EVALUATION OF FLUORESCENT PSEUDOMONADS FOR THE MANAGEMENT OF SHEATH ROT OF RICE

SUNDARAMOORTHY.M

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Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

DECLARATION

I hereby declare that this thesis entitled **"Evaluation of fluorescent pseudomonads for the management of sheath rot of rice"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,

20.11.04

SUNDARAMOORTHY.M
(2000-11-32)

CERTIFICATE

Certified that this thesis entitled **"Evaluation of fluorescent pseudomonads for the management of sheath rot of rice"** is a record of research work done independently by Mr. Sundaramoorthy, M. (2000-11-32) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Vellayani
22.11.04

Dr. K. K.SULOCHANA
(Chairman, Advisory Committee)
Associate Professor
Department of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram

APPROVED BY

Chairman

Dr. K.K.SULOCHANA
(Chairman, Advisory Committee)
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram

Members:

Dr. B. RAJAGOPALAN
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram

Dr. KAMALA NAYAR
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram

Dr. B. RANI
Assistant Professor
Soil Science and Agricultural Chemistry,
Cropping Systems Research Centre,
Karamana, Thiruvananthapuram

.

EXTERNAL EXAMINER

Dr. A CHANDRASHEKARAN
Professor, Plant Pathology,
TamilNadu Agriculture University,
Coimbatore.

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LIST OF ABBREVIATIONS

%	per cent
@	At the rate of
µl	Micro litre
µm	Micro metre
^{0}C	Degree Celsius
DAS	Days after sowing
CD	Critical Difference
cm	Centimetre
CRD	Completely Randomised Design
et al	And others
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
i.e.	that is
kg	Kilogram
kg/ha	Kilo gram per hectare
l	Litre
DAT	Days after transplanting
min	Minutes
ml	Milli litre
mm	Milli metre
rpm	Rotations per minute
sp.	Species
var.	Variety
viz	Namely

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of over half of the world's population. It provides income for millions of rice producers, processors and traders. Naturally there is a consistent demand for higher yields of rice grains through breeding and genetic manipulations, judicious use of fertilizers and water and control of pest and diseases.

Several diseases notably, sheath rot caused by *Sarocladium oryzae*, can cause serious losses of grain yield. A high degree of control of these diseases is possible by planting disease resistant varieties. But, only in few cases breeders have been successful in breeding cultivars with disease resistance that is durable. Even though chemical control is quite effective against this disease, their consistent use on a regular basis is undesirable from economic and environmental point of view. Another important point to be considered is the possible emergence of fungicide resistant pathogens. These considerations have prompted a search for biocontrol agents effective against this pathogen.

The discovery of plant growth promoting rhizobacteria (PGPR) had renewed the hopes of developing effective biological control systems, which would be ecology conscious, environment friendly and cost effective. Of the various antagonistic microbes so for reported, fluorescent pseudomonad and *Bacillus* spp. appear most promising. Various studies have clearly demonstrated the possibilities of using bacterial antagonists to control plant diseases.

In the present study fluorescent pseudomonad was chosen, since it has long reigned to be the foremost among several biocontrol agents particularly against rice pathogens because of their capacity to survive

under anaerobic soil conditions, in which rice is normally grown and also on phyllosphere of the crop.

Native fluorescent pseudomonads has to be isolated and test their efficacy in the management of the disease. In Kerala, practically no work has been conducted on the use of fluorescent pseudomonad as a biocontrol agent against sheath rot pathogen. Hence the present study was under taken with the following objectives.

- Isolation of the causal organism associated with the sheath rot disease.
- Purification and establishing the pathogenicity of the organism.
- Isolation of native fluorescent pseudomonads from rhizosphere and phyllosphere.
- *In vitro* testing of fluorescent pseudomonads against the pathogen.
- Evaluation of efficacy of selected isolates under green house condition.
- Field evaluation of promising isolate.
- Testing the metabolite of the selected isolate.

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Review of Literature

2. REVIEW OF LITERATURE

Rhizobacteria that colonize roots, mostly fluorescent pseudomonads (*Pseudomonas fluorescens* and *Pseudomonas putida*) have emerged as organisms with great potential in biological control (Dube, 1995) and plant growth promotion (Burr *et al*., 1978; Suslow *et al*., 1979; Schroth and Hancock, 1982). Among the many potential bacterial antagonists associated with the plant roots, the fluorescent pseudomonads have received prominent attention due to their abundance in plant rhizosphere and their ability to colonize roots of a wide range of crop plants. Fluorescent pseudomonads are the most widely studied bacteria for their potential to protect plants against diseases and to promote plant growth (Cook *et al*., 1988). For the past three decades, numerous strains of fluorescent pseudomonads have been isolated from the soil and plant roots by several workers (Austin *et al*., 1977; Mew and Rosales, 1986; Rosales *et al*., 1993; Rabindran and Vidhyasekaran, 1996). This unique group comprising of bacteria which are predominantly rhizosphere inhabitants can suppress many plant diseases due to their general biological activities including competition for space and nutrients, production of antibiotics, volatile and antimicrobial substance and compounds such as iron chelating siderophores and HCN (Rosales *et al*., 1995; Anith *et al*., 1999; Dave and Dube, 2000; Mondal *et al*., 2000). Other mechanisms include production of hydrogen cyanide (Defago *et al*., 1990) and degradation of toxins (Borowitz *et al*., 1992). Moreover, fluorescent pseudomonads produce plant growth promoting substances such as auxin and gibberellins and enhance plant growth and yield. Hence, they are collectively called as plant growth promoting rhizobacteria (PGPR) (Dubeikovsky *et al*., 1993).

2.1 BIOCONTROL OF PLANT PATHOGENS

Fluorescent pseudomonads have long reigned to be the foremost among biocontrol agents as they improve plant growth by suppressing

either major or minor pathogens of plants (Cook and Rovira, 1976; Weller, 1988; Defago *et al*., 1990). Cook and Rovira (1976) reported that the take-all disease of wheat caused by *Guamannomyces graminis* var. *tritici* was suppressed by certain strains of fluorescent pseudomonads that were isolated from natural soil. Austin *et al*. (1977) reported that *Pseudomonas fluorescens* isolated from leaves of *Lolium perenne* were antagonistic to the pathogen *Drechslera dictyoides*. *Pseudomonas cepaciae* isolated from conidia of *Bipolaris maydis* obtained from infected corn leaves successfully controlled *Cercospora* leaf spot of peanut and *Alternaria* leaf spot of tobacco (Blakeman and Fokkema, 1982).

Treatment with certain strains of fluorescent pseudomonads showed enhanced resistance in sugar beet to the fungus *Pythium ultimum*, which causes pre and post emergence damping off (Osburn *et al*., 1983) and seedling infection in cotton (Gutterson *et al*., 1986). Unnamalai and Gnanamanickam (1984) reported *Pseudomonas fluorescens* antagonistic to *Xanthomonas citri* causing citrus canker. Sukumar and Ramalingam (1986) reported reduction in mulberry leaf spot caused by *Cercospora moricola* on foliar application of *Pseudomonas maltophila*.

A strain of *Pseudomonas fluorescens* CHAO, isolated from a Swiss soil naturally suppressive to black root rot, caused by *Thielaviopsis basicola*, suppressed the disease in iron sufficient soils (Stutz *et al*., 1986). Mew and Rosales (1986) reported reduction in incidence and lesion size of sheath blight caused by *Rhizoctonia solani* on spraying bacterial suspension over rice plant. Suppression of *Sclerotium rolfsii* in groundnut and *Rhizoctonia solani* in rice by the strains of fluorescent pseudomonads under *in vitro* and *in vivo* conditions was reported (Ganesan and Gnanamanickam, 1987; Vasanthadevi *et al*., 1989).

Biological control of *Rhizoctonia solani* and *Sclerotium rolfsii* (causing stem rot of peanut) was achieved through *Pseudomonas fluorescens* (Savitry and Gnanamanickam, 1987). Sivamani *et al*. (1987) obtained

strains of *Pseudomonas fluorescens* antagonistic to the bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). Bacterized rice plants showed a substantial reduction of 40-60 per cent of bacterial blight severity (Anuratha and Gnanamanickam, 1987).

A fluorescent pseudomonad designated LECL suppressed the leaf pathogens *Septoria tritici* and *Puccinia recondita* (Levy *et al*., 1988). Seed treatment of chick pea with two strains of *Pseudomonas fluorescens* suppressed seed rot and pre emergence damping off caused by *Pythium ultimum* (Kaiser *et al*., 1989). *Pseudomonas* spp. especially *Pseudomonas fluorescens* have been widely reported to exert biocontrol of plant pathogens through various mechanisms (Schippers *et al*., 1987; Defago *et al*., 1990;Thomashow and Weller, 1990 and Young *et al*., 1991) Siderophores produced by *Pseudomonas* species have been implicated in the biological control of damping off of cotton (Loper,1988; Laha *et al*., 1992), root rot of wheat (Becker and Cook, 1988) caused by *Pythium ultimum* and potato seed piece decay by *Erwinia carotovora* (Xu and Gross, 1986) , suppression of several vascular wilts caused by *Fusarium oxysporum* (Kloepper *et al*., 1980b; Scher and Baker, 1982; Sneh *et al*., 1984) and in growth responses of potato (Bakker *et al*.,1987). *Pseudomonas fluorescens* proved to be effective against “take-all” of wheat and a variety of other root disease (Defago *et al*., 1990). Soil application of fluorescent pseudomonad strain E113 isolated from tulip roots at a concentration of 10^8 cfu g^{-1} of dry soil suppressed the root rot pathogen *Pythium ultimum* and was considered to be more effective than applying the bacterium to the bulbs (Weststeijn, 1990). Bacterization with *Pseudomonas* spp. strain WCS 417r protected carnation from fusarial wilt (Van peer *et al*., 1991). *Pseudomonas* sp. isolated from healthy leaves of cluster beans (*Cyamopsis tetragonaloba*) was inhibitory to the growth of the pathogen, *Xanthomonas campestris* pv. *cyamopsidis* (Saini and Parashar, 1991).

Seed bacterization with rhizosphere pseudomonad in common bean (*Phaseolus vulgaris* cv. *botanica*) resulted in reduction of disease incidence caused by *Pseudomonas syringae* pv. *phaseolicola* (Alstrom, 1991). Gnanamanickam and Mew (1992) reported strains 4-15 and 7-14 of *Pseudomonas fluorescens* afforded 59 and 47 per cent sheath blight reduction in rice variety UPLR-5 that received three sprays of 500 ml m^{-2} with bacteria (10^8 cfu ml^{-1}) in addition to seed treatment.

Pseudomonas fluorescens isolated from the surface of healthy cocoa pods were antagonistic to *Phytophthora palmivora in vitro and* in the field and were more effective than cupric oxide or chlorothalonil in controlling black pod (Galindo, 1992). Capper and Higgins (1993) proved the usefulness of *Pseudomonas fluorescens* (2-79 and 13-79) in the biocontrol of take all disease of wheat (*Gaeumannomyces* graminis). Myatt *et al*. (1993) opined that *Pseudomonas cepacia* and *Pseudomonas fluorescens* were effective in inhibiting *Phytophthora megasperma* f sp. *medicaginis*, the incitant of root rot of chickpea both in laboratory and in field. Sarathchandra *et al*. (1993) reported the inhibition of *Phytophthora nicotianae* var. *nicotianae* and four other fungal pathogens by fluorescent pseudomonads. Hagedorn *et al*. (1993) achieved improved seedling stand in cotton through furrow application of *Pseudomonas fluorescens* at the rate of 14.1 ml m^{-1} due to the suppression of seedling diseases by antagonistic fluorescent pseudomonads. Tosi and Zazzerini (1994) reported significant reduction in safflower rust when *Pseudomonas fluorescens* was used for seed treatment and as soil drench. *Pseudomonas fluorescens* and *Pseudomonas lindbergii* isolated from the phylloplane of *Poa pratensis* inhibited growth of *Sclerotinia homoeocarpa* and *Bipolaris sarokinia*. *Pseudomonas cepaciae* UPR 5C, which was a potent antagonist of *Macrophomina phaseolina* inhibited the growth of other fungal pathogens of *Phaseolus vulgaris* (Sanchez *et al*., 1994).

Treatment with *Pseudomonas aureofaciens* PA 147-2 afforded protection to asparagus seedlings against *Phytophthora megasperma* var.

sojae as indicated by the reduced disease incidence and severity (Carruthers *et al*., 1995). Application of *Pseudomonas fluorescens* to pear plants followed by inoculation of *Stemphylium vesicarium* causing brown spot disease resulted in 57 per cent less disease incidence and 88 per cent less disease severity than untreated control (Montesinos *et al*., 1996). Rice plants could be protected against blast, brown spot and bacterial blight pathogens by the application of *Pseudomonas fluorescens* (Nayar, 1996).

Sarma *et al*. (1996) found that rhizosphere bacteria especially the fluorescent pseudomonads were effective in checking the growth of *Phytophthora capsici* and in suppressing the expression of foot rot symptoms in black pepper under controlled conditions. Fluorescent pseudomonads were known to induce resistance against many pathogens (M' Piga *et al*., 1997; Van wees *et al*., 1997). Murakami *et al*. (1997) demonstrated the effectiveness of *Pseudomonas fluorescens* HP-72 for the control of *Rhizoctonia solani* in bent grass. Sindhan *et al*. (1997) reported that the bacterial blight disease in rice was controlled by the foliar spray of *Pseudomonas acidovorus*. Van Loon (1998) has emphasized the combination of different treatments and different biological control agents for better disease suppression. Seed bacterization with *Pseudomonas chlororaphis* strain MA-342 suppressed the seed borne diseases caused by *Pyrenophora graminis* (Johnson *et al*., 1998).

Infection of *Pyrenophora tritici* on wheat seeds was controlled by seed applied bio protectant of *Pseudomonas putida* (Da Luz *et al*., 1998). Several pathogens of tomatoes were infected by fluorescent pseudomonad spp. (Varshney and Chaube, 1999).

Magabala, (1999) isolated strains of fluorescent pseudomonads antagonistic to *Xanthomonas axonopodis* pv. *phaseoli.* Smitha (2000) identified an isolate of fluorescent pseudomonads that can act effectively

against foliar blight (*Rhizoctonia solani*) disease of amaranthus. Ramamoorthy and Samiyappan (2001) reported an isolate of *Pseudomonas fluorescens* isolate Pf1 effectively inhibited the mycelial growth of *Colletotrichum capsici* causing fruit rot in chilli, and seed treatment plus soil application of Pf1 effectively reduced the disease under green house conditions. Gan *et al*. (2001) reported reduction of *Rhizoctonia* infection on cucumber and tomato on seed treatment with *Pseudomonas aeruginosa* strain CR-54. *Pseudomonas* Plb-29, a resident phylloplane bacterium could be used as a potential biocontrol agent for the management of bacterial blight of cotton and it also inhibited the growth of *Xanthomonas campestris* pv. *malvacearum in vitro* (Saha *et al*., 2001). Kandan *et al*. (2002) reported *Pseudomonas fluorescens* (two native strains and one collection strain and their strain mixtures in all possible combinations) when applied as seed treatment, seedling root dip and foliar application significantly reduced the tomato spotted wilt virus (TSWV) disease in tomato. Heera (2002) reported successful biocontrol of sheath blight and bacterial blight of rice using the fluorescent pseudomonads isolated from the phylloplane and rhizosphere of healthy rice plants

2.2 GROWTH PROMOTING ACTIVITY OF FLUORESCENT PSEUDOMONADS

Fluorescent pseudomonads have emerged as the largest and potentially most promising group of plant growth promoting rhizobacteria for biocontrol of plant diseases (Kloepper and Schroth, 1978). Kloepper *et al*. (1980a) attributed the enhancement of plant growth to the yellow green fluorescent siderophores produced by fluorescent pseudomonads. Several fluorescent pseudomonads and bacilli have been used as seed or root inoculants for higher yield of crops like potato (Burr *et al*., 1978; Kloepper *et al*., 1980b), sugarbeet (Suslow and

Schroth, 1982), wheat (Brown.1974) and some other crops (Cooper, 1959; Kloepper and Schroth 1981; Yuen and Schroth, 1986).

Seed and root inoculation of rhizobacteria promoted plant growth by producing phytohormones like auxin and gibberellins (Loper and Schroth, 1986). Disease suppression by fluorescent pseudomonads depends mainly on competition for nutrients and space (Elad and Baker, 1985), its ability to colonize rhizosphere (Scher *et al*., 1988), to produce antibiotics viz., pyrolnitrin, pyoverdine, 2, 4- diacetyl phloroglucinol (Pierson and Thomashow, 1992; Maurhofer *et al*., 1995; Hammer *et al.,* 1995; Gardener *et al*., 2000), siderophores (fluorescent yellow green pigment) *viz*., *Pseudobactin* which chelates the iron thereby limiting the availability of iron necessary for the growth of pathogens (Loper, 1988), and lytic enzymes such as chitinases and β-1,3 glucanases which degrade chitin and glucan respectively, (Sikora,1992; Fridlender *et al*.,1993; Broadway *et al*., 1998; Velazhahan *et al*., 1999). In addition, production of hydrogen cyanide which degrade the toxin produced by the pathogen (Voisard *et al*., 1989; Borowitz *et al*., 1992; Duffy and Defago, 1997) and salicylic acid (Maurhofer *et al*., 1998) have also been involved in the suppression of soil borne pathogens.

