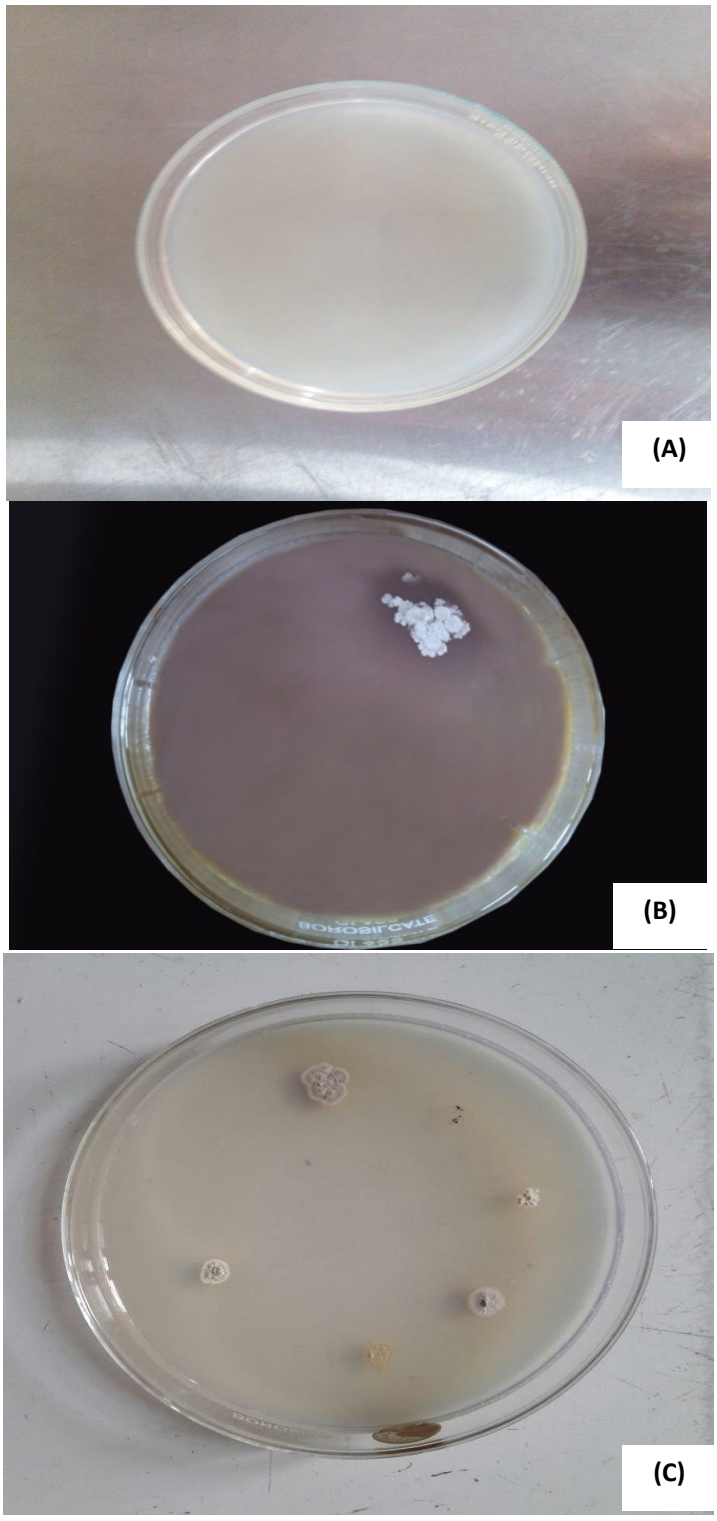


Plate 5: Growth on Dworkin and Foster (DF) minimal salt medium

A: DF medium containing neither Ammonium Sulphate nor ACC

B: DF medium containing Ammonium Sulphate

C: DF medium containing ACC

Plate 6: Antagonistic activity of actinomycete isolate against *Fusarium oxysporum*

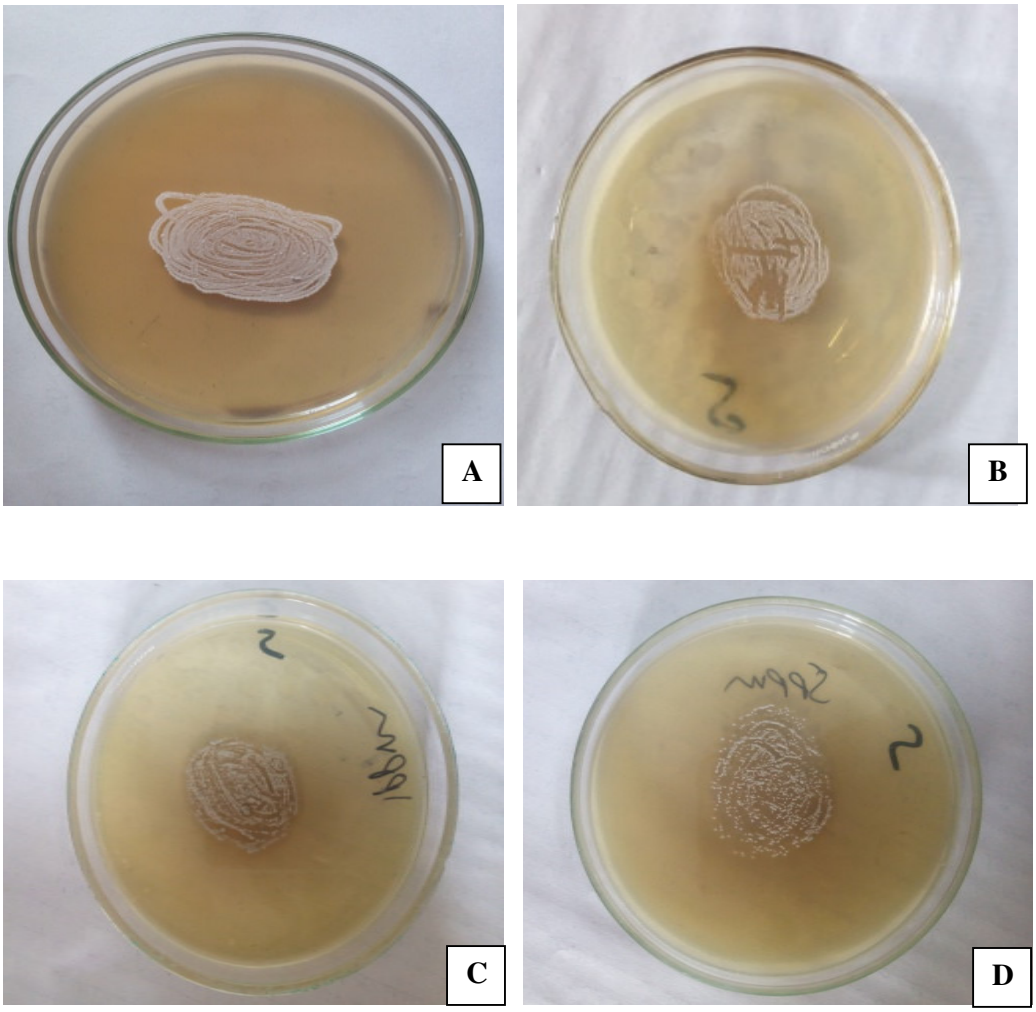


Plate 7: Effect of fungicide carbendazim on growth of actinomycete isolate *S. aureus*

[A] Control

[B] NA medium supplemented with 10ppm conc. of carbendazim

[C] NA medium supplemented with 25ppm conc. of carbendazim

[D] NA medium supplemented with 50ppm conc. of carbendazim

4.9 Scanning electron microscopic (SEM) studies of the antagonistic effect of potential actinomycete isolate Sc9 on fungal cell wall

Scanning electron microscopy was performed by co-culturing *Fusarium oxysporum* and Sc9 an isolate of *Gladiolus* rhizospheric soil. Scanning electron micrographs showed degradation of *Fusarium oxysporum* cell walls due to secretion of diffusible compounds by Sc9 as compared to control. The control plate of *Fusarium oxysporum* showed the presence of regular vegetative cells exhibiting smooth surface with overall intact morphology whereas fungal colony inoculated with Sc9 showed brittle, disrupted and damaged hyphae at the edges of the inhibited fungal colonies on the GYE plates (Figure 1). Tangum and Niamsup (2012) reported the breakage of the cell walls of *Fusarium oxysporum f.sp.lycopersici* mycelia growing towards *Streptomyces* sp. P4 as compared to control *Fusarium oxysporum*. The effect was investigated and compared with the control. He *et al* (2009) reported that endophytic bacteria obtained from *Epimedium brevicornu* degraded hypha of *Sclerotinia sclerotiorum* and the cytoplasm was extravagated outside from the fungal walls.

Many species of actinomycetes, particularly those belonging to the genus *Streptomyces,* are well known as antifungal agents that inhibit several plant pathogenic fungi (Khamna *et al,* 2009). The chitinolytic activity of *Streptomyces* sp. obtained from citrus and soybean plants showed high inhibition levels against fungi and the fungal hyphae exhibited a degraded appearance after chitinolytic A8 strain culture treatment. Therefore, there is an inhibitory role of chitinase to curb the growth of plant pathogenic fungi. The *C. sublineolum* hyphae surface-treated with A8 culture filtrate contained many holes, possibly corresponding to lysis zones. However, the hyphal surfaces of both *C.sublineolum* and *Pythium* sp. treated with A8 culture filtrate exhibited a slightly roughened surface, indicating little or no effect of hydrolytic enzymes on these structures (Quecine *et al,* 2008).

4.10 Presumptive identification of actinomycetes isolates

On the basis of PGP characteristic such as IAA, P-solubilization, siderophore production, gibberelllic acid, HCN and ACC deaminase activity, two (Sc9 and Sh2) best performing isolates were presumptively identified (Plate 8).

4.10.1 *Streptomyces aureus*: The colony of isolate Sc9 was white in color. In slide culture elaborate primary and secondary mycelium was observed. Spores were arranged in chains. The strain was Gram positive. The strain decomposed casein, starch, Tween-80, esculin, xanthine, hypoxanthine and tyrosine (Table 9).

4.10.2 *Micromonospora*: The colony of isolate Sh2 was white in color. In slide culture elaborate primary and secondary mycelium was observed. The single spore borne in dense clusters on repeatedly branched sporophores or are well dispersed throughout the mycelium. The strain was Gram positive. The strain decomposed casein, starch, Tween-80, esculin, xanthine, hypoxanthine and tyrosine (Table 9).

Table 9: Presumptive identification of the potential isolates of actinomycetes

Property	Sc9	Sh2
Colony color	White	White
Spore arrangement	Chain	Single
Spore color	Grey	White
Pigmentation	yellow	-
Genus	*Streptomyces aureus*	*Micromonospora*
Hydrolysis		
Casein	+	+
Starch	+	+
Decomposition		
Esculin	+	+
Tween-80	+	+
Xanthine	+	+
Hypoxanthine	+	+
Tyrosine	+	+

4.11 Evaluation of effectiveness of actinomycete isolates as potential plant growth promoters and antagonists against *Fusarium oxysporum* in *Gladiolus* under green house.

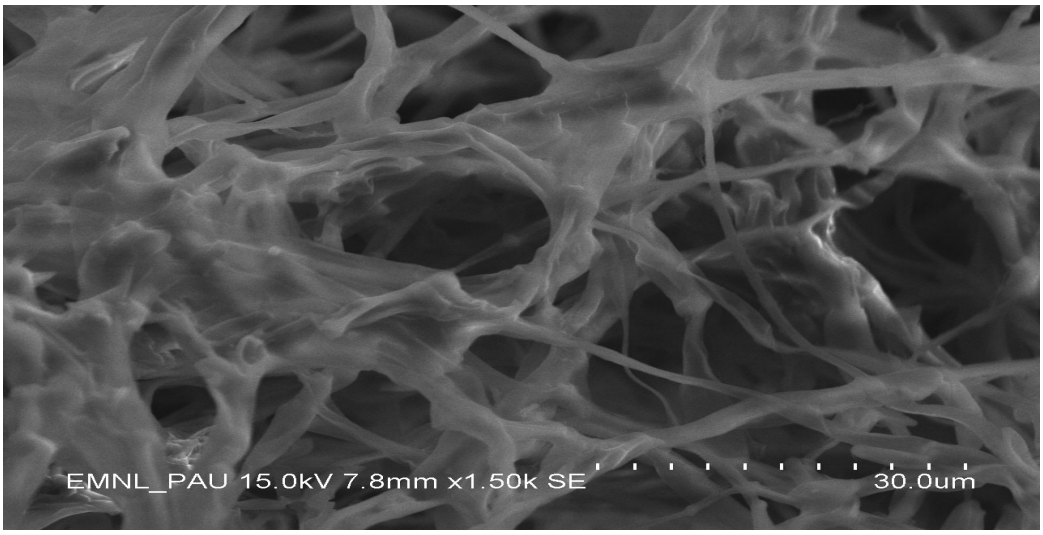
4.11.1 Corm Germination

The results in table 10 revealed that all the treatments were significantly different from each other. Maximum percent germination was observed in treatment T5 (NPK, 100%) and T6 (NPK + *S. aureus,* 100%) followed by T3 (*S. aureus* alone, 90 %) as compared to the treatment T4 (*S. aureus*+*Fusarium oxysporum*, 86%).