The introduced root colonizer, besides colonizing the root surface, must also survive in rhizosphere soil and compete with the resident flora (Weller, 1988). Podile and Dube (1988) obtained reduction in the infection of stem rot pathogens of pea nut, *Rhizoctonia solani* and *Sclerotium rolfsii*, when an isolate PN-3, isolated from pea nut rhizosphere was applied as seed coating during pot culture experiments. In field plots, rice varieties IR 50 and TKM 9 raised from bacteria treated seeds had 65-79 per cent less sheath blight than from untreated seeds (Vasanthadevi *et al*., 1989). Kaiser *et al*. (1989) reported increased emergence and yield of chickpea in soils naturally infected with *Pythium ultimum* when fluorescent pseudomonads were applied as seed treatment. Voisard *et al*. (1989) reported the contribution of HCN by *Pseudomonas fluorescens*

strain CHAO to the suppression of black root-rot of tobacco caused by *Thielaviopsis basicola*. Dube and Podile (1989) reported that a fluorescent pseudomonad protected peanut seedlings from *Rhizoctonia solani* and *Sclerotium rolfsii*.The disease incidence was reduced by 72 per cent and 50 per cent respectively when this strain was used as seed inoculant.

Development of rice blast decreased by 47 per cent in *Pseudomonas* treated rice seedlings of cv. UPLR i – 5 (Lee *et al*., 1990). *Pseudomonas* strains increased the biocontrol of black root rot as well as against the leaf pathogens, *Septoria tritici* and *Puccinia graminis* in wheat (Voisard *et al*., 1989; Flaishman *et al*., 1996). Gnanamanickam and Mew (1990) found that the efficient *Pseudomonas* strains produced two antiblast substances (antibiotic), which inhibited the conidial germination (70-100 per cent) of *Pyricularia oryzae* at 1.0 ppm concentration. Lim *et al*. (1991) reported that biocontrol of root rot caused by *Fusarium solani* by the bacterial antagonist *Pseudomonas stutzeri* was mainly by the production of the enzymes laminarinase and chitinase. Application of fluorescent pseudomonads as seed treatment alone or as seed treatment plus soil application has induced systemic resistance in cucumber plants against *Colletotrichum orbiculare* inciting anthracnose disease and resulted in reduction of disease incidence under field conditions (Wei *et al*., 1991; 1996).

Howie and Suslow (1991) reported a 70 per cent reduction of *Pythium* infection in cotton and about 50 per cent increase in emergence of seedlings by an antibiotic producing *Pseudomonas fluorescens*. *Pseudomonas fluorescens* increased the plant growth of rice and cotton by 25 and 40 per cent respectively when the bacterium was applied to seeds (Lin *et al*., 1992). The germination of maize seeds increased significantly by 30-60 per cent on treating seeds with plant growth promoting strains of *Pseudomonas aeruginosa* TNSK2 and *Pseudomonas fluorescens* ANP 15 was obtained by Hofte *et al*. (1991). Bacterization of chickpea and

soybean seeds with a siderophore producing fluorescent pseudomonad RBT 13 resulted in increased seed germination, growth and yield and reduced the number of wilted pea plants in wilt sick soil by 52 per cent (Dileepkumar and Dube, 1992). Gnanamanickam and Mew (1992) obtained slight increase in grain yield due to seed treatment with strains of *Pseudomonas fluorescens*. Seed treatment of rice seeds with fluorescent pseudomonad spp. reduced the intensity of sheath blight and promoted seedling growth (Lin *et al*., 1992). Gamaliel and Katan (1993) reported increased growth of tomato plants in non-solarized soils as a result of root colonization by fluorescent pseudomonads and inhibition of *Penicillium pinophilum*, a minor pathogen. Enhanced accumulation of chitinase was observed in tobacco and bean leaves in response to *Pseudomonas* spp. inoculation to roots (Zdor and Anderson, 1992; Maurhofer *et al*., 1994). Production of β-1, 3 glucanase by *Pseudomonas cepacia* was responsible for the reduction in number of plants infected by *Rhizoctonia solani, Sclerotium rolfsii* and *Pythium ultimum* (Fridlender *et al*., 1993). *Pseudomonas cepacia* strain UPR 5C, which was a potent antagonist of *Macrophomina phaseolina*, inhibited the growth of other fungal pathogens of *Phaseolus vulgaris* (Sanchez *et al*., 1994). Several biocontrol agents like *Pseudomonas* spp. belonging to plant growth promoting rhizobacteria (PGPR) has been shown to induce systemic resistance against several pathogens in plants (Maurhofer *et al*., 1994; M'Piga *et al*., 1997).

Increased chitinase activities in tobacco and in cucumber have been observed as a result of systemic resistance by fluorescent pseudomonads against *Pseudomonas syringae* pv. *tabaci* (Schneider and Ulrich, 1994). Nowak-Thompson *et al*. (1994) found that the antibiotic 2, 4-diacetyl phloroglucinol isolated from culture filtrates of *Pseudomonas fluorescens* inhibited the growth of phytopathogens *Pythium ultimum*, *Rhizoctonia solani* and *Erwinia carotovora* sub sp.*atroseptica*. Carruthers *et al.* (1995) obtained not only reduced root

infection by *Phytophthora megasperma* var. *sojae* in asparagus by inoculation with *Pseudomonas aureofaciens*, but also increased root length and plant weight in the presence of the pathogen. Leeman *et al*. (1995) obtained control of Fusarium wilt of radish by foliar application of *Pseudomonas fluorescens*. Seed treatment with *Pseudomonas fluorescens* strain WCS 417 has protected radish through induction of systemic resistance not only against the fungal root pathogen *Fusarium oxysporum* f.sp.*raphani*, but also against a virulent bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* and fungal leaf pathogens *Alternaria brassicola* and *Fusarium oxysporum* (Hoffland *et al*., 1996).

Montesinos *et al*. (1996) detected the antifungal activity of cell free culture filtrates of *Pseudomonas fluorescens* and *Erwinia herbicola* against *S.vesicarium*, the causal agent of brown spot of pear. *Fluorescent pseudomonas* spp. produce secondary metabolites with antibiotic activities like phenazine-1- carboxylic acid (PCA), 2,4 – diacetyl – phloroglucinol (DAPG), oomycin – A, pyocyanin, pyoluteorin and pyrrolnitrin were involved in suppression of soil – borne diseases (Thomashow and Weller, 1996). Many fluorescent pseudomonads are known to induce systemic resistance, which has been termed as induced systemic resistance (Pieterse *et al*., 1996) and in rice such antagonistic bacteria have been used against sheath blight (Mew and Rosales, 1986; Gnanamanickam *et al*., 1992; Krishnamoorthy and Gnanamanickam, 1997).

Pseudomonas aeruginosa strain 7 NSK 2 inoculated on the roots produced salicylic acid, which induced systemic resistance against *Botrytis cinerea* on beans (De Meyer and Hofte, 1997). Use of chitinase producing *Streptomyces* spp. and *Bacillus cereus* isolates used in combination with antibiotic producing *Pseudomonas fluorescens* and *Burkholderia* (*Pseudomonas*) *cepacia* isolates have shown a synergistic effect on the suppression of rice sheath blight incited by *Rhizoctonia solani* (Sung and Chung, 1997). Increased efficacy and enhanced biocontrol consistency against multiple pathogens can be achieved by

applying a combination of PGPR strains (Raupach and Kloepper, 1998). Fluorescent pseudomonads activate ISR against various fungal, bacterial and viral diseases (Liu *et al*., 1995 a, b; Maurhofer *et al*., 1998). Seed bacterization of chickpea, aubergine, soyabean and tomato with fluorescent pseudomonads isolated from rhizosphere of crop plants showed increased seed germination, shoot height, root length, fresh weight, dry weight and yield (Kumar, 1998). Epiphytic bacteria (*Pseudomonas vividiflava, Pseudomonas fluorescens*, *Pseudomonas putida*) produced high amount of IAA on leaf surface of pear and reduced severity of fruit russet (Lindow *et al*., 1998). Enzyme Linked Immunosorbent Assay (ELISA) studies indicated that *Pseudomonas* strains treatment restricted pathogenic fungal colonization when it was challenge inoculated (Viswanathan *et al*., 1998). Fluorescent pseudomonads have received much attention and have been used in many field and glass house tests due to the production of variety of secondary metabolites (Mathre *et al*., 1999) that suppress the pathogens directly or indirectly. Viswanathan and Samiyappan (1999) reported enhanced sett germination, tillering and further growth of the cane on sett treatment followed by soil application of PGPR belonging to fluorescent pseudomonads. Izhar *et al*. (1999) observed that seed treatment with *Pseudomonas fluorescens* enhanced the growth of cotton and reduced the infection by *Rhizoctonia solani*. Srivastava *et al*. (1999) examined two promising growth promoting strains GRP 3 (rhizoplane of soybean) and PRS 9 (rhizosphere of peas) of fluorescent pseudomonad, which promoted wheat growth in terms of root-shoot length and weight compared to uninoculated control. Rangeshwaran and Prasad (2000) reported increased growth in chickpea seeds treated with *Pseudomonas fluorescens*. Mishra and Sinha (2000) observed plant growth promoting activity of bacterial agents that improved rice seed germination and growth.

In addition to direct antagonism against pathogen and plant growth promotion, induced systemic resistance (ISR) by enhancement of

plant defense capacity by fluorescent pseudomonads is a new strategy for plant disease management (Ramamoorthy *et al*., 2001). Co-inoculation of the fluorescent pseudomonads and *Rhizobium leguminosarum* improved plant growth in terms of shoot height, root length and dry weight and also found to exhibit a wide range of antifungal activity against pathogens specific to pea (Kumar *et al*., 2001). Urashima and Hori (2003) recently studied plant growth promoting activity of several fluorescent pseudomonads using the small scale hydroponic culture bioassay system and reported 50 per cent increase in elongation of the spinach roots.

2.3 BIOCONTROL OF SAROCLADIUM

Sakthivel and Gnanamanickam (1986 a, b) reported that strains of *Pseudomonas fluorescens* isolated from citrus leaves and rice roots, were inhibitory to *Sarocladium oryzae* under *in vitro* conditions and produced 54 per cent reduction in lesion length when treated on IR 20 rice plants. Bacterization with *Pseudomonas fluorescens* resulted in the effective biocontrol of sheath rot of rice and stem rot of groundnut and enhanced plant growth and yield (Sakthivel *et al*., 1986, 1988). Sakthivel and Gnanamanickam (1987) isolated *Pseudomonas fluorescens* strains active against the sheath rot pathogen *Sarocladium oryzae* and evaluated them in green house and field experiments for disease suppression and grain yield in rice. Suparayono (1990) showed that the presence of antagonistic bacteria in the soil around rice plants or stubble inhibited the mycelial growth of sheath rot fungus. Eswaramurthy *et al*. (1995) and Radhika *et al*. (1995) reported effective control of *Sarocladium oryzae* on spraying *Pseudomonas fluorescens* and *Bacillus subtilis*. Pandiarajakumar, 1992 reported that pre-inoculation spraying of *Pseudomonas fluorescens* and *Bacillus* sp. significantly reduced the incidence of sheath rot over control. Radhika *et al.* (1995) screened seven isolates of *Pseudomonas fluorescens* (Pf1, Pf2, Pf18, Fp1, Fp6, Fp9and Fp11) against *Sarocladium oryzae* and

identified the isolate Pf2 and Fp1 exerted maximum inhibitory effect on the growth of pathogen. Ramanamma *et al*. (1994) isolated an antagonistic bacterium from the rice rhizosphere which produced an extra cellular yellow to green fluorescent pigment and showed large infection zone against *Sarocladium oryzae* and *Rhizoctonia solani*. Mini (1995) observed that indigenous strain of *Pseudomonas fluorescens* from Tamil Nadu had antagonistic effect on *Sarocladium oryzae* in rice and she also reported that species of *Chaetomium* could reduce the sheath rot pathogen under laboratory conditions. Mariyappan (1996) showed the antagonistic effect of *Trichoderma viride* and *Pseudomonas fluorescens* strains against sheath rot pathogen under *in vitro* condition. Krishnamurthy and Gnanamanickam (1998) assayed *Pseudomonas fluorescens* strain Pf 7-14 for antibiosis on PDA plates against rice pathogens *viz*., *Pyricularia oryzae* Sacc.,*Rhizoctonia solani* Kuhn., *Sarocladium oryzae* (Sawada) Gams and Hawksworth.

2.4 FORMULATION AND APPLICATION OF BACTERIAL ANTAGONIST

Cell suspension of the bacteria has been used for seed treatment, but the use of such suspension is impractical for large-scale field application (Mew and Rosales, 1986; Capper and Higgins, 1993). *Pseudomonas cepaciae* formulated as wettable powder effectively controlled pea nut leaf spot (Knudsen and Spurr, 1987) Seed treatment with liquid suspension of bacteria in the field experiment was unsatisfactory because it was difficult to apply suspension in the field and there was rapid loss of viability of bacteria in storage and to overcome this difficulty antagonistic bacteria were formulated with different carrier materials (Vidhyasekaran and Muthamilan, 1995). Different carrier formulations of the fluorescent pseudomonads have been developed by several workers (Kloepper and Schroth 1981; De freitas and Germida, 1992; Rabindran and Vidhyasekaran, 1996).

Capper and Higgins (1993) developed a peat based formulation of *Pseudomonas fluorescens* for the control of take-all disease of wheat. Good control of sheath blight was obtained when talc based formulation of fluorescent pseudomonads were applied as seed treatment, root bacterization and foliar spray (Rabindran, 1994; Muthamilan; 1994; Nayar, 1996). Muthamilan (1994) observed the reduction in the incidence of rice blast by seed treatment with peat based formulation of *Pseudomonas fluorescens* strain. Rabindran and Vidhyasekaran (1996) isolated *Pseudomonas fluorescens* from rhizosphere of different crops that inhibited the growth of *Rhizoctonia solani* and sheath blight pathogen and developed a peat based formulation of the most effective strain Pf ACR2 which is comparable to the commercially available fungicide carbendazim in efficacy. Samiyappan (1988) reported that *Pseudomonas fluorescens* strains grown on King B broth for 48 h mixed with peat soil at the rate of 50 ml broth 100 g^{-1} soil gave good control of black gram root rot when applied to soil. Talc based formulation of *Pseudomonas fluorescens* was prepared by mixing a 48 h old culture of bacteria grown in King's B broth with sterilized talc @ 400 ml kg^{-1} along with five gram of sticker carboxy methyl cellulose (CMC) (Vidhyasekaran and Muthamilan, 1995; Nayar, 1996). Karunanithi (1996) formulated *Pseudomonas fluorescens* with different carrier materials like peat, talc, lignite, kaolin, talc + peat, and talc + lignite and found that the talc supported the longer survival of *Pseudomonas fluorescens*. Seed treatment with powder formulation of fluorescent pseudomonad at the rate of 4 g kg^{-1} of seeds followed by soil application with 2.5 kg ha^{-1} at 0,30, and 60 days after sowing showed enhanced resistance to pigeon pea. The methods of application include seed treatment (Vanpeer and Schippers, 1988; Liu *et al*., 1995 a, b; Rosales and Mew, 1997), seedling root dip (Maurhofer *et al*., 1994; Nayar, 1996), soil application (Samiyappan, 1988; Vidhyasekaran *et al*., 1997) and foliar application (Mew and Rosales, 1986; Chatterjee *et al*., 1996). Combination of

individual treatments can also be practiced (Rabindran and Vidhyasekaran, 1996; Vidhyasekaran *et al*., 1997; Nandakumar, 1998). *Pseudomonas fluorescens* that inhibited the growth of *Rhizoctonia solani*, the rice sheath blight pathogen were isolated from the rhizosphere of different crops by Rabindran and Vidhyasekaran (1996). Both seed and soil application increased the yield compared to seed treatment alone (Vidhyasekaran *et al*., 1997)

The seed treatment with peat based formulation of *Pseudomonas fluorescens* strain showed encouraging results for the control of sheath blight of rice (Rabindran and Vidhyasekaran, 1996; Sivakumar and Narayanaswamy, 1998). The talc based powder formulation containing antagonistic bacterium was effective against chickpea wilt, pigeon pea wilt, rice blast, and rice sheath blight (Rabindran and Vidhyasekaran, 1996; Vidhyasekaran *et al*., 1997; Vidhyasekaran and Muthamilan 1999} and sugarcane red rot (Viswanathan and Samiyappan, 1999). The development of powder formulation of the bacteria with a shelf life of more than eight months (Vidhyasekaran and Muthamilan, 1999) and an efficient method of application may be highly useful for large-scale field application of the product and effective control of the disease. Meena *et al*. (2000) obtained effective control of late leaf spot of groundnut (*Cercosporidium personatum*) by seed treatment followed by foliar application of talc based formulation of *Pseudomonas fluorescens* and increase in phenolic content and activity of lytic enzymes was observed. Shanmugam *et al*. (2001) reported that a fluorescent pseudomonad isolate Pf1 effectively inhibited the mycelial growth of *Macrophomina phaseolina* causing black gram root rot under *in vitro* condition, in the green house and field conditions. Seed treatment and soil application of talc based formulation of the isolate Pf1 effectively controlled the root rot incidence and increased the yield. Nandakumar *et al*. (2001) observed that the

application of *Pseudomonas fluorescens* as bacterial suspension or talc based formulation reduced sheath blight disease in rice.

A satisfactory control of banded leaf and sheath blight (*Rhizoctonia solani* f.sp. *sasakii* Exner.) was obtained by seed treatment with peat-based formulation of *Pseudomonas fluorescens* (Sivakumar *et al*., 2000). Seed treatment along with the foliar spray of talc based formulation of *Pseudomonas fluorescens* reduced the disease intensity of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and increased the yield of the rice crop (Vidhyasekaran *et al*., 2001). They developed peat based formulation of the effective strain of Pf ALR2 and the effective dose of the formulation was assessed for seed treatment, soil application and foliar spray. In field trials its efficacy was comparable to that of the commercially available fungicide carbendazim. Kloepper and Schroth (1981) observed that population of PGPR did not decline in the talc mixture with 20 per cent xanthan gum after storage for two months at 4^0C. Hagedorn *et al*. (1993) reported satisfactory survival of specific strains of fluorescent pseudomonads multiplied on 10 per cent tripticase soy broth when mixed with pre-neutralised sterile peat at pH 6.8 Vidhyasekaran and Muthamilan (1995) studied the efficacy of various carriers sustaining the population of antagonist, and demonstrated the survival of bacteria in talc-based formulation up to eight months, but the population started to decline after one month of storage. Krishnamoorthy and Gnanamanickam (1998) observed increased shelf life and enhanced viability of *Pseudomonas putida* in a formulation containing methyl cellulose and talc in 1:4 proportions for up to 10 months. Gasoni *et al*. (1998) reported the survival of *Bacillus cereus* and *Pseudomonas fluorescens* in peat/vermiculite/dung formulation for at least 150 days and effectively controlled *Rhizoctonia* damping off in green house trials.

2.5 BIOCHEMICAL CHARATERISTICS OF BACTERIAL ISOLATES

The identification scheme of Stolp and Gadkari (1981) suggested spore production test, and biochemical tests like gelatin liquefaction, arginine dihydrolase, oxidase tests, levan production, catalase test etc for the identification of species of fluorescent pseudomonads. The antagonism could be identified based on the gram reaction and characters like colony type, pigment production on Kings A and Kings B media (King *et al*., 1954). Gorden and Paleg (1957) formulated a method to assess the production of Indole Acetic Acid (IAA) by different fluorescent pseudomonads in the medium. De Ley and Van Muylem (1963) observed that the GC content of genus plb-29 *Pseudomonas* ranged from 57-70 per cent and identified it as a strain of *Pseudomonas* species. Lelliot *et al*. (1966) described the LOPAT test which involved levan production, oxidase test, potato rotting, arginine dihydrolase and tobacco hypersensitive tests to see the pathogenic or saprophytic nature of the organisms. Morphological features, staining reaction, physiological characters and bio chemical properties were required for the identification of species of fluorescent pseudomonads (Schaad, 1980). Members of *Pseudomonas putida* produce yellowish green fluorescent pigment on King's B medium and cannot liquefy gelatin (Stolp and Gadkari, 1981)

Ramaswamy *et al*. (1992) determined the species of antagonistic bacteria based on the guanine–cytosine (GC) content of the bacteria .Hildebrand *et al*. (1992) identified *Pseudomonas fluorescens* and *Pseudomonas putida* among 20 fluorescent pseudomonads isolated from rhizosphere soil of different crops using King's B medium based on gelatin liquefaction, arginine dihydrolase, nitrate reduction and various carbon source utilization. The saprophytic pseudomonads association with plants can be assigned generally to one of the three species *viz*., *Pseudomonas fluorescens*, *Pseudomonas putida* complex (Schaad, 1992; Rosales *et al*., 1993). Plb–29 a phylloplane bacterium exhibited positive

catalase, oxidase, and arginine dihydrolase activities but was unable to produce levan from sucrose and also unable to utilise lactose as carbon source, which are the characteristics of most pseudomonads (Holt *et al*., 1994). Maurhofer *et al* (1994) reported that ISR by *Pseudomonas fluorescens* strain CHAO against tobacco mosaic virus in tobacco was associated with accumulation of β-1.3- glucanase and endochitinases. Bio chemical analysis of rice plants raised from seeds treated with *Pseudomonas fluorescens* showed an early and higher induction of peroxidase (PO), phenylalanine ammonia lyase (PAL) and chitinase (Nayar, 1996). In tomato, *Pseudomonas fluorescens* strain 63-28 induced the accumulation of chitinase and prevented the infection of *Fusarium oxysporun* f.sp.*radicis lycopersici* (M' Piga *et al*., 1997). ISR by fluorescent pseudomonads involves strengthening the physical and mechanical strength of the host cell wall and causing biochemical or physiological changes leading to synthesis of defense proteins (Maurhofer *et al*., 1998; Viswanathan and Samiyappan, 1999). De Meyer *et al*. (1999) reported that rhizosphere colonization by *Pseudomonas aeruginosa* 7 NSK 2 activated phenylalanine ammonia lyase (PAL) in bean roots and increased the salicylic acid levels in leaves. First work on the taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida* were based on phenotypic characters such as metabolic tests, fatty acid composition and protein profiles and these studies led to the subdivision of *Pseudomonas fluorescens* and *Pseudomonas putida* into 5 and 2 biovars, respectively (Bossis *et al*., 2000). Accumulation of phenolics, phenylalanine ammonia lyase, per oxidase, polyphenol oxidase, β-1, 3 glucanase and chitinase by *Pseudomonas fluorescens* isolate Pf1 in chilli leaves collectively contributed to induced resistance in chilli leaves against *Colletotrichum capsici* (Ramamoorthy and Samiyappan, 2001). Yeole *et al*. (2001) reported tests like $FeCl_2$ test, CAS assay, CAS agar plate and spectrophotometric assay for identification of siderophore production by fluorescent pseudomonads. Sivakumar and Sharma (2003) reported that the

Pseudomonas fluorescens induced resistance against *Rhizoctonia solani* f. sp. *sasakii* by activating various defense related enzymes and increasing phenolic content. Heera (2002) identified the species of isolates P11 and P33 among 33 different isolates of fluorescent pseudomonads which showed highest *in vitro* antagonism against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. o*ryzae* as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar I based on the biochemical tests *viz*., levan formation, gelatin liquefaction and utilization of various carbon and nitrogen sources.

Materials and Methods

3. MATERIALS AND METHODS

3.1. ISOLATION AND CULTURING OF SHEATH ROT PATHOGEN IN RICE

Panicles of rice plants showing typical symptoms of sheath rot disease were collected from naturally infected rice plants of Cropping Systems Research Centre (CSRC), Karamana. The sheath portions showing characteristic symptoms along with some healthy portion were cut into small bits of 0.5 cm and washed thoroughly in sterilized distilled water. The pieces were then surface sterilized with 0.1 per cent mercuric chloride solution for one to two min and again washed in two to three repeated changes of sterilized distilled water. These bits were then placed on sterile petridishes previously poured with 15 ml Potato Dextrose Agar (PDA) medium by tissue segment method and incubated under laboratory conditions (28 ± 2^0C). On third day when mycelial growth was observed, bits were aseptically transferred to PDA slants. The culture was then purified by frequent transferring of hyphal tips. The isolated fungal culture was identified and confirmed by observing the mycelial and conidial characteristics under the microscope. This pure culture of the fungus was used throughout the study.

3.2 MORPHOLOGICAL CHARACTERS OF THE FUNGAL PATHOGEN

The fungus associated with the sheath rot disease was brought into pure culture following the standard techniques. Detailed morphological characters of the pathogen like length and breadth of the mycelium, size of conidium, colour of the mycelium during different stages were studied following the slide culture technique (Riddel, 1950).

Sterile plain agar medium was poured in sterilized petridishes to a thickness of 2 mm and after solidification blocks of 6 mm square pieces

were cut out using sterile needle. One square was placed in the centre of each sterile glass slide and all the four sides of the block were inoculated with small culture bits of the fungus. A cover slip was placed on the top of the inoculated agar block and slides were kept in moist petridish chamber (sterile petridish with wet sterilized filter paper at the bottom on which two glass rods were kept to serve as support for the slide). The dish with the slide was then incubated at room temperature for two to three days. After this period, the cover slip was lifted gently and a drop of 95 per cent alcohol was placed in the centre and before drying, the cover slip was mounted using lactophenol cotton blue on another slide. The square of the agar was removed from the original culture slide and another mount was prepared in a similar manner without any disturbance to the fungal growth on the slide. These slides were then examined under microscope and the morphological characteristic of pathogen was studied.

3.3 CULTURAL CHARACTERISTICS OF *SAROCLADIUM ORYZAE*

3.3.1 Growth of *Sarocladium oryzae* on Different Media

Growth of *Sarocladium oryzae* on different solid media *viz*., potato dextrose agar medium, carrot agar medium, oat meal agar, King's B medium, Czapek's agar and liquid media like paddy leaf extract broth, potato dextrose broth, King's B broth, Czapek's broth and Richard's broth was done. The detailed composition of these media is given in Appendix I.

3.3.1.1 Growth of Sarocladium oryzae on Different Solid Media

Different solid media like potato dextrose agar medium, carrot agar medium, oat meal agar medium, King's B medium and Czapek's agar medium were prepared, sterilized and poured at the rate of 15 ml per dish and allowed to solidify. From a seven day old pure culture of

Sarocladium oryzae, five mm circular mycelial discs were cut out using a sterile cork borer and placed at the centre of the petridish using a sterilized fungal loop. The plates were then incubated at room temperature (28 ± 2^0C). Three replications were maintained for each medium. The mean radial growth and the growth characters of the fungus in different solid media were observed and recorded.

3.3.1.2 Growth of Sarocladium oryzae on Different Liquid Media

Different liquid media like paddy leaf extract broth, potato dextrose broth, King's B broth, Czapek's broth and Richard's broth were used for this study. Fifty ml each of the broth was taken in 250 ml conical flasks and sterilized. These sterilized flasks were inoculated with mycelial discs of 5 mm size cut out from actively growing culture of the fungus and incubated at room temperature (28±2^0). After two weeks of incubation, the culture was filtered through previously weighed Whatman No.1 filter paper and dry weight of the biomass was determined. Three replications were maintained for each isolate.

3.4 PATHOGENICITY TEST

The pure culture of the *Sarocladium oryzae* isolated was used for detailed pathogenicity test as follows. The rice variety Aiswarya at boot leaf stage was selected for this study. The inoculation was done by inserting a bit of pure culture behind the boot leaf sheath and lower two leaf sheaths. The inoculated plants were maintained under humid conditions by providing polythene cover for six days. Plants, which were inoculated with sterile distilled water maintained under identical conditions served as control.

The plants were observed for the development of symptoms and observations were recorded from the fifth day onwards. The nature of infection, the colour and the size of the lesions were observed from fifth day onwards. The fungus was then reisolated from the sheath portions

exhibiting typical symptoms of sheath rot from the artificially infected plants. The characters of the pathogen obtained from reisolation were compared with those of the original pathogen.

3.5 ISOLATION OF FLUORESCENT PSEUDOMONADS

3.5.1 From Rhizosphere

Fluorescent pseudomonads were isolated from rhizosphere of healthy plants collected from Rice Research Station, Moncompu; Regional Agriculture research Station, Vytilla; Croping System Research Centre (CSRC), Karamana and College of Agriculture, Vellayani by dilution plate technique (Johnson and Curl, 1972).

One gram of root sample with soils adhering to it were taken and washed with sterile distilled water. Then it was taken in 250 ml Erlen Meyer flask containing 100 ml of sterilized distilled water and shaken at 150 rpm for 20 min. The serial dilutions were prepared and one ml of the aliquot from the dilutions 10^6 and 10^7were transferred to sterilized petridishes poured with King's B medium agar (King's *et al*., 1954) and gentle rotation was given at both clockwise and anticlockwise direction to enable uniform spreading of the aliquot. Then it was kept for incubation at 28 ± 2^0 C for 48 h. Typical colonies appearing with characteristics of fluorescent pseudomonad spp. were transferred and maintained on KMB slants at 24 ± 2^0 C.

3.5.2 From Phyllosphere

Leaf samples, collected from disease free plants were used for isolation of fluorescent pseudomonads by dilution plate technique as mentioned under 3.5.1. Here dilutions of 10^3 and 10^4 from serial dilution were used for plating with King's B medium agar. To obtain endophytic isolates of the bacterium, 10 g leaf sample was taken and surface sterilized with 0.1 per cent mercuric chloride for one min, rinsed in sterile distilled water and ground with sterilized pestle and mortor. From

the extract, serial dilutions were prepared and one ml of aliquot from 10^3 and 10^4 dilutions were transferred to sterilized petridish over which 15 ml molten KMB agar was poured, and incubated for 48 h. After incubation, isolates of those colonies, which exhibited fluorescence around it, were selected and maintained in KMB agar medium for further studies.

3.6 *IN VITRO* TESTING OF FLUORESCENT PSEUDOMONADS AGAINST *SAROCLADIUM ORYZAE*

All the 20 fluorescent pseudomonad isolates obtained from rhizosphere and phyllosphere of rice plants were tested for antagonistic property against the sheath rot pathogen *Sarocladium oryzae* by dual plate method (Johnson and Curl, 1972).

Solidified KMB agar was taken in sterilized petridishes and kept as such for cooling. Then each isolate of fluorescent pseudomonad was streaked on the medium 1.5 cm away from the edge prior to pathogen inoculation from three sides, so that the isolate was streaked in a triangular shape. Then a five mm disc of the *Sarocladium* culture cut out from the edge of the pure culture was placed at the centre of the triangle. Three replications were maintained for each isolate. Petridishes inoculated with pathogen alone served as control. Inhibition zone was measured using the formula

I = (C-T)

C – Growth of pathogen in control plates (mm)

T – Growth of pathogen in dual culture (mm)

I – Inhibition of mycelial growth (mm)

Based on the inhibition zone developed, the isolates were numbered from 1 to 20.

3.7 PREPARATION OF TALC BASED FORMULATION OF FLUORESCENT PSEUDOMONADS

The pseudomonad isolates selected from *in vitro* studies were made into talc based formulation to enable different methods of application *viz*., seed treatment, seedling root dip and foliar spray at green house and field experiments.

3.7.1 Mass Multiplication of Fluorescent Pseudomonads

Six pseudomonad isolates *viz*., Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 selected based on the inhibition zone developed under *in vitro* studies were used for the green house studies. They were grown separately on King's B agar medium. King's B broth was prepared and autoclaved at 1.5 kg cm^{-2} for 20 min. A loopful of each isolate was inoculated into the respective broth and incubated at room temperature (28 ± 2^0C). The bacterial population was adjusted to 4 x 10^9 cfu ml^{-1} after 48 h of incubation.

3.7.2 Preparation of Talc based Formulation of the Isolates

Talc based formulation of the respective isolate was prepared by following the method of Vidhyasekaran and Muthamilan (1995) as detailed below.

The six isolates *viz*., Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 were formulated using talc as carrier material as follows.

Hundred g of talc powder was taken in polypropylene bags and its pH was adjusted to neutral by adding four g of calcium carbonate. Then one g of carboxy methyl cellulose (CMC) was added to the combination and kept it for autoclaving at 1.5 kg cm^{-2} pressure for one h on two successive days. After sterilization 40 ml of 48 h old respective

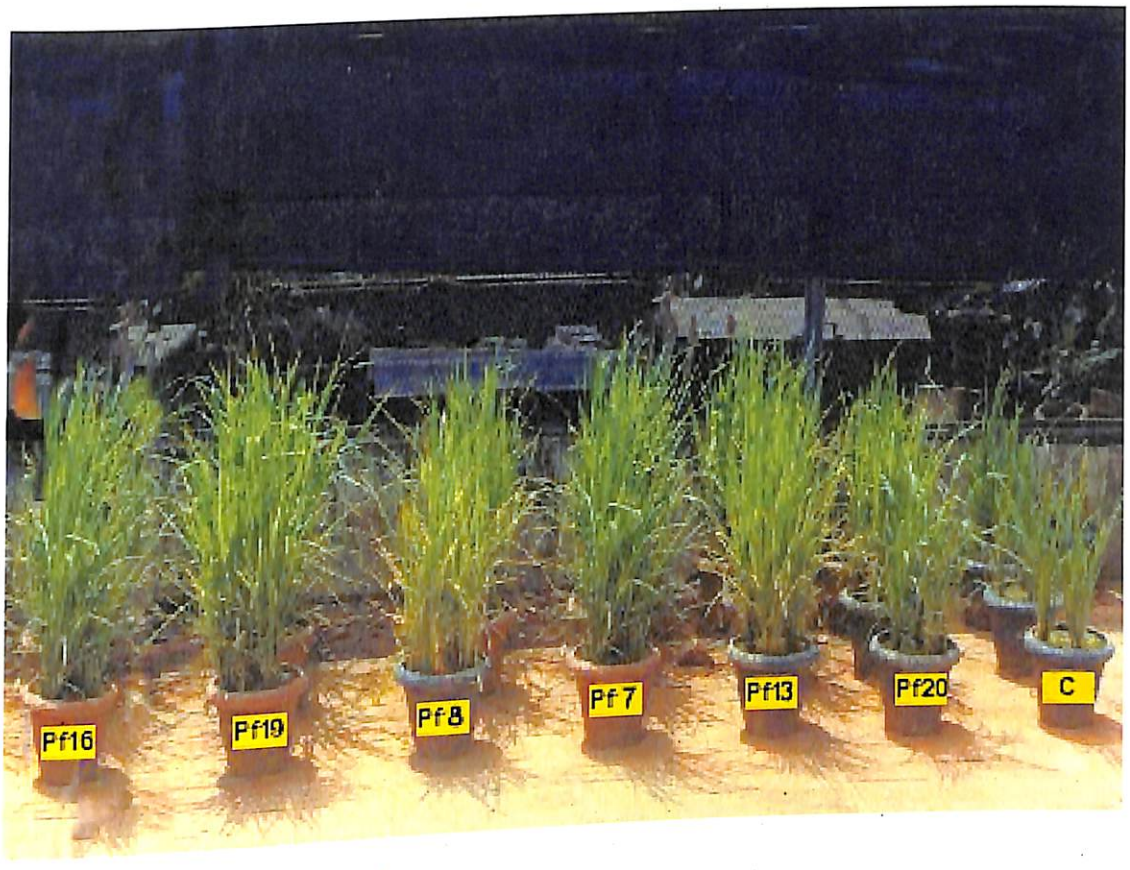
Pseudomonas isolate broth was added to it and mixed thoroughly under aseptic conditions. A polypropylene bag containing sterilized talc without any *Pseudomonas* isolate served as control. These polypropylene bags were stored at room temperature (28 ± 2^0C) with the labels bearing date of preparation, isolate number etc. This formulated product was used for the experiments under pot culture and field studies.

3.7.3 Survival of pseudomonad isolates Under Room Temperature

At 15 days interval, one g sample each was taken and serially diluted in sterile distilled water up to 10^7 dilution and one ml aliquot was pipetted out, poured in sterilized petridishes to which the King's B medium was poured gently rotated and incubated at room temperature (28±2^0C). The number of colonies were counted after 24 h and recorded.