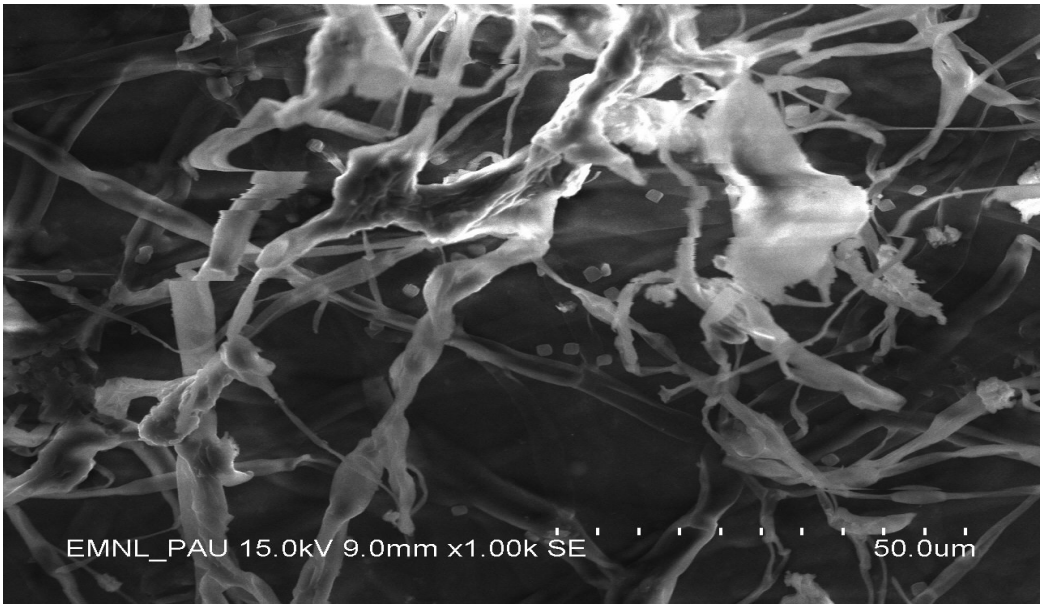
Table 10: Effect of actinomycete isolates on corm germination in green house

Treatments		Corm germination @ 10 DAS
Treatment 1	Control	60%
Treatment 2	*Fusarium oxysporum*	40%
Treatment 3	*S. aureus* (Sc9)	90%
Treatment 4	*S. aureus* +*Fusarium oxysporum*	86%
Treatment 5	NPK	100%
Treatment 6	NPK + *S. aureus*	100%
$p \geq 0.05$		1.85

Results are in accordance with Srividya *et al* (2012) who reported 100% germination index in treatment of the chilli seeds with co-inoculation of *Sreptomyces* isolate 9p. and also showed 75% reduction in seed mortality as compared to the seed treated with pathogen *Colletotrichum* alone. Similarly, Kamboj (2014) reported 86.66% germination in treatment of the musk melon seeds with co-inoculation of the actinomycete isolate O9 and the pathogen



(A)



(B)

Figure 1: ***Fusarium oxysporum*** **control (A),** ***F. oxysporum*** **infected with actinomycete isolate Sc9 (B)**

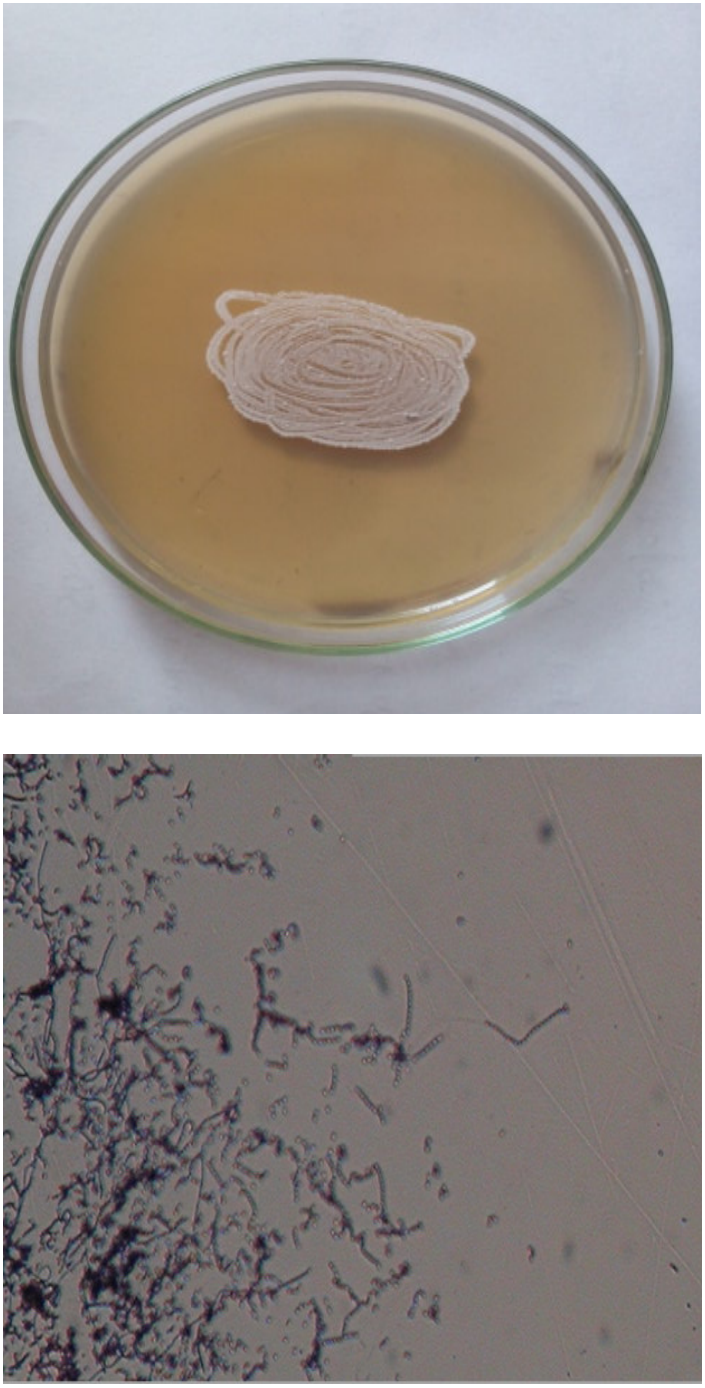


Plate 8: Morphological characterstic of actinomycete isolate *S. aureus* (Sc9)

F.oxysporum as compared to the treatment of the seed with *F.oxysporum* alone (66.66%).

4.11.2 Root length

Data on root length presented in Table 11 showed significant difference in all the treatments at 60 days. Maximum root enhancement was recorded in NPK + *S. aureus* (11.8 at 60 DAS), followed by *S. aureus* (10.5 cm plant^{-1} at 60 DAS) (Plate 9). Root length was measured to be less in treatment with *Fusarium oxysporum* (7.8 cm plant^{-1} at 60 DAS) as compared to *S. aureus* + *Fusarium oxysporum* (9.7 cm plant^{-1} at 60 DAS). Under greenhouse conditions in tomato, when *Streptomyces flavofuscus* was inoculated alone, it significantly enhanced the plant height and root volume (Kamal and Sharma 2014).