3.8 EVALUATION OF EFFICACY OF SELECTED PSEUDOMONADS ISOLATES AGAINST *S. ORYZAE* UNDER GREEN HOUSE CONDITION

Six fluorescent pseudomonad isolates selected based on the inhibition zone under *in vitro* screening *viz*. Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 were used for this study. The rice variety Aiswarya was selected for the experiment. The experiment was conducted in a Completely Randomized Design (CRD) with three replications each (Plate 1). Control plants were maintained without any *Pseudomonas* treatment. Bacterial inoculation was given as seed treatment, seedling root dip and foliar application as given below. Seedlings for the experiment was raised separately by seed treatment with respective talc based bacterial formulation (Vidhyasekaran and Muthamilan, 1995). At the time of transplanting (21 days after sowing), seedling root dip was given, followed by foliar application of respective formulated product at boot leaf stage and at one week later. Fertilizer application was done as per package of practices recommended for rice by Kerala Agricultural University. Artificial inoculation of the pathogen was given to incite the disease at boot



(Isolates Pf 16, Pf 19, Pf 8, Pf 7, Pf 13, Pf 20)

Plate 1. Performance of different pseudomonad isolates on growth of rice plants under greenhouse condition

leaf stage. The disease intensity was measured using 0 – 9 scale of Standard Evaluation System for rice (IRRI, 1980), 10 days before harvesting. Based on the efficiency of the isolates to suppress the disease, one promising isolate was selected for further field evaluation (Plate1).

Different biometric observations such as plant height, number of tillers, number of panicles, shoot and root length, grain and straw yield and 1000-grain weight were also recorded and presented.

3.8.1 Seed Treatment

Ten gram of the respective formulated product per kg of seed was made into slurry and mixed with the seed. This was kept under shade for 30 min. After shade drying, the seeds were soaked in water for 8 to 10 h, then the excess water was drained off and incubated in dark for 24 h for inducing germination. Then they were taken for sowing.

3.8.2 Seedling Root Dip

Aqueous suspension of talc-based formulation of the respective bacterial isolates was prepared at a concentration of two per cent. Rice seedlings in bundles were dipped in the prepared suspension for one hour ensuring that the roots alone were completely immersed in the inoculum, before transplanting them to pots under green house condition.

3.8.3 Foliar Spray

Two per cent suspension of the talc based formulation of the respective bacterial isolates was prepared, allowed to settle for one hour filtered through muslin cloth, and filtrate was sprayed on the plants at boot leaf stage and at one week later.

3.8.4 Artificial Inoculation of Pathogen

The artificial inoculation of sheath rot pathogen *Sarocladium oryzae* was done by growing the pathogen by ‘single grain culture’ technique (Estrada *et al*., 1979) as follows.

Twenty gram paddy seeds were taken in 100 ml conical flask to which 25 ml water was added, and then sterilized at 1.5 kg cm^{-2} for 20 min. After cooling, a bit of 24 h old pure culture of *Sarocladium oryzae* was cut out using sterilized needle and transferred to the conical flask under aseptic condition, and allowed to grow at room temperature till it completely covered the paddy seeds. Then artificial inoculation was done by inserting single grain culture in between the leaf sheath and culm and covered by wet cotton and tied by polythene rope. Observation for the symptom expression was made after 48 h of inoculation.

3.8.5 Disease Scoring

The intensity of the disease was recorded 10 days before harvest from individual plants grown in the pots. The intensity was recorded using 0 – 9 scale of Standard Evaluation System for rice diseases (IRRI, 1980)(Plate 2).

Grades	Description
0	No incidence of disease
1	Lesions limited to lower one fourth of leaf sheath area
3	Lesions present in lower half of leaf sheath area
5	Lesions present on more than half of leaf sheath area, slight infection on lower (3^{rd} or 4^{th}) leaves
7	Lesions present on more than three fourth of leaf sheath, severe infection on lower leaves and slight infection on upper leaves (flag and second leaf)
9	Lesions reaching top of the tillers, severe infection on all leaves and some plants get killed

0 – No lesion,

1 – Lesion at one fourth of leaf sheath area

3 – Lesion at lower half of the leaf sheath area

5 – Lesion at more than half of leaf sheath area

7 – Lesion at more than three fourth of leaf sheath area

9 – Lesion reaching top of the tillers

Plate. 2 Score chart for sheath rot disease

Disease Index (DI) was calculated using the following formula as suggested by Mayee and Datar (1986).

$$DI = \frac{\text{Total numerical ratings x 100}}{\text{Total no. of hills observed x 9}}$$

3.9. EFFICACY OF DIFFERENT PSEUDOMONAD ISOLATES ON THE BIOMETRIC OBSERVATIONS UNDER GREEN HOUSE CONDITION

The influence of six-selected pseudomonad isolates *viz*., Pf16, Pf19, Pf8, Pf7, Pf13, and Pf20 on various biometric characters of rice plant like root and shoot length, number of tillers, number of panicles and plant height were recorded. The grain and straw yield were recorded at the time of harvest.

3.9.1 Evaluation of Six Selected Pseudomonad Isolates for Root and Shoot Length of Rice Plants

The six pseudomonad isolates selected were evaluated for their influence on the root and shoot length 20 days after sowing. For this , seedlings from respective isolates were pulled out carefully and the root and shoot lengths were measured and recorded. Similar measurements were also recorded at 60 DAT.

3.9.2 Evaluation of Different Pseudomonad Isolates on the Number of Tillers

The number of tillers was recorded at active tillering stage and at panicle initiation stage from random hill selected from each pot and it was expressed in number of tillers $hill^{-1}$.

3.9.3 Evaluation of Selected Pseudomonad Isolates on Plant Height

The plant height was measured 45 days after transplanting from the respective treatments and recorded in cm.

3.9.4 Evaluation of Selected Pseudomonad Isolates on Grain and Straw Yield

The crop was harvested separately, threshed, cleaned and weight of the grain and straw were recorded and expressed in g.

3.9.5 Evaluation of Six Selected Pseudomonad Isolates for 1000-Grain Weight

The plants grown in pots after giving respective pseudomonad treatments were harvested separately, dried and cleaned. One thousand grains from each isolate was counted and weighed. The weight was expressed in g.

3.9.6 Evaluation of Selected Isolates on the Number of Panicles

The number of panicles was counted from random hills selected from a pot at panicle emergence stage and was expressed as number of panicles $hill^{-1}$.

3.10 Field Evaluation of Promising Fluorescent Pseudomonad Isolate (Pf16)

Evaluation of the most promising isolate obtained from green house experiment was further evaluated under field conditions. The experiment was laid out during the kharif season of 2002 at Cropping System Research Centre (CSRC), Karamana in Randomized Block Design (RBD) (Plate 3) with nine treatments and three replications (Plate 3). The size of the individual plot was 3 x 2 m^2. The rice variety 'Aiswarya' was used at a spacing of 20 x 15 cm. Seedlings were raised as per the technical programme and transplanting was done at 21 days after sowing. In the field, application of fertilizer and other manures were followed as per the

Plate 3. General view of field experiment at CSRC, Karamana

package of practices recommended for rice by Kerala Agricultural University.

The treatment details are given below.

T_1 – Seed treatment with formulated product at the dose of 10 g kg^{-1} of seed

T_2 – Seedling root dip (SRD) with thick slurry of the formulated product

T_3 – Foliar spray (FS) with two per cent of the formulated product (at boot leaf stage and one week later)

T_4 – Seed treatment + seedling root dip (Doses as in T_1 and T_2)

T_5 – Seed treatment + foliar spray (Doses as in T_1 and T_3)

T6 – Seedling root dip + foliar spray (Doses as in T_2 and T_3)

T_7 – Seed treatment + seedling root dip + foliar spray (doses as in T_1, T_2 and T_3)

T_8 – Treated check using carbendazim (0.1 per cent)

T_9 – Untreated check.

Pseudomonas treatment was given as seed treatment, seedling root dip and foliar spray. Seed treatment at the rate of 10 g kg^{-1} seed with the talc based formulated product of promising isolate Pf16 followed by seedling root dip in two per cent of the formulated product at the time of transplanting and foliar application at boot leaf stage and at one week later was given.

Artificial inoculation of the *S. oryzae* was given at boot leaf stage and the disease scoring was done as per the method given under 3.8.5. Observations on disease suppression and other parameters like plant height, root and shoot length, number of tillers, number of panicles,

grain and straw yield and 1000 grain weight were recorded. The data were analyzed statistically.

3.11 CHARACTERIZATION OF BACTERIAL ISOLATES ANTAGONISTIC TO THE RICE PATHOGEN *SAROCLADIUM ORYZAE*

The cultural, physiological and biochemical characteristics of the best three pseudomonad isolates selected from *in vitro* screening was studied under laboratory condition. The cultural tests like Gram's reaction, physiological tests like growth at different temperature and biochemical tests like levan formation, gelatin liquefaction, and utilization of alcohols, n-butyl amine, propylene glycol, and phenyl acetylene were studied.

3.11.1 Cultural Characters of the Isolates

Colony characters of the best three pseudomonad isolate *viz*., Pf16, Pf19 and Pf8 were studied from a 24 h old culture of the bacterium grown on KMB agar (Appendix 1). The growth of the bacteria in single colony was observed, cell morphology was studied by Gram staining and the slides were observed under oil immersion objective of the microscope.

3.11.1.1 Colony Characters

Colony characteristics of the selected pseudomonad isolates were studied by plating bacteria on King's B medium agar. The bacterial isolates were streaked on KMB poured in petriplates and incubated at room temperature. After 24 h, observation was made for its shape, elevation, margin and texture of the colonies.

3.11.1.2 Gram Staining

The smear of the test isolates were prepared, and fixed on a clean microscopic slide. It was stained with crystal violet green (Appendix II) for 30 sec. The excess stain was removed by rinsing in tap water. The smear was then flooded with Gram's iodine (Appendix II) and allowed to react for 30 sec, after which it was again rinsed off with water. The

preparation was then decolourised with 95 per cent ethanol, rinsed with water and counter stained with safranin (Appendix II) for 30 sec. The smear was rinsed with tap water, blotted dry and examined under oil immersion objective of a microscope.

3.11.1.3 Motility Test

The motility of the bacterial isolates was tested by stab culture method in semi solid nutrient agar (Appendix II). These tubes containing sterile melted nutrient agar was cooled in an upright position and inoculated by thrusting the inoculation needle containing the bacterial culture through the centre of the medium. The test tubes were incubated at 28 ± 2^0C and observed for the nature of growth from the line of inoculation.

3.11.2. Biochemical Characteristics of Bacterial Isolates

A series of biochemical tests were conducted under laboratory conditions to identify the species of the pseudomonad isolates (Schaad, 1992).

3.11.2.1 Levan Formation

The media prepared for this test was peptone beef extract agar containing five per cent sucrose (Appendix III). The sterilized media was poured into the petridish and a dilute suspension of the bacterial isolates was streaked over the medium and observation for the growth character was made after 48 h of inoculation.

3.11.2.2 Gelatin Liquefaction

The media used for this test was nutrient gelatin agar (Appendix III). After preparation, the medium was poured into the cleaned and dried test tube to a height of 4 cm and sterilized at 1.5 kg cm^{-2} pressure

for 20 min. The test tubes were kept at room temperature as such for two days to confirm its sterile condition. Then a 48 h old culture of each pseudomonad isolate was stab inoculated in the properly sterilized gelatin columns. The tubes were kept under incubation and observation for liquefaction of gelatin column was made at one week interval for one month.

3.11.2.3 Utilization of Alcohol

The basal medium used in this test was Hayward's medium (Hayward, 1964) (Appendix III). Ninety ml of the sterilized Hayward's medium was taken in a 250 ml conical flask and autoclaved at 1.5 kg cm^{-2} pressure for 20 min. Ten per cent solution of ethanol was prepared in sterile distilled water and sterilized by filtration. Ten ml of the filtered solution was added to 90 ml of melted Hayward's medium and poured into the sterilized test tubes to a height of four cm. The medium was inoculated with the test isolates using sterilized bacterial loop. The inoculated tubes were incubated and observation recorded at weekly intervals up to a period of one month. Change in colour of the medium from blue to yellow indicated utilization of ethanol.

3.11.2.4 Utilization of Propylene Glycol, n- Butyl amine, Phenyl acetate

Utilization of all these chemicals was tested using Hayward's medium containing one per cent of each of these chemicals as described under 3.11.2.3.

3.11.3 Physiological Characters of the Isolates

It was studied by growing the selected isolates Pf16, Pf19, Pf8, under varied temperatures *viz*., 4^0C and 41^0C. The test was done in King's B medium broth (Appendix I). Five ml of the King's B medium broth was poured into test tubes and autoclaved for 1.5 kg cm^{-2} pressure for 20 minutes. The test isolates were inoculated into the broth and kept

at 4^0C, 41^0C along with control. The absorbance of the inoculated broth was measured at regular intervals for four days.

3.12 TESTING THE METABOLITE OF SELECTED PSEUDOMONAD ISOLATE

The metabolite of the best isolate Pf16 selected from green house studies was tested against the pathogen as detailed below.

A loopful of 48 h old culture of the isolate Pf16 was transferred to a 200 ml of the sterilized medium taken in Erlenmeyer flask and incubated at 28 ± 2^0C for seven days. The bacterial suspensions were centrifuged at 10,000 rpm at 20^0C for 15 min. The supernatant was collected and filtered using bacterial proof filter to obtain partially purified filtrates. The metabolites isolated were tested against the pathogen as described below.

Two ml of metabolite extracted (partially purified culture filtrate) from the pseudomonad isolate Pf16 was added to the flask containing 50 ml of KMB agar autoclaved (1.5 kg cm^{-2} for 20 min.) melted and cooled to 45^0C. They were thoroughly mixed by gently swirling the flasks and poured aseptically into sterilized Petri dishes. Five mm dia mycelial disc of seven-day-old culture of the pathogen (*Sarocladium oryzae*) was placed in the centre of each plate. King's B medium agar plates without culture filtrates and inoculated with the pathogen served as control. Three plates were incubated at 28 ± 2^0C for seven days and the mean diameter of the growth of the pathogen was recorded.

Results

4. RESULTS

4.1 ISOLATION OF CAUSAL ORGANISM

The causal organism of sheath rot disease in rice, *Sarocladium oryzae* Gams and Hawksworth was isolated from the naturally infected rice plants collected from paddy fields of Cropping Systems Research Centre (CSRC), Karamana, Thiruvananthapuram. The disease was characterized by the formation of brown oblong lesions on the flag leaf sheath, surrounded by light yellow halo initially. On maturity, the lesions turned dark brown with grey centres. Panicles were partially choked and grain discolouration was noticed (Plate 4).

4.2. MORPHOLOGICAL CHARACTERS OF FUNGAL PATHOGEN

The fungus *Sarocladium oryzae* on pure culture in PDA medium formed a pinkish white to pink coloured colony (Plate 5), with septate mycelium and the thickness of the hypha was 2.67 µm in size. The conidiophore measured 39.76 x 2.51µm in size. The conidium was hyaline, cylindrical and single celled, attached singly at the tip of the branches. The conidium measured 6.16 x 1.57 µm in size (Plate 6).

4.3 CULTURAL CHARACTERISTICS OF FUNGAL PATHOGEN

Cultural characteristics of the pathogen *Sarocladium oryzae* was studied by growing it in different solid and liquid media.

4.3.1. Growth of *Sarocladium oryzae* on Different Solid Media

The effect of *Sarocladium oryzae* on different solid media was studied. Carrot agar medium gave the maximum radial growth of 8.78 cm, followed by oat meal agar (8.66 cm) and Czapek's agar (8.62 cm). Oat meal agar (8.66) and Czapek's agar medium (8.62 cm) were on par. In King's B medium, the growth was 7.17 cm. But the potato agar medium formed mycelial growth better than Kings B medium. The results were represented in Table 1 and Fig 1.

Plate 4. Rice plants showing typical symptoms of sheath rot disease

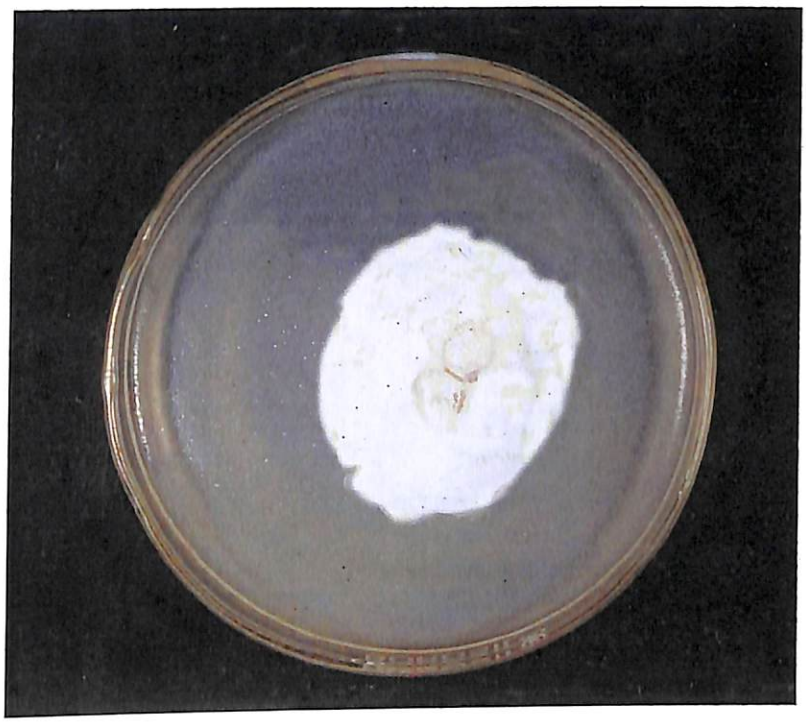


Plate 5. Growth of *Sarocladium oryzae* on potato dextrose agar medium

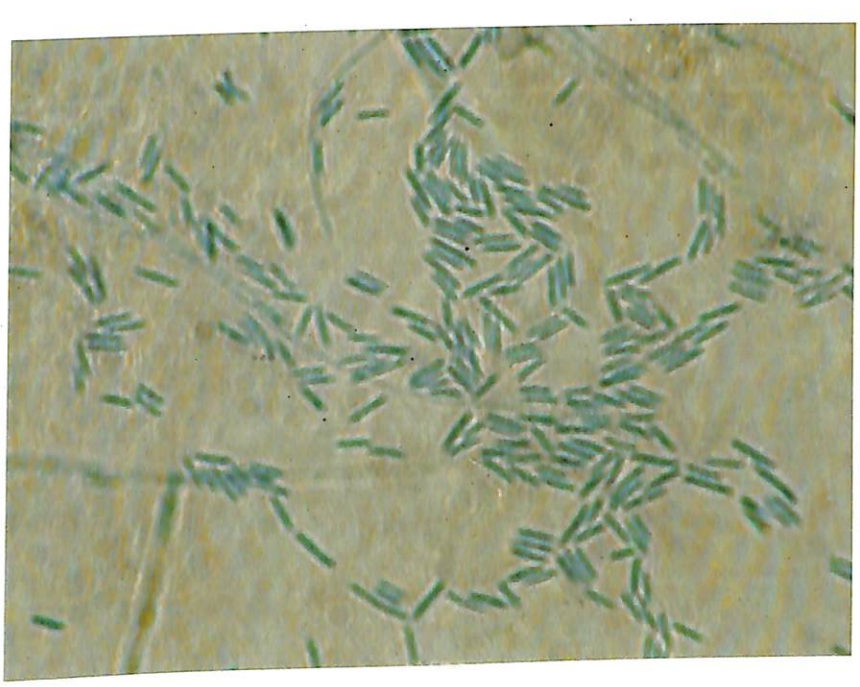


Plate 6. Conidia of *Sarocladium oryzae*

4.3.2 Growth of *Sarocladium* on Different Liquid Media

Among the five different liquid media tested, maximum dry weight of biomass was obtained in Richard's broth having a mycelial weight of 0.3870 g followed by potato dextrose broth (0.3319 g), Czapek's broth (0.3305 g) and paddy leaf extract broth (0.2595 g). King's B broth was least respondent with mycelial weight of 0.1409 g. (Table 2, Fig 2).

4.4 PATHOGENICITY TEST

The pathogen *Sarocladium oryzae* isolated from naturally infected plant produced typical sheath rot symptoms, when inoculated on rice cultivar Aiswarya at boot leaf stage. Reisolation yielded the same pathogen with growth characters similar to that of original culture (Plate 7).

4.5 ISOLATION OF FLUORESCENT PSEUDOMONADS

Twenty different isolates of fluorescent pseudomonads were isolated from healthy rice plants collected from places like Karamana, Vellayani, Moncompu, and Vytilla covering the southern parts of the State. Of these eleven isolates were obtained from rhizosphere and nine from phyllosphere. The stock cultures of these isolates were maintained at 4^0C in a refrigerator.

4.5.1 Isolation of Fluorescent Pseudomonads from Rhizosphere

Typical colonies of fluorescent pseudomonads were obtained from 10^6 and 10^7 dilutions on Kings medium B agar. The colonies formed were circular to ovoid slightly convex raised with entire margin. The individual colonies produced fluorescent pigment around them, which were clearly visible under UV light.

Table 1. Growth characters of *Sarocladium oryzae* in different solid media

Sl. No.	Medium used	Mycelial growth, cm (7 days after inoculation)
1	PDA	8.42
2	Carrot Agar Medium	8.78
3	Czapek's Agar	8.62
4	King's Medium B Agar	7.17
5	Oat meal Agar	8.66
	CD (0.05)	0.15

Table 2 Growth characters of *Sarocladium oryzae* in different liquid media

Sl. No.	Broth used	Average dry weight of biomass, g
1	Potato Dextrose Broth	0.3319
2	Paddy Leaf Extract	0.2595
3	Czapek's broth	0.3305
4	King's B Broth	0.1409
5	Richard's Broth	0.3870
	CD (0.05)	0.019

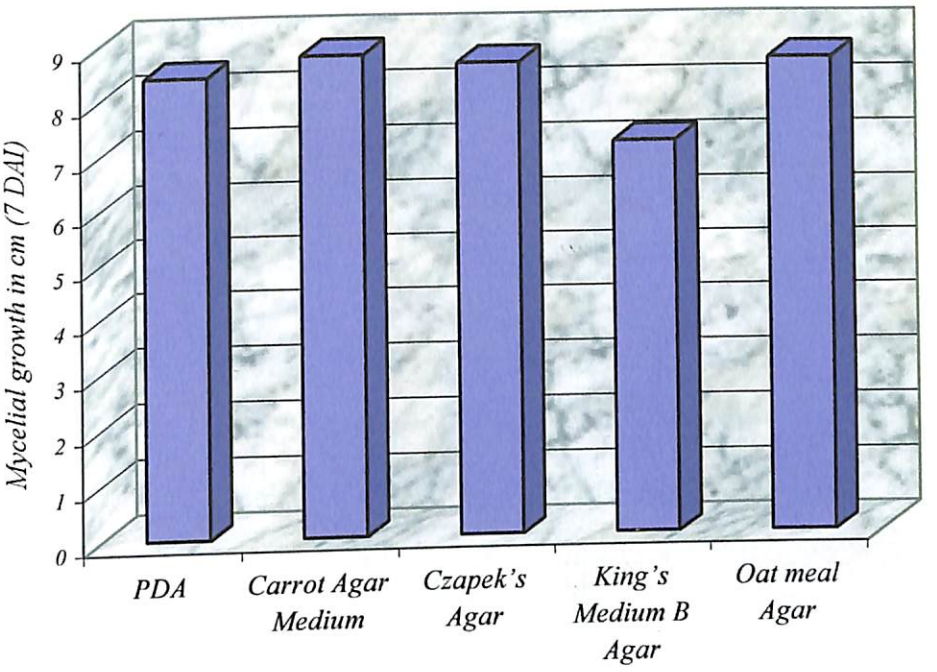


Fig. 1 Growth of *Sarocladium oryzae* in different solid media

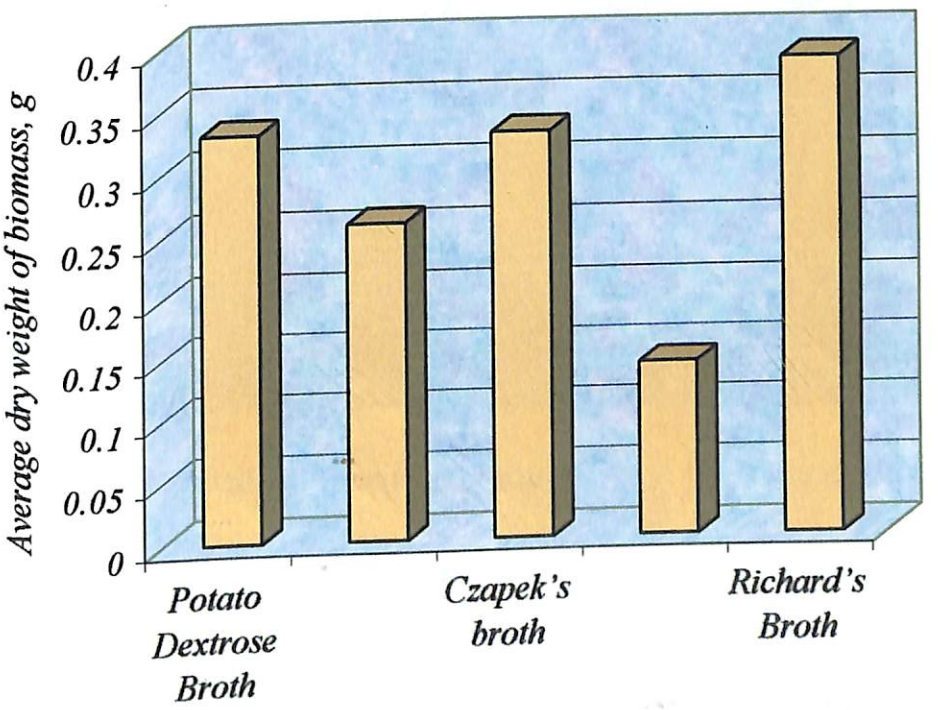


Fig. 2 Growth of *Sarocladium oryzae* in different liquid media

T - Inoculated with *Sarocladium oryzae* C – Uninoculated

Plate 7. Plants inoculated with *Sarocladium oryzae* and uninoculated plants

4.5.2 Isolation of Fluorescent Pseudomonads from Phyllo sphere

The isolation was done at dilutions of 10^3 and 10^4 on King's B medium agar. Colonies showed typical characteristics of fluorescent pseudomonads as described under 4.5.1.

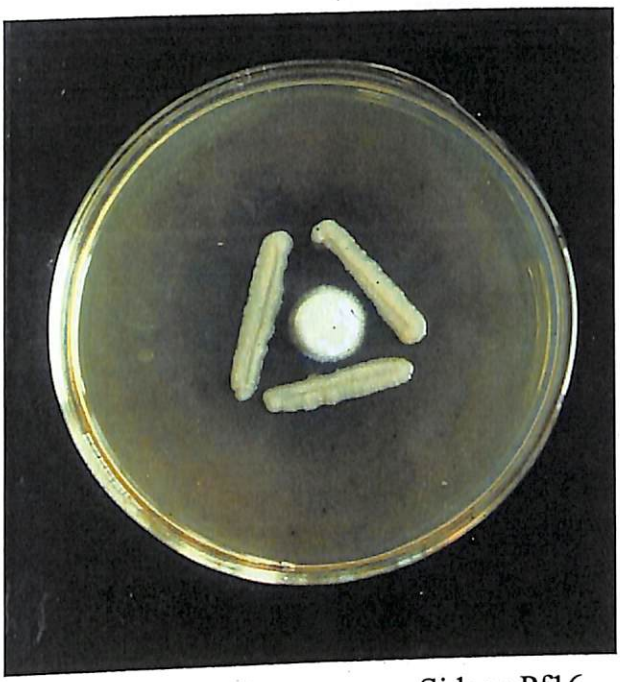
4.6 *IN VITRO* TESTING OF FLUORESCENT PSEUDOMONADS AGAINST *SAROCLADIUM ORYZAE*

Twenty isolates of fluorescent pseudomonads spp. collected from different locations were numbered from Pf1 to Pf20. They were screened for their antagonistic property against *Sarocladium oryzae*. In King's B medium agar, the maximum inhibition zone of 11.3 mm was obtained for Pf16 in dual plate culture against *Sarocladium oryzae* (Table 3,Plate 8). Next to Pf16, the isolate Pf19 was effective in inhibiting the pathogen and produced an inhibition zone of 9 mm .The isolates Pf8, Pf7, Pf13 and Pf20 produced inhibition zones of 7.9 mm, 7.1 mm, 7.1 mm and 6.8 mm respectively (Plate 9). Inhibition zone of all the other remaining isolates were between 2.3 to 6.6 mm. In PDA medium Pf16 formed a slight inhibition zone of 3.2 mm followed by Pf20 (1.2mm) and Pf3 (0.5 mm) 4.7 SHELF LIFE OF FLUORESCENT PSEUDOMONAD

Survival of fluorescent pseudomonad (Pf16) in talc based formulation at room temperature was studied. The bacterial formulation with the isolates Pf16 was prepared as described under 3.7. Population count was found decreasing from 42.13 x 10^7 to 19 x 10^7 cfu g^{-1} from the first day of observation to the last day. The population count on the first day of preparation was 42.13 x 10^7. After 15 days it was 40.69 x 10^7 cfu g^{-1} and finally at 90^{th} day, it was 19 x 10^7 cfu g^{-1} (Table 4,Fig 3). The percentage reduction in population in Pf16 was 3.42 on the 15^{th} day. As the number of days under storage increased, the population of the bacterial cells decreased correspondingly. After 30 days, the reduction of bacterial population was 10.59 per cent over initial population. It further came down to 36.70 per cent at 75 days after preparation. The population decreased more than half (54.90 per cent) at the end of the third month.

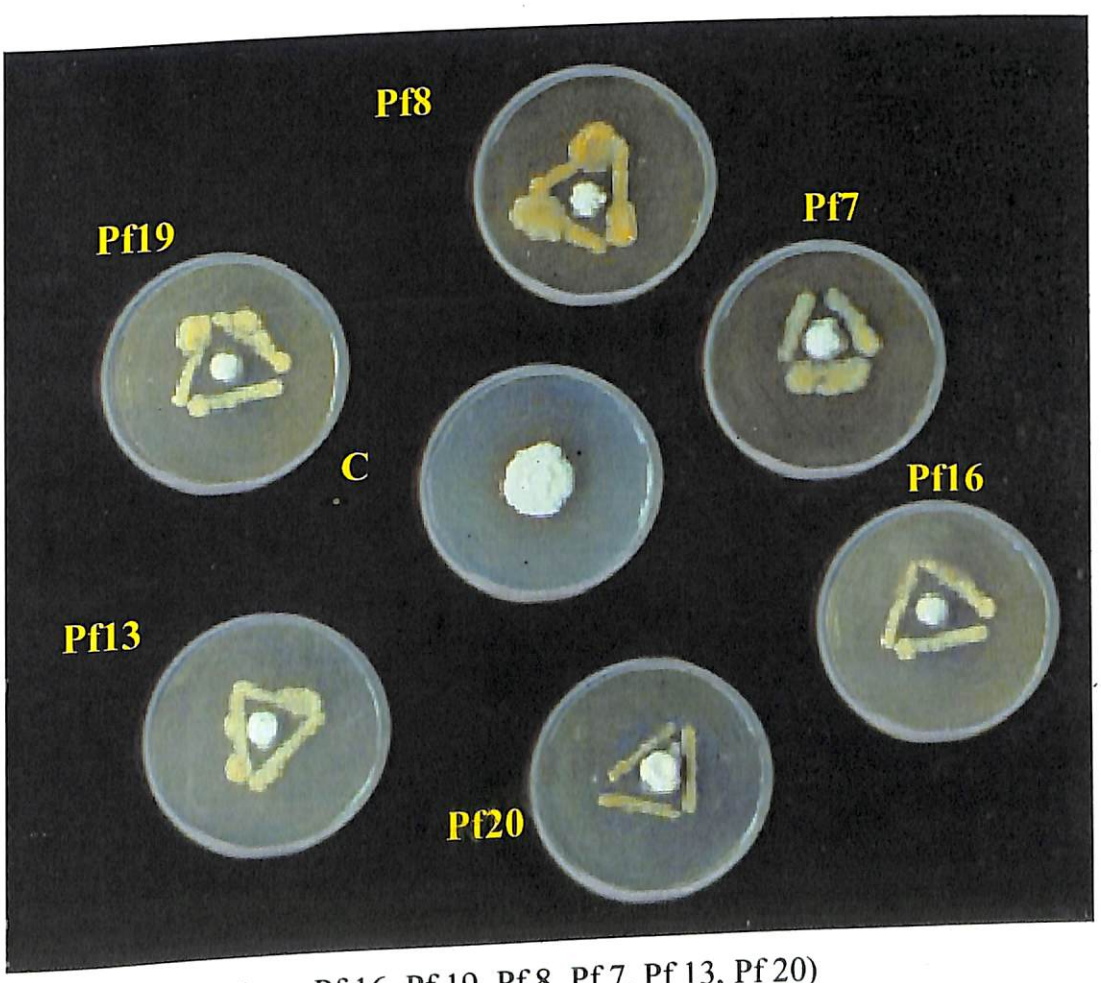
Table 3. Inhibition zone of fluorescent pseudomonad isolates against *Sarocladium oryzae* on KMB agar medium under *in vitro* conditions after 48 h

Sl. No.	Isolate No.	Inhibition zone, mm	
		KMB	PDA
1	Pf 16	11.3	3.2
2	Pf 19	9.0	0.0
3	Pf 8	7.9	0.0
4	Pf 7	7.1	0.0
5	Pf 13	7.1	0.0
6	Pf 20	6.8	1.2
7	Pf 15	6.6	0.0
8	Pf 4	5.7	0.0
9	Pf 2	5.3	0.0
10	Pf 10	4.8	0.0
11	Pf 14	4.4	0.0
12	Pf 1	4.3	0.0
13	Pf 3	4.1	0.5
14	Pf 5	4.0	0.0
15	Pf 6	3.8	0.0
16	Pf 18	3.3	0.0
17	Pf 17	3.2	0.0
18	Pf 9	3.0	0.0
19	Pf 11	2.8	0.0
20	Pf 12	2.3	0.0



Centre : *Sarocladium oryzae*, Sides : Pf16

Plate 8. Effect of fluorescent pseudomonad isolate Pf16 inhibiting the growth of *Sarocladium oryzae*



(Isolates Pf 16, Pf 19, Pf 8, Pf 7, Pf 13, Pf 20)

Plate 9. Dual culture testing of various pseudomonad isolates against *Sarocladium oryzae* under *in vitro* condition