Table11: Effect of isolates on root length

Treatments	**Root length (cm plant^{-1})**	
	30 DAS	**60 DAS**
Control	2.0	6
Fusarium oxysporum	2.5	7.8
S. aureus (Sc9)	7.5	10.5
S. aureus +Fusarium oxysporum	4.5	9.7
NPK	3.8	9.5
NPK + *S. aureus* (Sc9)	4.2	11.8
p≥0.05	0.2	0.4

4.11.3 Shoot length

The results of shoot length were observed to be significantly different in all the treatments at 30 and 60 DAS (Table 12). The maximum shoot length was recorded in the treatments with NPK + *S. aureus* (82.5 cm plant^{-1} at 60 DAS). But shoot length in *S. aureus* + *Fusarium oxysporum* was recorded to be 63 cm plant^{-1} at 60 DAS. Ara *et al* (2012) reported plants treated with *Streptomyces* strain AS-2shown maximum plant height, weight and minimum disease index (13.9 cm, 3.8 and 0.58 g respectively) after 30 DAS.

Table12: Effect of isolates on shoot length

Treatments	**Shoot length (cm plant^{-1})**	
	30 DAS	**60 DAS**
Control	12.5	72
Fusarium oxysporum	12.5	66
S. aureus (Sc9)	44.1	70
S. aureus +Fusarium oxysporum	37.5	63
NPK	34.5	52.5
NPK + S. aureus	27.4	82.5
p≥0.05	0.5	1.2

4.11.4 Root and shoot fresh weight

Root fresh weight was observed to be significantly maximum in the treatments with *S. aureus* (6.62 gm plant^{-1}), *S. aureus* with *Fusarium oxysporum* (5.24gm plant^{-1}) when compared with *Fusarium oxysporum* (1.12gm plant^{-1}) (Table 13).

The results of shoot fresh weight were recorded to be significantly different in all the treatments (Table 14). The shoot fresh weight was observed maximum in NPK + *S. aureus* (22.2gm plant^{-1}) followed by *S. aureus* with *Fusarium oxysporum* (20.1gm plant^{-1}) as compared to treatment with *Fusarium oxysporum* (8.6gm plant^{-1}) (Table 13). The actinomycete antagonists considerably improved fresh and dry weight of shoot and root, length of root and shoot height of maize as compared to the control *Cephalosporium maydis*, a causal agent of late wilt disease of maize (El-Mehalawyi *et al* 2004).

Table 13: Effect of isolates on root and shoot fresh weight

Treatments	**Fresh root weight (gm plant^{-1})**	**Fresh shoot weight (gm plant^{-1})**
Control	0.17	9.5
Fusarium oxysporum	0.15	8.6
S. aureus (Sc9)	0.24	18.3
S. aureus +Fusarium oxysporum	0.21	20.1
NPK	0.19	17.9
NPK + S. aureus	1.77	22.2
p≥0.05	0.3	0.4

4.11.5 Root and shoot dry weight

The root dry weight was recorded in the treatments at 15 and 30 days were non-significantly different from each other (Table 14). Maximum was observed in NPK + *S. aureus* (0.8gm plant^{-1}). The root dry weight was more in the treatment with *S. aureus*+*Fusarium oxysporum* (0.6 gm plant^{-1}) as compared to treatment with *Fusarium oxysporum* (0.1gm plant^{-1}). Some actinomycete isolates significantly increased root and shoot dry weight. Such increases have been reported in cauliflower, wheat and potato. This phenomenon may be related to the production of growth regulators by actinomycetes (Kloepper 1993).

The maximum shoot dry weight was recorded in *S. aureus* (7.73gm plant^{-1}) and NPK + *S. aureus* (5.06gm plant^{-1}). The shoot dry weight was observed minimum in the treatment with *Fusarium oxysporum* (1.71gm plant^{-1}) as compared to *S. aureus* with *Fusarium oxysporum* (4.68gm plant^{-1}) (Table 14). Aly *et al* (2012) revealed that soil inoculation with *Azotobacter vinelandii* and *Streptomyces* sp. enhanced root depth, shoot length and dry

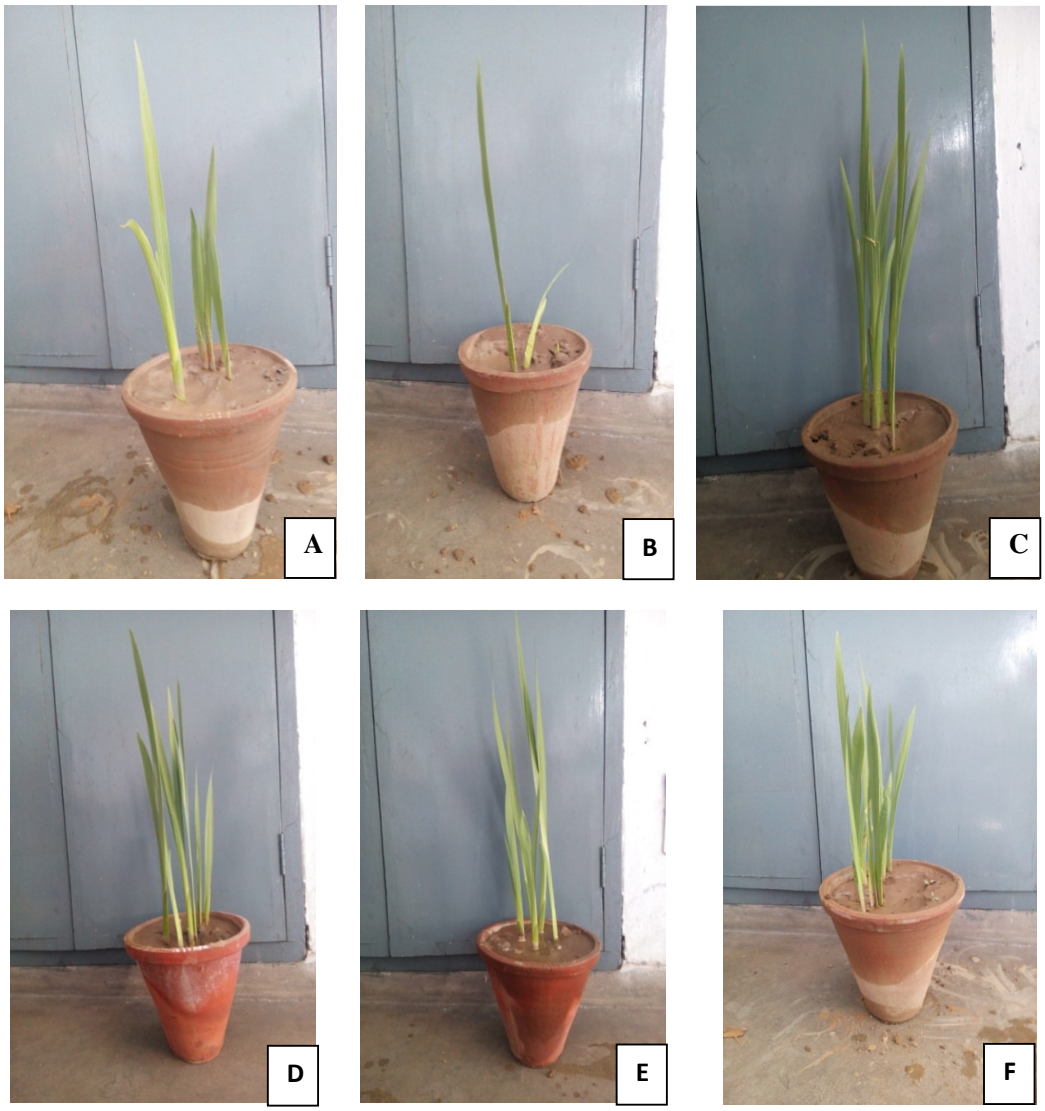


Plate 9: Effect of different treatments on shoot length of *Gladiolus* plant after 30 days

[A] Control

[B] Treated with *F. oxysporum*

[C] Treated with potential actinomycete isolate *S. aureus*

[D] Treated with recommended NPK

[E] Treated with recommended NPK + *S. aureus*

[F] Treated with *F. oxysporum* + *S. aureus*

weights of root and shoot.

Table 14: Effect of actinomycete isolates on root and shoot dry weight

Treatments	Dry root weight (gm plant^{-1})	Dry shoot weight (gm plant^{-1})
Control	0.1	1.82
Fusarium oxysporum	0.1	1.71
S. aureus (Sc9)	0.5	7.73
S. aureus +*Fusarium oxysporum*	0.6	4.68
NPK	0.4	3.17
NPK + *S. aureus*	0.8	5.06
p≥0.05	0.1	0.5

4.11.6 Incidence and Inhibition of disease development (%) in *Gladilous* plants

Maximum wilt incidence (%) was observed in treatment with *Fusarium oxysporum* (60%) (Table 15 and Plate 10 and 11). There was no wilting in the treatments with inoculation of *S. aureus*, NPK and NPK + *S. aureus*. Incidence of disease was reported in *S. aureus* with *Fusarium oxysporum* (26%). Maximum inhibition of disease was observed in *S. aureus* with *Fusarium oxysporum* (80%). Taechowisan *et al* (2003) isolated *Streptomyces aureofaciens* CMUc 130 from tissues of *Zingiber officinale* Rose (*Zingiberaceae*) which was showing antagonism against *Colletotrichum musae* and *Fusarium oxysporum*, the causative agents of anthracnose of banana and wilt of wheat. Cao *et al* (2005) reported that about 37.5% of *S. griseorubro violaceus* showed antifungal activity against *Fusarium oxysporum,* a proportion larger than other actinomycetes texa.

Table 15: Effect of different treatments on inhibition of disease development (%)

Treatments	Incidence of Disease (%)	Inhibition of disease (%)
Fusarium oxyporum	60	-
S. aureus+ *Fusarium oxyporum*	26	80
CD @ 5%	1.2	1.2

Streptomyces griseoviridis strain K61 and *Streptomyces lydicus* WYEC 108 were applied to control important plant pathogens such as *Fusarium oxysporum, Alternaria brassicicola* and *Botrytis cinerea* (Crawford *et al* 2005). Chamberlain and Crawford (1999) reported the antifungal activity of *Streptomyces hygroscopius* isolates, YCED9 and WYE53 against *Fusarium oxysporum* and *Rhizoctonia solani.*

Riaz *et al* (2007) reported 100% disease incidence and 20% plant mortality, with reductions in shoot and root biomass of 63 and 100%, respectively, when *Gladiolus*

grandiflorus corms grown in a pot culture system were inoculated with *F. oxysporum f. sp. gladioli*.

Five strains of *Streptomyces* sp. (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) isolated from herbal vermi-compost were reported as having potential for biocontrol of *Fusarium* wilt in chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and the application of *Streptomyces griseoviridis* (Mycostop) reduced the percentage of disease caused by *Fusarium oxysporum* f. sp. *radiciscucumerinum.* (Gopalakrishnan *et al* 2011).

Salma Z (2013) reported two bacterial strain for the control of *Fusarium oxysporum* in *Gladiolus* under green house conditions, *Bacillus* strain RSD8 recorded 11.1% disease incidence, whereas *Pseudomonas* strain HCS2 did not show any disease symptoms. Costa *et al* (2013) reported two *Streptomyces* isolates in cucumber (*Cucumis sativa* L.) for the control of *P. aphanidermatum* under greenhouse conditions. Isolate 16R3B was able to reduce 71% disease incidence and isolate 14F1D/2 reduced the disease incidence by 36%. Kanini *et al* (2013) reported the ability of *Streptomyces rochei* ACTAI55I to protect tomato seeds from the pathogenic effect of *Fusarium oxysporu*m. The results found under greenhouse conditions with the isolates *S. aureus* proved their potential as a biocontrol agents to damping off the Fusarium wilt caused by *Fusarium oxysporum* in *Gladiolus*.

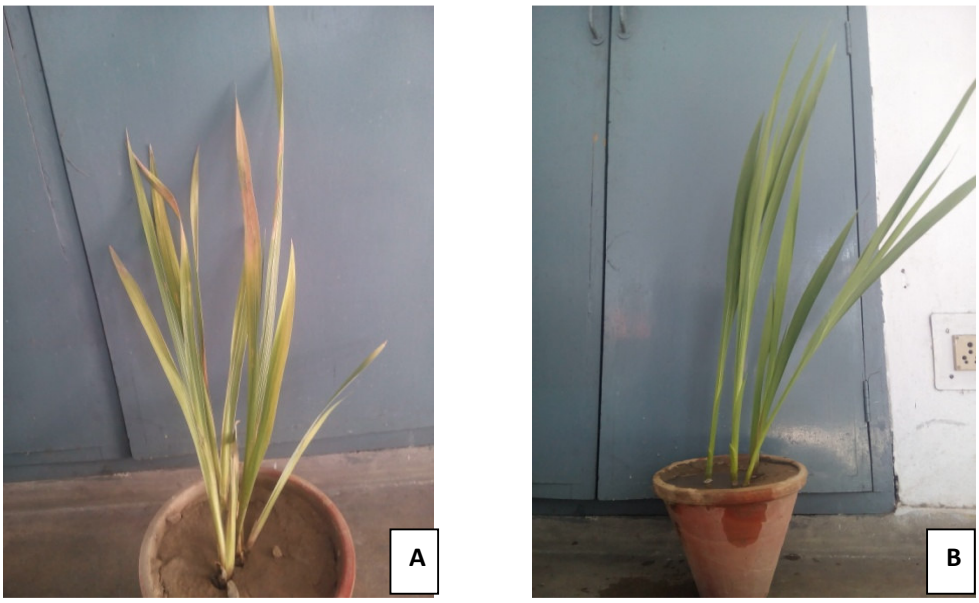


Plate 10: Wilting in different plants of *Gladiolus*
[A] Treated with *F. oxysporum*
[B] Treated with *F. oxysporum* + *S. aureus*