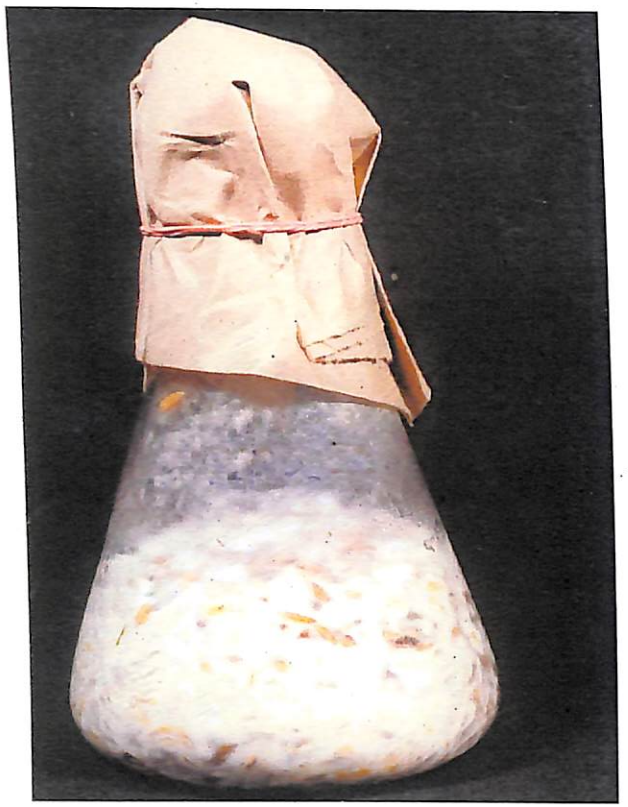


Plate 10. Mass multiplication of *Sarocladium oryzae* in paddy grains

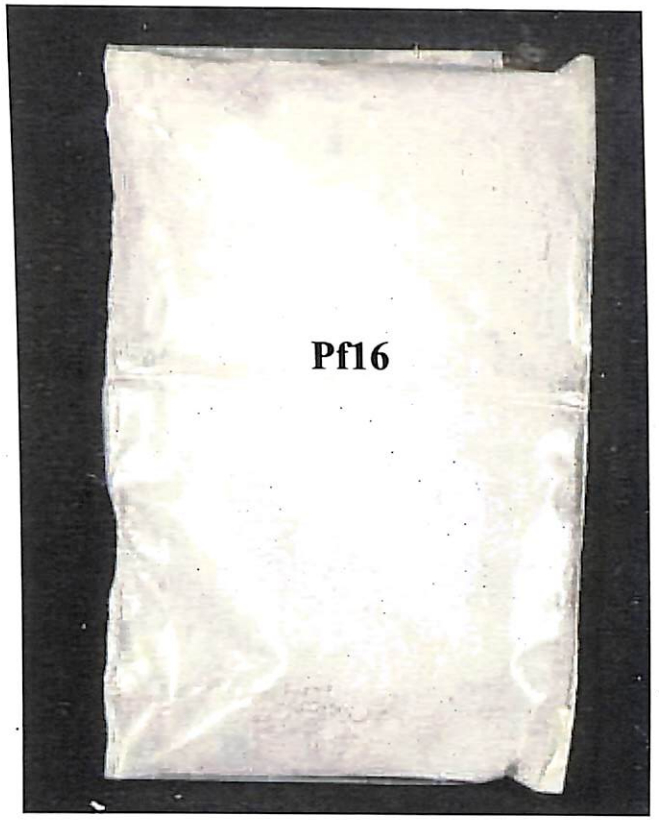


Plate 11. Talc based formulated product of isolate Pf 16

Table 4. Survival of fluorescent pseudomonad sp. (Pf16) in talc based formulation at room temperature (28±2°C)

Days after preparation	cfu g^{-1} (x 10^7)	Percentage reduction over initial population
0	42.13	-
15	40.69	3.42
30	37.67	10.59
45	34.00	19.30
60	30.67	27.20
75	26.67	36.70
90	19.00	54.90

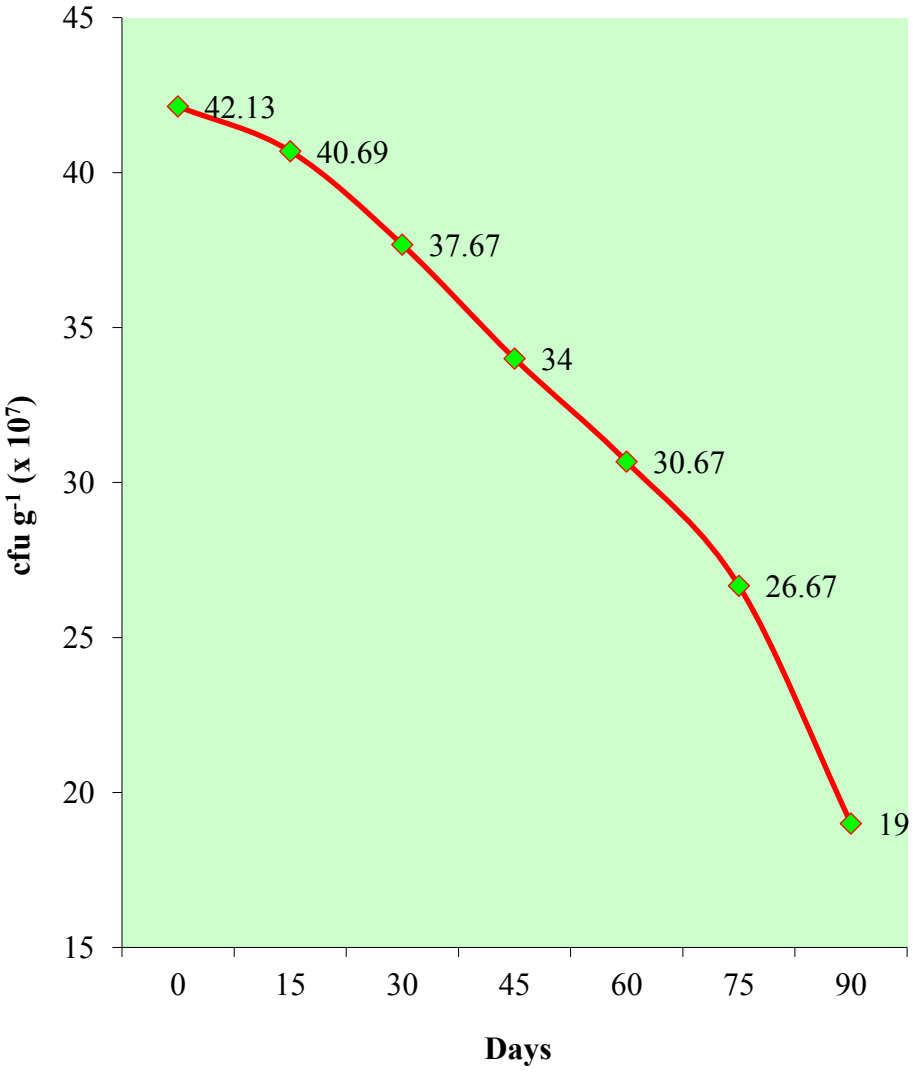


Fig. 3 Survival of fluorescent *Pseudomonad* sp. (Pf16) in talc based formulation

4.8 EVALUATION OF EFFICACY OF SELECTED PSEUDOMONAD ISOLATES AGAINST *S. ORYZAE* UNDER GREEN HOUSE CONDITION

Evaluation of the six best pseudomonad isolates selected from *in vitro* studies based on the inhibition zone formation was tested under green house condition. The talc based pseudomonad isolates Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 were applied as seed treatment, seedling root dip and foliar spray as given under 3.8.1 to 3.8.3. Pathogen inoculation was done at boot leaf stage, and the disease intensity was calculated ten days before harvest. The lowest disease index was recorded for the plants treated with Pf16 (1.67) followed by Pf19 (3.33). The isolate Pf8 and Pf7 have the disease index value of 15.18 and 19.63 respectively (Table 5) and two treatments were statistically on par. The highest value was recorded in Pf20 (28.70) whereas control plants scored a disease intensity of 57.04. The different isolates varied in their growth promoting activity in different manner.

4.9 EFFICACY OF DIFFERENT PSEUDOMONAD ISOLATES ON THE BIOMETRIC OBSERVATIONS UNDER GREEN HOUSE CONDITION

Various biometric observations like shoot and root length, number of tillers, number of panicles, plant height, grain and straw yield and 1000 grain weight were recorded for all the six isolates under green house condition.

4.9.1 Evaluation of Six Selected Pseudomonad Isolate on Shoot and Root Length of Rice Plants

The shoot and root length in response to the six pseudomonad isolates were recorded at two stages *i*.e., one at 20 DAS and second at 60 DAT. At 20 DAS, the maximum shoot length was recorded in Pf16 (18.33 cm) followed by Pf19 (17.51 cm) and Pf8 (17.19 cm). Minimum shoot length was recorded in the isolate Pf20 (14.82), whereas in control it was 14.36 cm. Similarly, the root length was also recorded at 20 DAS.

Table 5. Effect of different pseudomonad isolates on disease suppression

Sl. No.	Isolate No.	Disease index	Per cent reduction over control
1	Pf 16	1.67	97.07
2	Pf 19	3.33	94.16
3	Pf 8	15.18	73.39
4	Pf 7	19.63	65.59
5	Pf 13	28.52	50.00
6	Pf 20	28.70	49.68
7	Control	57.04	-
	CD (0.05)	8.38	-

Table 6. Effect of different fluorescent pseudomonad isolates on shoot and root length under green house conditions

Sl. No.	Isolate No.	Shoot and root length at 20 DAS			
		Shoot length, cm	Per cent increase over control	Root length, cm	Per cent increase over control
1	Pf 16	18.33	27.97	4.92	36.11
2	Pf 19	17.51	21.68	5.20	44.44
3	Pf 8	17.19	20.28	5.41	50.00
4	Pf 7	15.42	7.69	5.39	47.22
5	Pf 13	16.84	17.48	4.50	25.00
6	Pf 20	14.82	3.50	4.40	22.00
7	Control	14.36	-	3.66	-
	CD (0.05)	2.34		1.92	

Here, the maximum root length was recorded in Pf8 (5.41) followed by Pf7 (5.39) and Pf19 (5.20). In control, the value was 3.66 cm (Table 6, Plate 12).

The shoot and root length was recorded again at 60 DAT, when all the three different types of pseudomonad applications were completed. The maximum shoot length at 60 DAT was recorded in Pf19 (80.62) followed by Pf7 (80.16). The lowest value was recorded in Pf8 (75.27), whereas in control it was 79.19 cm. Maximum root length at 60 DAT was recorded in PF13 (11.97) followed by PF19 (10.71 cm) and Pf20 (10.73). In control the value recorded was 10.22 (Table 7).

In terms of percentage increase over control, at 20 DAS shoot length in the case of isolate Pf16 was 27.97 followed by PF19 (21.68), Pf8 (20.28) (Table 6). In the case of root length, the percentage increase over control for Pf8, 20 DAS was 50.00 followed by Pf7 (47.22). Statistical analysis of the data showed that at 20 DAS, the shoot length was significant over control in Pf16 treated plants, followed by Pf19, Pf8 and Pf13. Isolates Pf7 and Pf20 were on par.

4.9.2 Evaluation of Six Selected Pseudomonad Isolates on Number of Tillers

The number of tillers was statistically significant over control both at active tillering stage and panicle initiation stage in all the isolates under study. At active tillering stage the number of tillers was maximum in Pf16 (7.95), followed by Pf20 (6.79), whereas minimum number of tillers was recorded for Pf13 (5.76) (Table 4.8). In control the value was 4.74. At panicle initiation stage also the number of tillers was maximum for Pf16 (11.97), the next highest value was in Pf7 (11.49). The minimum number of tillers was recorded in Pf8 (7.88). In control it was 7.40. At both the stages of observation *viz*., at active tillering stage and at panicle initiation stage, the isolates Pf16 and Pf20 were on par. Similarly the isolates Pf16 and Pf19 were on par at active tillering stage, but during the panicle initiation stage it differed. The increase in number of tillers in

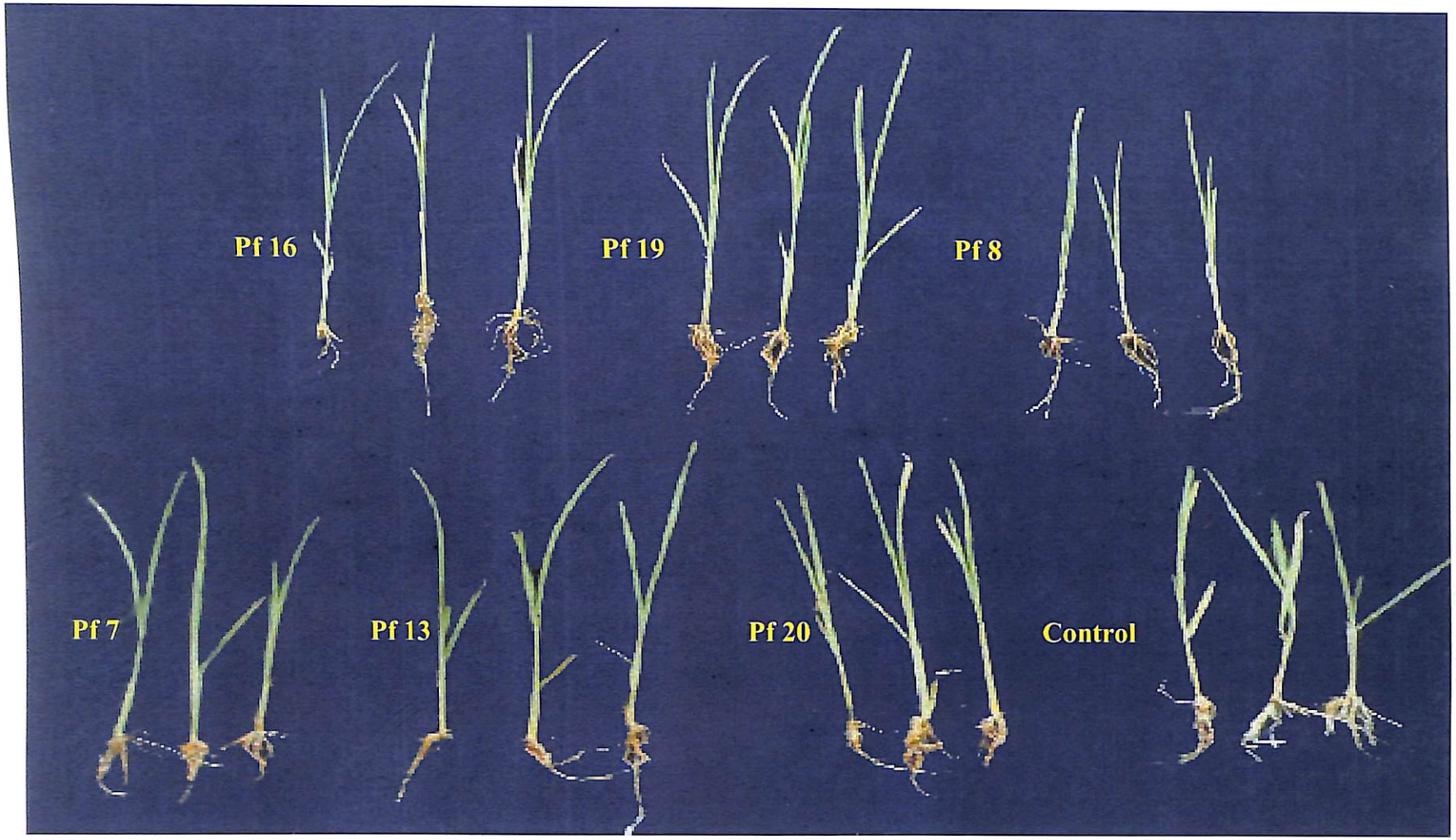


Plate 12. Effect of different pseudomonad isolates on shoot and root length in rice plants

Table 7. Effect of different fluorescent pseudomonad isolates on shoot and root length under green house conditions

Sl.No.	Isolate No.	Shoot and root length at 60 DAT			
		Shoot length, cm	Per cent increase over control	Root length, cm	Per cent increase over control
1	Pf 16	78.72	-0.59	10.38	1.57
2	Pf 19	80.62	1.81	10.79	5.58
3	Pf 8	75.27	-4.95	10.70	4.70
4	Pf 7	80.16	1.22	10.39	1.67
5	Pf 13	75.66	-4.46	11.97	17.12
6	Pf 20	78.19	-1.26	10.73	5.00
7	Control	79.19	-	10.22	-
	CD (0.05)	1.58	-	2.32	

Table 8. Effect of different fluorescent pseudomonad isolates on number of tillers in rice plants under green house condition

Sl. No.	Isolate No.	Number of tillers			
		Active tillering stage	Per cent increase over control	Panicle initiation stage	Per cent increase over control
1	Pf 16	7.95	67.08	11.97	60.89
2	Pf 19	6.79	43.25	8.86	19.09
3	Pf 8	6.38	34.60	7.88	5.91
4	Pf 7	6.37	34.39	11.49	53.76
5	Pf 13	5.76	21.52	10.61	42.61
6	Pf 20	6.99	47.47	10.39	39.65
7	Control	4.74		7.40	
	CD (0.05)	1.36		2.07	

terms of percentage over control was 67.08 in Pf16 followed by Pf20 (47.47). Least percentage was recorded in Pf13 (21.52) (Table 8).

4.9.3 Evaluation of Six Selected Pseudomonad Isolates on the Plant Height

The plant height at 45 days after transplanting was significant for all the treatments over control. The highest plant height was recorded for the plants treated with Pf16 (59.41 cm) followed by Pf 13 (58.84 cm), Pf19 (58.04) and Pf8 (57.70). Statistically these four treatments were on par. The lowest plant height was recorded for Pf7 (54.60 cm), which was on par with the control. Except Pf7, all the other isolates were significantly superior over control. In terms of percentage increase over control, in the isolate Pf16 it was 15.23 followed by Pf13 (14.14) and Pf19 (12.59) (Table 9).

4.9.4 Evaluation of Six Selected Pseudomonad Isolates on the Number of Panicles

Effect of application of pseudomonad isolates on the number of panicles was recorded at panicle emergence stage of the crop. Number of panicles was highest for Pf19 (11.44) followed by Pf16 (10.43) and Pf7 (10.32). Statistically all these isolates were on par, whereas the lowest number of panicles was recorded for Pf8 (6.08), which was on par with control. In terms of percentage increase in number of panicles over control it was 105.02, 86.92, 85.30 for the isolates Pf19, Pf16 and Pf7 respectively (Table 9).