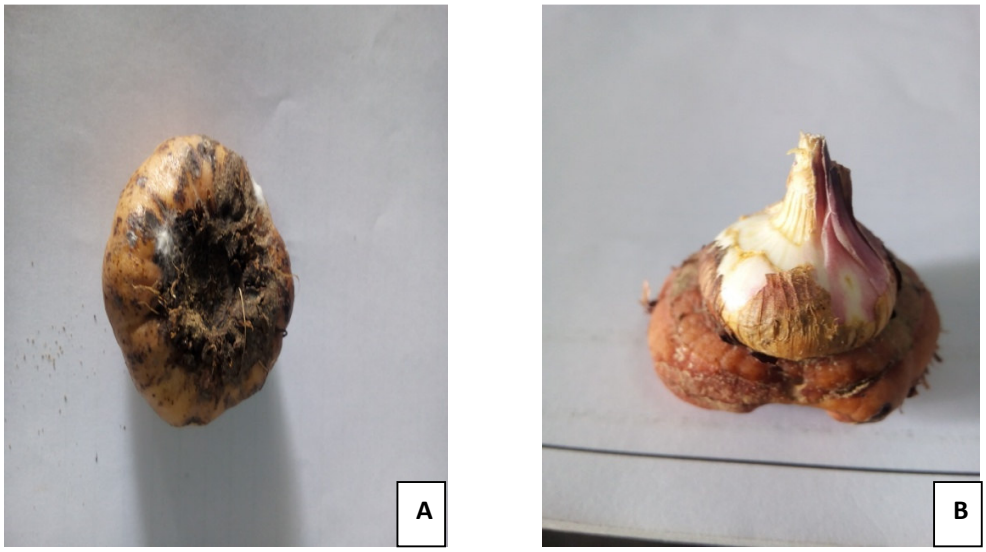


Plate 11: Effect of *Fusarium oxysporum* on corm of *Gladiolus*
[A] Treated with *F. oxysporum*
[B] Treated with *F. oxysporum* + *S. aureus*

CHAPTER V

SUMMARY

Actinomycetes represent a high proportion of the soil microbial biomass. Plant rhizospheric soils are a major habitat for actinomycetes. These actinomycetes also gain entry inside host tissues via wounds or openings hence termed as endophytic actinomycetes. Most actinomycetes in soil belong to the genus *Streptomyces* and have the potential to generate a diverse range of secondary metabolites. The association of actinomycetes with plants establishes many advantages such as the production of anti-microbials and extracellular enzymes which help in plant protection against abiotic and biotic stresses.

Gladiolus is a perennial flowering plant with medicinal values. Excessive use of chemical fertilizers is leading to diminishing reserve of high quality raw material and increasing energy costs. So actinomycete bio-inoculants can be used to reduce dependence on chemical fertilizer which would lead towards sustainable agriculture.

A total of 90 isolates were obtained from rhizospheric soil and roots of Gladiolus plants. Out of which 40 isolates were from rhizospheric soil and 50 from roots of Gladiolus plant. The population density of actinomycetes in rhizospheric soil was found to be 2.5×105 cfu gm-1.

Thirty isolates out of 90 were observed to produce the phytohormone IAA. From which 16 were from rhizospheric soil and 14 were from roots of Gladiolus. Indole acetic acid production ranged from 4.3-79.9 µg/ml. Maximum IAA production was reported in Sc9 (79.9 µg/ml), followed by Sb3 (68.9 µg/ml) and Sh2 (68.3 µg/ml) in root actinomycetes. Whereas IAA production without tryptophan was maximum in isolate Sb3 (27.4µg/ml), followed by Sc9 (19.6µg/ml) and Sh2 (17.5µg/ml).

Eighteen (20%) out of 90 isolates exhibited phosphate solubilization in the range of 6.1-64.5mg/100ml. The maximum amount of phosphate solubilization was shown by Sc9 (64.5mg/100ml), followed by Sm9 (47.9mg/100ml) and Sh2 (43.7mg/100ml) and minimum by Sd1 (6.1mg/100ml).

Twelve isolates were producing hydroxamate type of siderophore in the range of 5.5 to 39.4µg/ml and nine isolates produced catechol type of siderophore ranging between 12.1-35.3µg/ml. Maximum hydroxamate type of siderophore was produced by Sc9 (39.4µg/ml) followed by Sc16 (34.4µg/ml) and Sh2 (34.1µg/ml). On the other hand, maximum catechol type of siderophore production was exhibited by Sc9 (35.3µg/ml) followed by Sc3 (34.8 µg/ml) and Sc12 (30.7µg/ml).

Fifty out of 90 isolates were observed to produce the gibberellic acid. Gibberellic acid production ranged from 12.5-50.6 µg/ml. Maximum gibberellic acid production was

reported in Sc9 (50.6 µg/ml), followed by Sd10 (45.2µg/ml) and Sh2 (36.2µg/ml). Minimum gibberellic acid production was shown by Sc7 (8.1µg/ml).

Ten isolates were found to produce HCN. Out of which, Sc9, Sm6 and Rd9 were highest producers of HCN.Five rhizospheric actinomycetes namely, Sc9, Sc16, Sb2, Sc20 and Sc7 showed ACC deaminase activity.

Out of the 90 isolates obtained from roots and rhizospheric soil of Gladiolus plants, 12 were displaying antagonistic activity against *Fusarium oxysporum*. Isolate Sc9 exhibited maximum percent inhibition of 81.2±0.2% against *F. oxysporum*, followed by Sh2 (72.7+0.1%) and Sc16 (69.4+0.1%).

On the basis of plant growth promoting traits and antifungal activity,isolate Sc9 was selected for greenhouse studies. Isolates Sc9 was presumptively identified as *Streptomyces aureus* on the basis of morphological and biochemical characteristics.

The pot experiment on *Gladiolus* conducted in greenhouse revealed maximum percent germination with isolate NPK + *S. aureus* (100%), followed by *S. aureus* (90%) and minimum was recorded in treatment with *Fusarium oxysporum* (40%). Maximum enhancement in root length was recorded in NPK + *S. aureus* (11.8 at 60 DAS), followed by *S. aureus* (10.5 cm plant^{-1} at 60 DAS).as compared with *Fusarium oxysporum* (7.8 cm plant^{-1} at 60 DAS). Shoot length was recorded to be maximum in treatment with NPK + *S. aureus* (82.5 cm plant^{-1} at 60 DAS). Whereas it was found to be minimum with *Fusarium oxysporum* (85.6 cm plant^{-1} at 60 DAS).

Root fresh weight was observed to be more in the treatments of isolate *S. aureus* (6.62 gm plant^{-1}) and *S. aureus* with *Fusarium oxysporum* (5.24gm plant^{-1}) when compared with *Fusarium oxysporum* (1.12 gm plant^{-1}). Maximum shoot fresh weight was observed in isolate *S. aureus* with *Fusarium oxysporum* (15.6 gm plant^{-1}) and minimum in treatment with *Fusarium oxysporum* (4.9 gm plant^{-1}).

Maximum root dry weight was observed in NPK + *S. aureus* (0.8gm plant^{-1}) and *S. aureus* +*Fusarium oxysporum* (0.6 gm plant^{-1}) as compared to treatment with Fusarium *oxysporum* (0.1 gm plant^{-1}). Similarly, maximum shoot dry weight was recorded in isolate *S. aureus* (7.73gm plant^{-1}) and NPK + *S. aureus* (5.06gm plant^{-1}) as compared to treatment having *Fusarium oxysporum* (1.71 gm plant^{-1})

Maximum wilt incidence (%) was observed in the treatment with *Fusarium oxysporum* (60%). Incidence of disease was observed in *S. aureus* with *Fusarium oxysporum* (26%). Maximum inhibition of disease was observed in *S. aureus* with *Fusarium oxysporum* (80%).

Hence, from the present study, it is concluded that actinomycete strains can be considered for isolation of secondary metabolites which may be important for various

biocontrol applications. In the future, use of biocontrol agents such as actinomycetes will probably be one of the important tactics for plant disease management as they allow the reduced use of pesticides that are potential pollutants of the environment.

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APPENDIX-I

Media composition

Following media were used in the present study:

1. Nutrient agar (NA) gl^{-1}

Beef extract	3.0
Peptone	5.0
NaCl	5.0
Agar	20.0
pH	7.0

2. Glycerol agar gl^{-1}

Yeast extract	2.0
Glycerol	5.0 (ml)
Agar	20.0

3. Glucose yeast extract agar (GYE) gl^{-1}

Glucose	10
Yeast extract	5.0
Peptone	5.0
Agar	20.0
pH	6.8-7.2

4. National Botanical Research Institute Bromophenol Blue (NBRI-BPB) medium (Nautiyal 1999) gl^{-1}

Glucose	10.0
Tricalcium phosphate	5.0
$MgCl_2$	5.0
$MgSO_4$	0.25
KCl	0.2
Ammonium sulphate	0.1
Bromophenol blue	0.025
Agar	20.0

5.	Luria broth	gl^{-1}
	Tryptone	10
	Yeast extract	5.0
	NaCl	10
	pH	6.8-7.0
6.	Pikovskya broth	gl^{-1}
	Glucose	10g
	$Ca_3\ PO_4$	5.0
	$(NH_4)2SO_4$	0.5
	NaCl	0.2
	$MgSO_4\ .2\ H_2O$	0.1
	KCl	0.2
	Yeast extract	0.5
	$MnSO_4$	0.001
	$FeSO_4$	0.001
	pH	6.8-7.2
7.	Dworkin and Foster medium (DF) gl^{-1}	
	KH_2PO_4	1.36
	NH_2PO_4	2.73
	$MGSO_4.7H_2O$	0.2
	$CaCl_2.\ 2H_2O$	0.7
	$FeSO_4.\ 7H_2O$	0.2
	$CuSO_4.\ 5\ H_2O$	0.04
	$MnSO_4.\ H_2O$	0.02
	$ZnSO_4$	0.02
	$H_3\ BO_4$	0.003
	$CaCl_2$	0.007
	$Na_2\ MoO_4$	0.004
	Glucose	10
	Ammonium - Sulphate	2
	ACC	3 mM
	pH	7±2

8.	Starch Casein agar (SCA)	gl^{-1}
	Starch	10
	Casein	0.3
	NaCl	2.0
	K_2HPO_4	2.0
	$CaCO_3$	0.002
	$MgSO_4$. 7 H_2O	0.05
	$FeSO_4.7H_2O$	0.01
9.	Yeast malt extract agar (YMEA)	gl^{-1}
	Yeast extract	4.0
	Malt extract	10.0
	Dextrose	4.0
	pH	6.8-7.2