4.9.5 Evaluation of Six Selected Pseudomonad Isolates on Grain and Straw Yield

The grain yield was highest for Pf16 (33.20) followed by Pf13 (31.87), Pf7 (31.67), Pf8 (31.23) and Pf20 (31.10) (Table 10; Fig 4). All the isolates were significantly superior over control. The percentage

Table 9. Effect of different pseudomonad isolates on plant height and number of panicles hill^{-1}

Sl. No.	Isolate No.	Plant height (45 DAT), cm	Per cent increase over control	Number of panicles	Per cent increase over control
1	Pf 16	59.41	15.23	10.43	86.92
2	Pf 19	58.04	12.59	11.44	105.02
3	Pf 8	57.70	11.91	6.08	8.96
4	Pf 7	54.60	5.92	10.32	85.30
5	Pf 13	58.84	14.14	7.77	39.25
6	Pf 20	55.50	7.66	6.94	20.79
7	Control	51.55		5.58	
	CD (0.05)	3.69		3.09	

Table 10. Effect of different fluorescent pseudomonad isolates on grain and straw yield under green house condition

Sl. No.	Isolate No.	Grain and straw yield, g			
		Grain yield hill^{-1}	Per cent increase over control	Straw yield hill^{-1}	Per cent increase over control
1	Pf 16	33.20	30.20	58.57	32.58
2	Pf 19	29.47	15.69	52.10	17.87
3	Pf 8	31.23	22.35	54.17	22.62
4	Pf 7	31.67	22.75	55.45	25.34
5	Pf 13	31.87	25.10	55.13	24.66
6	Pf 20	31.10	18.04	51.20	15.84
7	Control	25.50	-	44.20	-
	CD (0.05)	2.48		7.52	

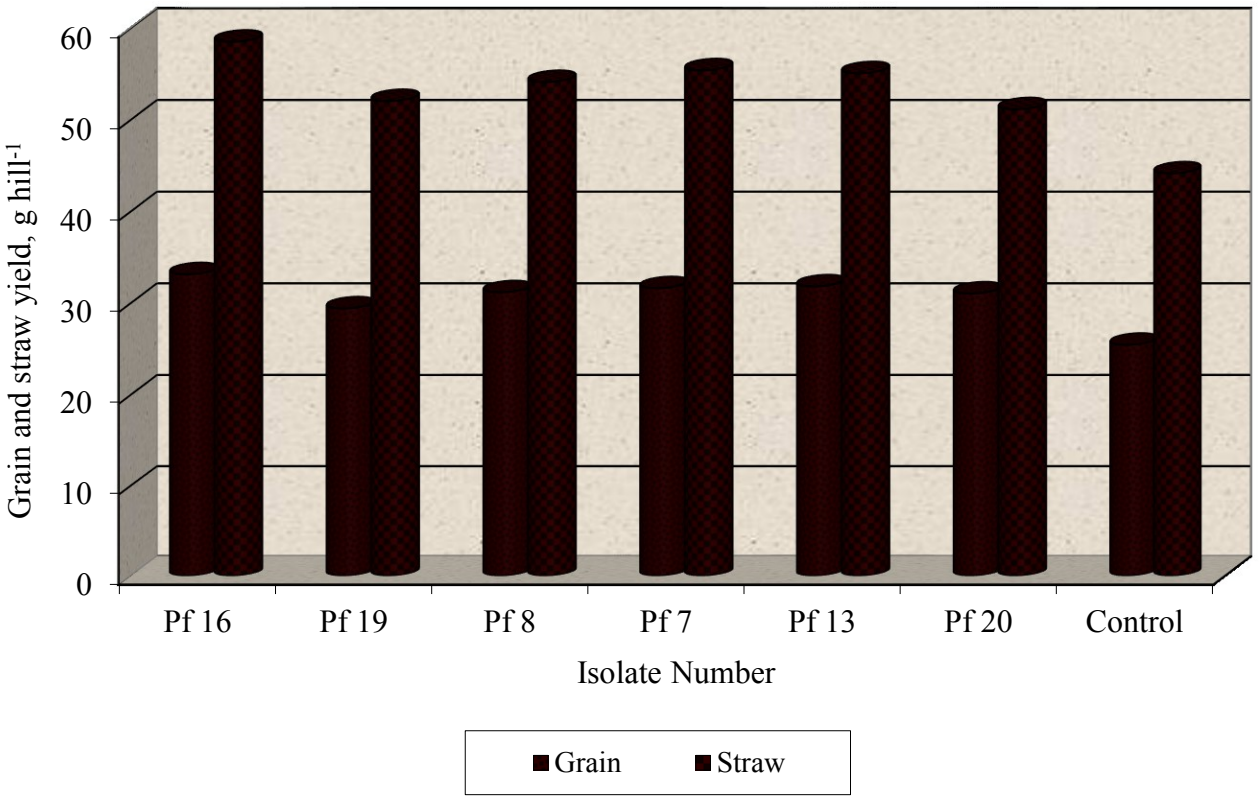


Fig. 4 Effect of different fluorescent pseudomonad isolates on grain and straw yield in plot culture experiment

increase over control was 30.20 for Pf16, 25.10 for Pf13 and 22.75 for Pf7.

For straw yield also Pf16 yielded maximum (58.57) followed by Pf7 (55.45), Pf13 (55.13) and Pf8 (54.71). The lowest value of 51.20 was recorded for Pf20. But all the isolates are statistically on par among treatments and significantly superior over control (44.20). The increase in percentage of straw yield over control in the case of Pf16 was 32.58, which was followed by Pf7 and Pf13 (25.34 and 24.66 respectively).

4.9.6 Evaluation of Six Selected Pseudomonad Isolates on 1000-Grain Weight

Thousand-grain weight was maximum for the isolates Pf7 (22.83) followed by Pf16 (22.32) (Table 11). In isolates Pf19 and Pf8, the thousand grain weight values were less than those obtained in control. In terms of percentage increase over control, the isolates Pf7 and Pf16 recorded 8.15 and 5.73 respectively. The isolate Pf7 was found to be significantly superior over control. All the other isolates were on par.

4.10 FIELD EVALUATION OF PROMISING ISOLATE OF FLUORESCENT PSEUDOMONADS (Pf16) AGAINST SHEATH ROT DISEASE

From *in vitro* and *in vivo* evaluation of different Pseudomonad isolates, Pf16 was found to be the best one, in the following aspects *i*.e., inhibition zone formation, grain and straw yield, shoot length, plant height etc. Considering all these factors Pf16 was selected and it was further evaluated under field conditions.

The talc-based formulation of this isolate (Pf16) was applied in different combinations as seed treatment, seedling root dip and foliar spray. After all the treatment as per the technical programme, artificial inoculation with the pathogen (*Sarocladium oryzae*) was done at boot leaf stage and the disease intensity was recorded. The lowest disease index (9.27) was recorded for those plots which received all the three methods of *Pseudomonas* treatment with Pf16 *viz*., seed treatment + seedling root dip + foliar spray (Table 12). Those plots in which

Plate 13. Field affected with sheath rot disease

Table 11. Effect of different pseudomonad isolates on 1000-grain weight

Sl. No.	Isolate No.	Thousand grain weight, g	Per cent increase over control
1	Pf 16	22.32	5.73
2	Pf 19	20.85	-1.23
3	Pf 8	20.37	-3.51
4	Pf 7	22.83	8.15
5	Pf 13	21.19	0.38
6	Pf 20	20.43	-3.22
7	Control	21.11	-
	CD (0.05)	1.44	

Table 12. Effect of different treatment combinations of promising isolates Pf16 on disease suppression

Sl. No.	Treatments	Disease index	Per cent decrease over control
1	ST	11.92	27.55
2	SRD	20.93	25.56
3	FS	41.85	25.83
4	ST + SRD	24.23	27.27
5	ST + FS	16.11	28.39
6	SRD + FS	30.18	26.03
7	ST + SRD + FS	9.27	28.30
8	Chemical	19.26	27.59
9	Control	55.36	27.85
	CD (0.05)	9.78	

Pseudomonas treatment was given as seed treatment, the disease index was 11.92 followed by those plots, which received seed treatment and foliar application (16.11). All the treatments were statistically significant over control.

The three treatments *viz*., seed treatment, seed treatment + foliar spray and seed treatment + seedling root dip + foliar spray were on par in suppressing the disease intensity.

4.10.1 Evaluation of Promising Isolates (Pf16) on Shoot and Root Length

The evaluation of shoot and root length was made at 60 days after transplanting (DAT). At 60 DAT plots that received seedling root dip and foliar spray together gave maximum shoot length of 84.58 cm followed by seed treatment + seedling root dip + foliar spray (79.72 cm) (Table 13). In carbendazim treated plots the shoot length recorded was 78.74 cm, which was on par with control. The observation on root length recorded maximum in the treatment, which received seed treatment (13.93 cm) and foliar spray (ST + FS) (13.93 cm), followed by seed treatment and foliar spray (13.36 cm), seed root dip + foliar spray (SRD + FS) (12.79 cm), carbendazim (12.78 cm) and seed treatment + seedling root dip + foliar spray (ST + SRD + FS) (12.23 cm). The root length recorded for control was 10.79 cm. Both shoot length and root length were not significant over control for any of the treatments at 60 DAT (Table 13).

4.10.2 Evaluation of Promising Isolate on Number of Tillers

The effect of different treatments on the number of tillers was studied. This study was done both at active tillering stage and at panicle initiation stage. Those plots which received seed treatment + seedling root dip + foliar spray (ST + SRD + FS) as their treatments recorded maximum number of tillers (7.02) at active tillering stage, followed by seedling root dip (SRD) (6.70), and carbendazim (6.46) whereas lowest

Table13. Effect of the promising isolate Pf 16 on shoot and root length at 60 DAT

Sl. No.	Treatments	Shoot length, cm	Per cent increase over control	Root length, cm	Per cent increase over control
1	ST	76.94	10.20	12.13	12.42
2	SRD	77.94	11.63	11.73	8.71
3	FS	76.53	9.60	13.36	23.82
4	ST + SRD	75.34	7.91	11.45	6.12
5	ST + FS	74.19	6.24	13.93	29.10
6	SRD + FS	84.58	7.43	12.79	18.53
7	ST + SRD + FS	79.72	14.18	12.23	13.34
8	Chemical	78.74	14.18	12.78	18.44
9	Control	70.82	-	10.79	-
	CD (0.05)	9.86		2.51	

Table 14. Effect of promising isolates pf16 on number of tillers at field condition

Sl. No.	Treatments	Number of tillers			
		At active tillering stage	Per cent increase over control	Panicle initiation stage	Per cent increase over control
1	ST	5.88	27.55	10.91	38.98
2	SRD	6.70	45.34	10.07	28.28
3	FS	5.63	22.13	10.23	30.31
4	ST + SRD	6.05	32.32	11.32	44.20
5	ST + FS	5.44	18.00	7.87	0.25
6	SRD + FS	5.68	23.21	7.98	27.13
7	ST + SRD + FS	7.02	52.28	11.67	48.66
8	Chemical	6.46	40.13	10.59	34.90
9	Control	4.62	-	7.85	-
	CD (0.05)	0.90		0.59	

number of tillers were recorded in seed treatment + foliar spray (ST+FS) (5.44). Control plots recorded 4.62 (Table 14)

These three treatments *viz.,* seed treatment+ seedling root dip + foliar spray (ST +SRD+FS), seedling root dip (SRD) and chemical treatments were on par. A total of 52.28 per cent increase in no.of tillers over control was recorded for seed treatment + seedling root dip + foliar spray (ST+SRD+FS) followed by seedling root dip (SRD) (45.34) and chemical treatment (40.13).

At panicle initiation stage also seed treatment + seedling root dip + foliar spray (ST + SRD + FS) gave maximum value (11.67) among the treatments with respect to number of tillers followed by seed treatment and seedling root dip (ST + SRD). The lowest among the treatments was recorded in seed treatment + foliar spray (ST + FS), that is 7.87. In control the value was 7.85. There was an increase of 48.66 per cent in number of tillers over control in seed treatment + seedlings root dip + foliar spray (ST + SRD + FS), 44.20 per cent for seed treatment + seedling root dip (ST + SRD) and 38.98 per cent for seed treatment alone (ST). In general at both the stages of the crop *i*.e., at active tillering and panicle initiation, the combined application of seed treatment + seedling root dip + foliar spray (ST + SRD + FS) was significantly superior to all other treatments (Table 14).

4.10.3 Evaluation of Promising Isolate Pf16 on Plant Height at 45 DAT

Plant height at 45 days after transplanting (DAT) was recorded for all the treatments (Table 15). Seed treatment + seedling root dip + foliar spray (ST + SRD + FS) recorded maximum height of 60.58 cm followed by seed treatment + seedling root dip (ST + SRD) 60.54 cm and seed treatment (ST) 60.10 cm. The chemical (carbendazim) applied plots recorded the minimum plant height among treatments (56.4 cm). Control plots recorded 53.26 cm. The percentage increase over control with respect to plant height in different treatments is as follows. In seed

treatment + seedling root dip + foliar spray (ST + SRD + FS) it was 13.74 in seed treatment + seedling root dip (ST + SRD) it was 13.67 and in seed treatment alone (ST) 12.84. Statistically also these treatments *viz*., seed treatment + seedling root dip + foliar spray (ST + SRD + FS), seed treatment + seedling root dip (ST + SRD), seed treatment (ST), foliar spray (FS) and seedling root dip (SRD) were significantly superior over control. The other treatments were on par with control.

4.10.4 Evaluation of Different Methods of Application of Promising Isolate on Number of Panicles

The number of panicles per hill was counted at panicle emergence stage. The number of panicles was maximum (11.27) for plots given with seed treatment + seedling root dip + foliar spray (ST + SRD + FS) followed by seed treatment + foliar spray (ST + FS) (11.04) and seed treatment + seedling root dip (ST + SRD) (10.97) (Table 15). The lowest number was recorded in the treatment seedling root dip (SRD) (9. 44), whereas in control, it was 7.56. The percentage increase over control was 48.81 in seed treatment + seedling root dip + foliar spray (ST + SRD + FS) followed by 46.3 in seed treatment + foliar spray (ST + FS), 45.11 in seed treatment + seedling root dip (ST + SRD). All the above three treatments were statistically significant over control.

4.10.5 Effect of Pseudomonad Isolates (Pf16) on Grain and Straw Yield

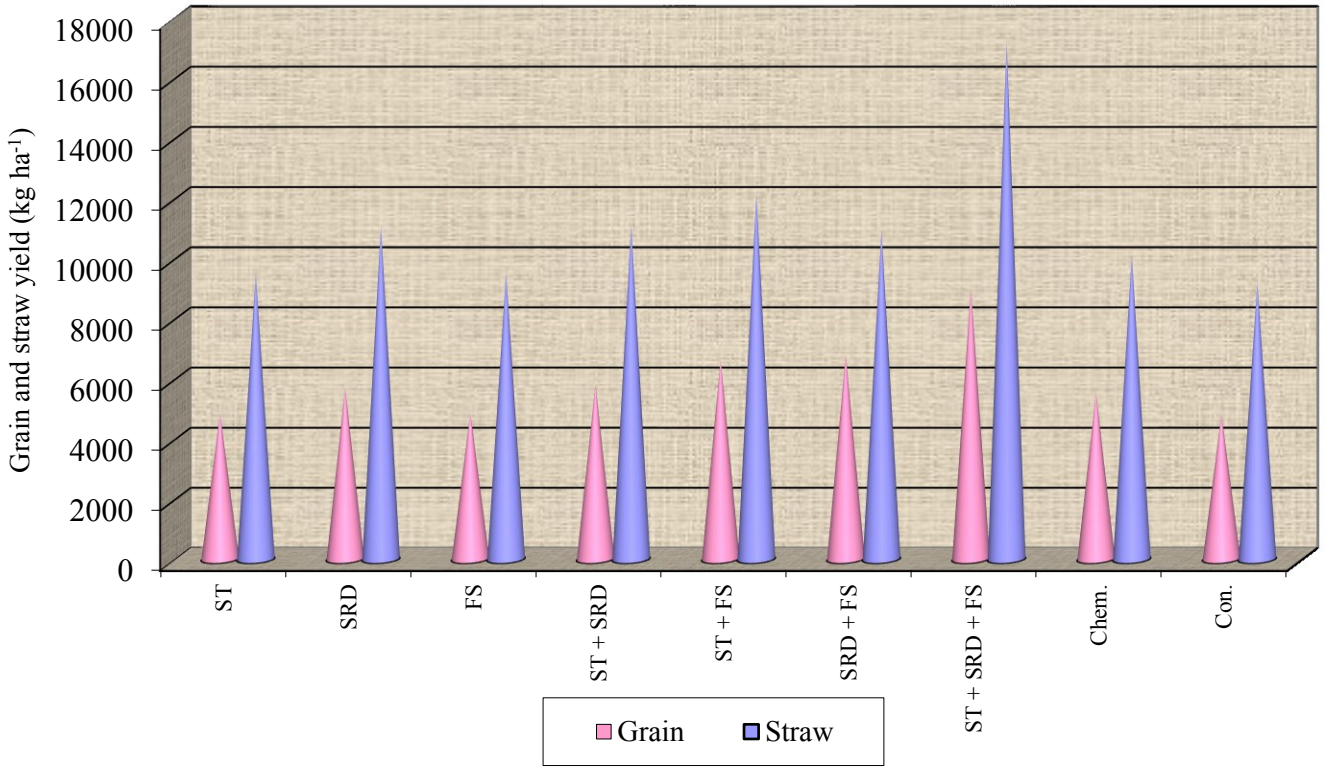
Grain yield was maximum (5.43 kg) for the plots that received seed treatment + seedling root dip + foliar spray (ST + SRD + FS) followed by seedling root dip + foliar spray (SRD + FS) (4.10 kg) and seed treatment + foliar spray (ST + FS) (4.00 kg) . The lowest grain yield was recorded in treatment that received foliar spray (FS) 2.93 kg and in seed treatment (ST) 2.93 kg. In control plots grain yield was 2.90 kg, whereas the grain yield of other treatments *viz*., Seedling root dip (SRD), seed treatment + seedling root dip (ST + SRD) and carbendazim treated plots recorded values between 2.9 to 3.5 (Table 16,Fig 5). The increase

Table.15 Effect of promising isolate on plant height (at 45 DAT) and number of panicle

Sl.No.	Treatments	Plant height, cm	Per cent increase over control	Number of panicles	Per cent increase over control
1	ST	60.10	12.84	10.58	39.85
2	SRD	59.41	11.55	9.44	24.87
3	FS	59.82	12.32	10.24	35.45
4	ST + SRD	60.54	13.67	10.97	45.11
5	ST + FS	58.57	9.97	11.04	46.03
6	SRD + FS	58.36	9.58	10.03	32.67
7	ST + SRD + FS	60.58	13.74	11.12	48.81
8	Chemical	56.41	5.91	10.47	38.49
9	Control	53.26	-	7.56	-
	CD (0.05)	5.28		1.27	

16. Effect of the promising isolate Pf16 on grain and straw yield

Sl. No.	Treatment	Grain yield, kg	Per cent increase over control	Straw yield, kg	Per cent increase over control
1	ST	4883.33	1.03	9500.00	3.64
2	SRD	5716.66	17.24	11116.66	21.82
3	FS	4883.33	1.03	9550.00	3.64
4	ST + SRD	58330.00	20.69	11116.66	21.82
5	ST + FS	6666.66	37.93	12166.66	32.73
6	SRD + FS	6833.33	41.38	10950.00	20.00
7	ST + SRD + FS	9050.00	86.21	17216.00	87.27
8	Chemical	5550.00	13.80	10116.66	10.91
9	Control	4833.00		9216.66	-
	CD (0.05)	1169.80		1914.00	



ST: Seed treatment, SRD: Seedling root dip, FS: Foliar spray, Chem: Chemical (Carbendazim), Con : Control

Fig. 5 Effect of the promising isolate Pf16 on grain and straw yield in field experiment

in grain yield for seed treatment seedling root dip + foliar spray (ST + SRD + FS) over control was 86.21 per cent, whereas all other treatments were below 50 per cent *i*.e., in seedling root dip + foliar spray (SRD + FS) 41.38 and seed treatment + foliar spray (ST + FS) 37.93 etc.