APPENDIX II

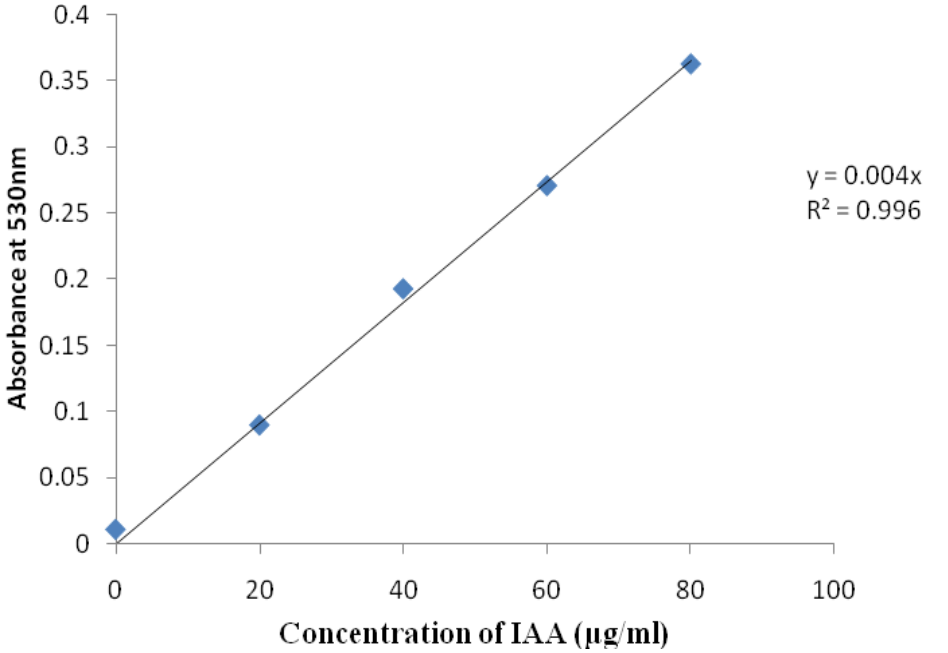

Fig 1: Standard curve for Indole acetic acid estimation

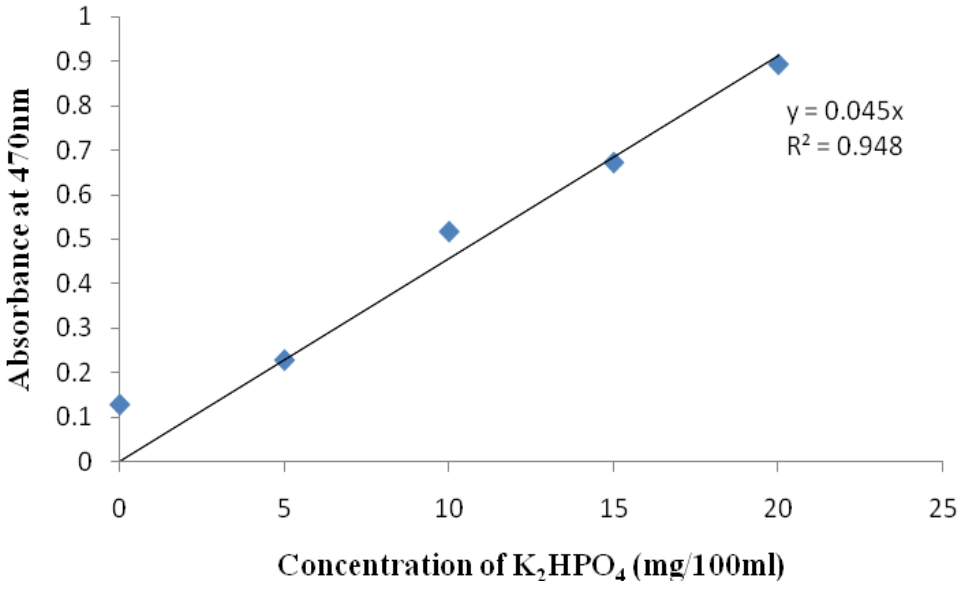

Fig 2: Standard curve for Phosphate estimation

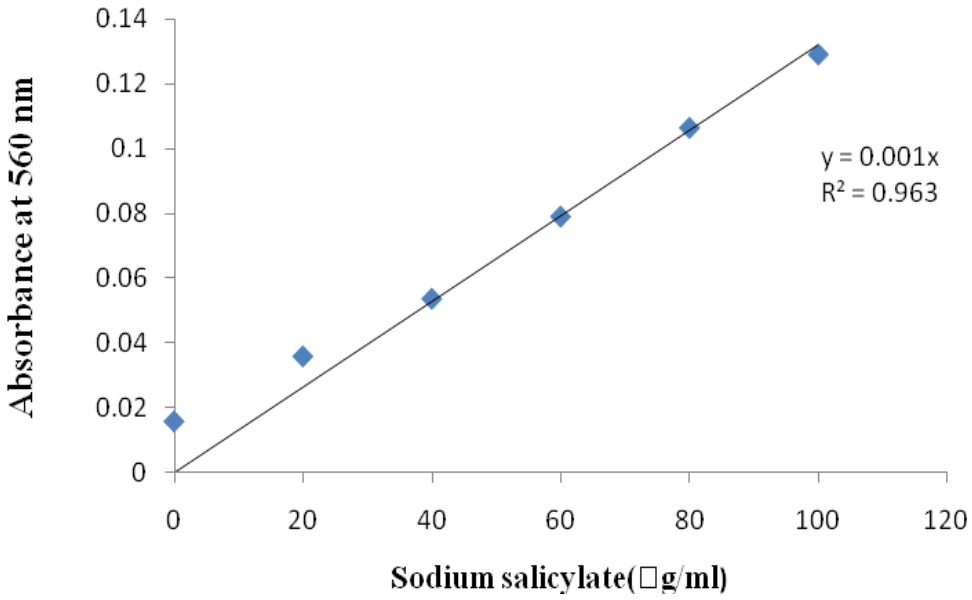

Fig 3: Standard curve for catechol type siderophore estimation

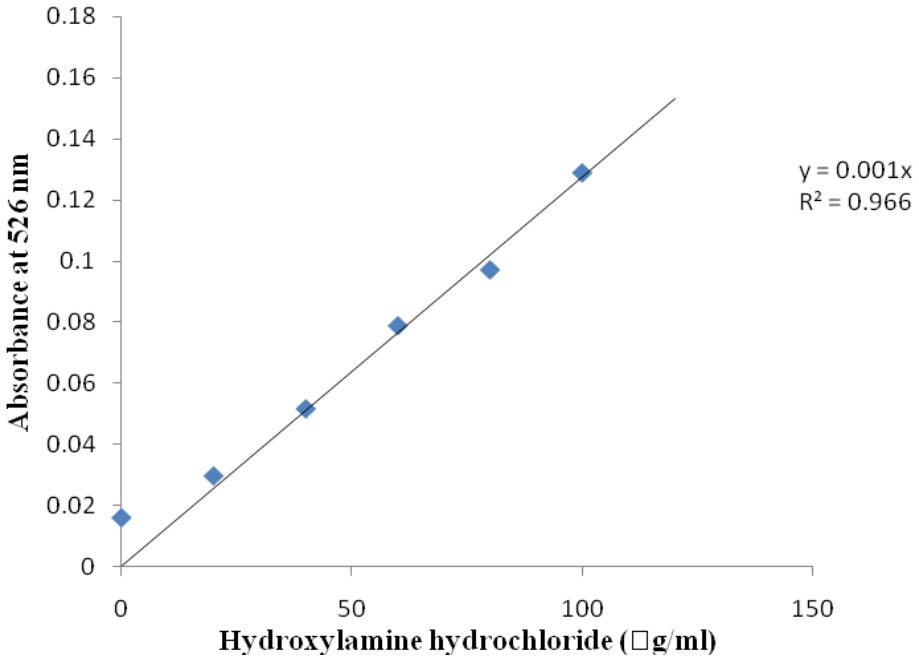

Fig 4: Standard curve for Hydroxamate siderophore estimation

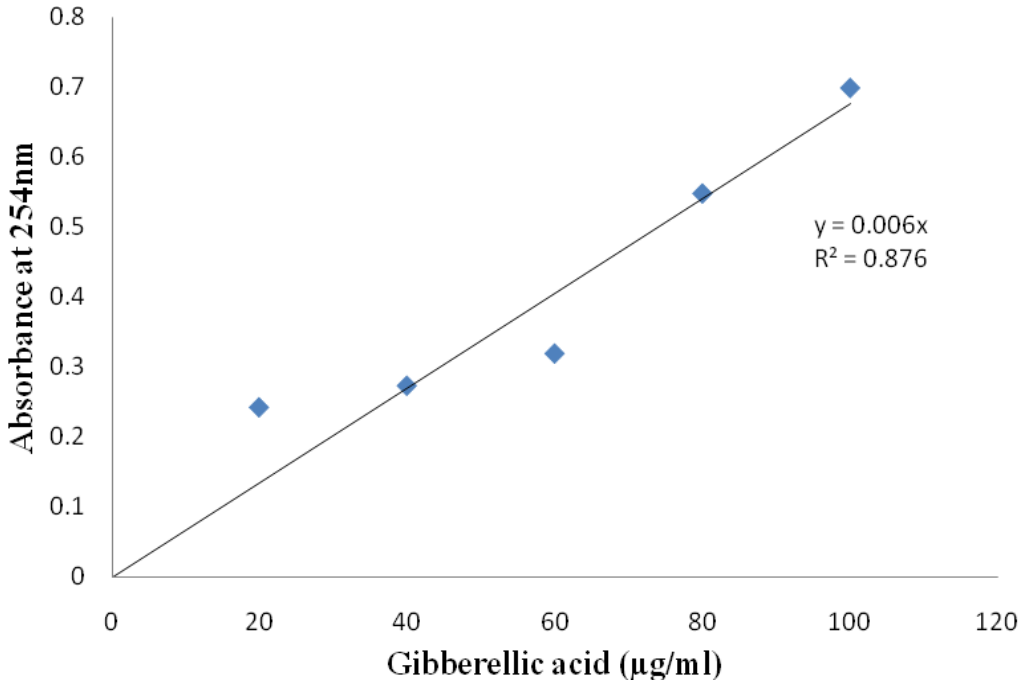

Fig 5: Standard curve for Gibberellic acid production

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