Straw yield was also maximum in seed treatment + seedling root dip + foliar spray (ST + SRD + FS) 10.23 kg, followed by seed treatment + foliar spray (ST + FS) 7.30 kg, seedling root dip (SRD) 6.67 kg, seed treatment + seedling root dip (ST + SRD) 6.67 kg and seedling root dip + foliar spray (SRD+ FS) 6.57 kg and the value recorded in control was 5.53 kg. In terms of percentage increase over control, in seed treatment + seedling root dip + foliar spray (ST + SRD + FS) it was 87.27, whereas the increase over control by other treatments was in the following order. Seed treatment + foliar spray (ST + FS) 32.73 per cent, seed treatment + seedling root dip (ST + SRD) 21.82 per cent, and seedling root dip + foliar spray (SRD + FS) 20 per cent.

When grain and straw yield data were analyzed, seed treatment + seedling root dip + foliar spray (ST + SRD + FS) was statistically significant over control whereas the performance of seed treatment (ST), foliar spray (FS) and carbendazim treated plots were on par with the control.

4.10.6 Evaluation of Promising Isolate Pf16 on 1000-Grain Weight

The thousand grain weight was maximum in plots which received seed treatment + foliar spray (ST + FS) 28.39 g followed by seed treatment + seedling root dip + foliar spray (ST + SRD + FS) 28.30 g, carbendazim 27.59 g, seed treatment (ST) 27.55 g and seed treatment + seedling root dip (ST + SRD) 27.27 g. The lowest value was in seedling root dip (SRD) 25.56 g whereas in control was 27.85 g. (Table 17).

Table 17. Effect of promising isolate Pf16 on 1000-grain weight

Sl. No.	Treatment	1000 grain weight, g	Per cent increase over control
1	ST	27.55	-1.08
2	SRD	25.56	-8.22
3	FS	25.83	-7.25
4	ST + SRD	27.27	-2.08
5	ST + FS	28.39	1.94
6	SRD + FS	26.03	-6.54
7	ST + SRD + FS	28.30	1.62
8	Chemical	27.59	-0.93
9	Control	27.85	-
	CD (0.05)	0.93	

Table 18. Characterisation of isolates Pf16, Pf19 and Pf8 based on biochemical tests under laboratory conditions

Test	Pf 16	Pf 19	Pf 8
Levan formation	+	-	+
Gelatin liquefaction	+	+	+
Growth at 4°C	+	-	+
Growth at 41°C	-	+	-
Utilisation of ethanol	+	+	-
Utilisation of propylene glycol	+	+	-
Utilisation of N-butyl amine	-	-	-
Utilisation of phenyl acetate	-	-	-

4.11 CHARACTERISATION OF BACTERIAL ISOLATES ANTAGONISTIC TO THE RICE PATHOGEN *SAROCLADIUM ORYZAE*

The cultural, physiological and biochemical characteristics of the best three pseudomonad isolates were studied.

4.11.1 Cultural Character of the Isolates

All the three isolates, Pf16, Pf19 and Pf8 studied were gram negative, slightly curved and rod shaped. Colonies formed were white to cream to yellow coloured, pigmented and produced extra cellular fluorescent pigments around them. The motility was possible by the presence of one to several polar flagella.

4.11.2 Biochemical Characteristics of the Bacterial Isolates

Biochemical characteristics of the isolates Pf16, Pf19 and Pf18 were given under Table 18.

The isolate pf16 showed positive growth for levan formation, gelatin, liquefaction, utilization of ethanol and propylene glycol but Pf19 showed negative growth for leaven formation, but other two characters were similar to Pf16. Whereas Pf8 showed negative growth for the utilization of ethanol, propylene glycol, and positive growth for levan formation and gelatin liquefaction. All the three isolates showed negative growth for n-butyl amine and phenyl acetate.

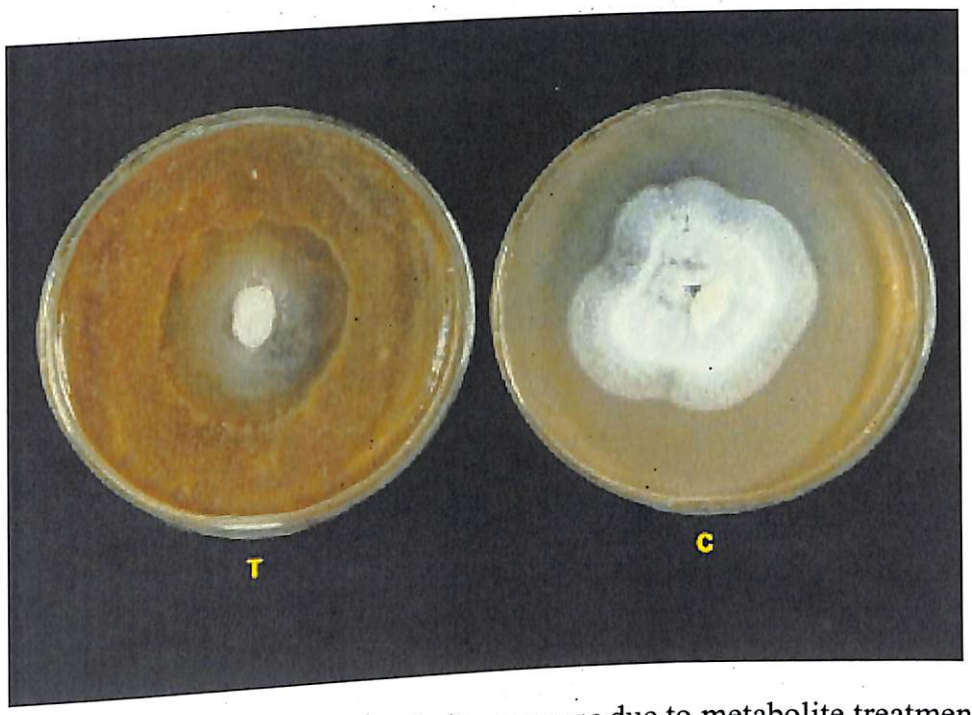
4.11.3 Physiological Characters of the Isolate

The isolate Pf16 and Pf8 showed positive growth only at 4^0C whereas Pf19 showed positive growth only at 41^0C.

From the above tests, the isolates Pf16 can be assumed for *Pseudomonas fluorescens* biovar II and Pf19 and Pf8 can be assumed to be *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar I.

4.12 TESTING THE METABOLITE OF THE SELECTED PSEUDOMONAD ISOLATE

The metabolite of the best isolate, Pf16 extracted by the procedure mentioned under 3.12 formed a clear zone of inhibition around the pathogen (*Sarocladium oryzae*) at the centre of the disc whereas control plate showed unrestricted growth of *Sarocladium oryzae* (Plate 14).



T – Restricted growth of *Saracladium oryzae* due to metabolite treatment
C – Unrestricted growth of *Saracladium oryzae* not treated with metabolite

Plate 14 Effect of the metabolite produced by Pf16 on the growth of *Saracladium oryzae*

Discussion

5. DISCUSSION

Sheath rot of rice incited by *Sarocladium oryzae,* (Sawada). Gams and Hawksworth is a disease gaining attention of farmers in the rice growing tracts of southern states especially Kerala in recent time, since it causes severe yield loss. The constraints in disease control especially those involving chemicals due to their broad-spectrum toxicity, ecological hazards and their expensive nature have necessitated alternative methods of control. For over several years, biological control has been gaining importance and recognition as an alternative method in the control of various diseases, since it has been demonstrated to possess excellent fungicidal properties and specific to targeted pathogen and safe to environment too. So, for the management of sheath rot pathogen biological control was considered as a useful alternative management strategy. Moreover breeding for sheath rot resistance has not solved the problem, due to the fact that inherent level of host resistance in rice due to sheath rot pathogen is very low. However, research on the management of foliar diseases, using fluorescent pseudomonads has been initiated only a few years back (Austin *et al*., 1977; Blakeman and Fokkema, 1982; Mew and Rosales, 1986; Levy *et al*., 1988; Velazhahan *et al*., 1999; Smitha, 2000; Nandakumar *et al*., 2001). Although intensive studies are being carried out in India for the control of plant pathogens using fluorescent pseudomonades, practically not much work has been reported from Kerala on the management of crop disease using this group of bacterium.

Keeping this in view the present study was carried out and the results of the investigation are discussed below.

In the present study the rice sheath rot pathogen *Sarocladium oryzae*, was isolated from naturally infected rice plants collected from Cropping System Research Centre (CSRC), Karamana, Thiruvananthapuram. Pathogenicity was proved and the pure culture was maintained on PDA.

The identity of the pathogen was done by comparing the characters reported by other workers (Sawada, 1922; Tasugi and Ikeda, 1956; Chen, 1957; Ou, 1963; Jiminez and Panizo, 1977; and Krishnakumaran Nair, 1986).

The morphology of the *Sarocladium oryzae* isolated was studied and a slight variation was seen in morphology with respect to conidiophore length, size of conidia and colour of the colony, when compared to morphological characters described by previous workers. Tasugi and Ikeda (1956) obtained conidial measurement of 2.1 – 8.5 x 0.5 – 1 µm and 1.8 – 13 x 1 – 1.6 µm from host plant and culture respectively. Ou (1972) obtained mycelium of 1.5 – 2.0 µm dia and conidia of 4.0 to 9.0 x 1.0 to 2.5 µm. Nair and Sathyarajan (1976) described the *Sarocladium oryzae* with mycelial thickness of 1.25 – 2.0 µm and conidial measurement of 3.5 – 7.0 x 1.0 – 1.5 µm from host and 4.0 – 8.0 x 1.0 x 1.5 µm from culture.

Cultural characteristics of the *Sarocladium oryzae* were studied by growing it on different solid and liquid media in the present study. Among the different solid media tested, carrot agar medium was the best one which supported maximum growth of mycelium (8.78 cm) and the best liquid medium which supported maximum growth (in terms of dry weight of biomass) was Richard's broth (0.3870 g). Different workers obtained differential growth of *Sarocladium* fungus on the same set of media used. This may be due to difference in the response of the fungus to the carbon and nitrogen sources present in the media as suggested by Chen (1957). According to Prabhakaran *et al*. (1973), it was PDA, which supported better growth of *Sarocladium oryzae* over other media. Mohan and Subramanian (1978) reported PDA and oats agar medium as the best solid medium and Czapek's Dox broth as the best liquid medium. Shahjahan *et al*. (1977) reported PDA as the best medium, over glucose yeast extract agar, corn meal agar, lima bean agar and malt extract agar. Alagarsamy *et al*. (1994) and Revathy *et al*. (2002) reported that Czapek's Dox broth supported maximum mycelial dry weight among different broth cultures tested.

In the present study, a search for potential fluorescent pseudomonad isolates for the management of *Sarocladium oryzae* was carried out. Among the 20 different isolates of fluorescent pseudomonads collected from different locations of Kerala the isolate Pf16 was found to be the best one. The fluorescent pseudomonads were collected from the healthy rice plants following Cook and Baker (1983). Of the total 20 isolates of fluorescent pseudomonades collected during the present study, six best isolates were forwarded for further study under green house condition based on inhibition zone formation following dual culture technique (Johnson and Curl, 1972; Utkhede and Rahe, 1983; Laha and Venkataraman, 2001; Ramamoorthy and Samiyappan, 2001). In this study antagonistic bacterium was streaked equally away from *Sarocladium oryzae* at the centre of the petridish containing KMB agar in a form of triangle. This method was earlier used successfully by Prashanthi *et al*. (2000) against *Macrophomina phaseolina* under *in vitro* condition. Usually the *in vitro* antagonism of bacteria can be taken as clue for their capacity to suppress the pathogen *in vivo,* and such isolates can be selected for further evaluation. In this study isolates Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 were selected in this manner for further evaluation under green house conditions. Several workers have reported the positive correlation between *in vitro* antibiosis and biocontrol. (Alconero, 1980; Uthkhede, 1984; Galindo, 1992) on the other hand lack of correlation between *in vitro* antibiosis and biocontrol was observed during the screening of bacterial antagonist against take all pathogen in wheat and turf grass (Wong and Baker, 1984) and *Stemphylium vesicarium* on pear. From the six isolates under green house condition one best isolate was selected (Pf16) based on the capacity to reduce the disease severity, biometric characters mainly grain yield, accordingly Pf16 was selected for further evaluation under field conditions.

Similar type of *in vitro* studies, pot culture experiment and field experiment with talc based formulation of *Pseudomonas fluorescens* was done successfully against fruit rot of chilli *Colletotrichum capsici*

(Ramamoorthy and Samiyappan, 2001) and against blackgram root rot *Macrophomina phaseolina* (Shanmugam *et al*., 2001). High degree of disease suppression by fluorescent pseudomonads against sheath rot of rice was also reported by many workers (Sakthivel and Gnanamanickam, 1986 a,b; Sakthivel *et al*., 1988; Suparayono *et al*., 1990; Mini, 1995; Radhika *et al*., 1995; Nayar, 1996).

For the pot culture experiment, all the selected isolates were made into talc based formulation following the method of Vidhyasekaran and Muthamilan (1995). Various biometric observations *viz*., shoot and root length, number of tillers, number of panicles, grain and straw yield, 1000-grain weight along with disease index was taken. In the field experiment, using the talc based formulation of the Pf16 isolate different types of treatment *viz*., seed treatment, seedling root dip, foliar spray and combination of either of the two methods and all the three methods together were followed. The plots which received the combination of all the three treatments gave maximum disease suppression, and also enhanced the growth parameters like number of tillers, number of panicles, grain and straw yield, so the use of promising fluorescent pseudomonad isolate Pf16 improved the crop health and growth. Application of talc based formulation of fluorescent pseudomonads as seed treatment + seedling root dip + foliar spray was effective in controlling the sheath blight of rice (Kamala and Vidhyasekaran, 1997; Heera, 2002).

Talc based formulation of the fluorescent pseudomonads were used in the earlier studies also (Vidhyasekaran and Muthamilan, 1995; Shanmugham *et al*., 2001; Ramamoorthy and Samiyappan, 2001; Heera, 2002). Earlier the disease suppression using fluorescent pseudomonads were carried out using cell suspension of the bacteria only (Mew and Rosales, 1986; Sakthivel and Gnanamanickam, 1987; Devi *et al*., 1989; Lee *et al*., 1990; Gnanamanickam and Mew, 1992). Bacterial suspension was used for seed treatment, soil application and foliar spray .For this two-day-old culture was required, which is impractical to supply to farmers regularly.

The efficacy of fluorescent pseudomonads as cell suspension in the control of rice sheath blight (Mew and Rosales, 1986) and groundnut root rot has been reported already. Moreover, bacteria as cell suspension cannot be used for larger area (Capper and Higgins, 1993) and with the passage of time, the cell suspension applied to the soil may get denatured by soil microorganisms or get fixed to the clay particles (Lam and Gaffney, 1993) and hence the effect seem to be overwhelmed by the pathogen. Therefore, it became necessary to develop a formulation, which supports the longer shelf life without any loss in efficacy. In this aspect different workers tried different carrier materials to formulate a useful commercial product of fluorescent pseudomonads (Kloepper and Schroth, 1981; De Freitas and Germida, 1992). Talc as a carrier material proved to be an effective formulation, which supported longer shelf life of bacterial cells (Vidhyasekaran *et al*., 1997). In this study the shelf life of promising isolate Pf16 on talc-based product was studied for over a period of 90 days,and it was found that it retained nearly 45 per cent of the initial population on 90^{th} day. Muthamilan (1994) recorded the survival of bacterial cells in talc based formulation even up to 240 days of storage, but the population started declining after one month of storage. Krishnamoorthy and Gnanamanickam (1998) reported shelf life of *Pseudomonas putida* upto a period of ten months in the formulation containing talc. Sivakumar *et al*. (2000) observed that *Pseudomonas fluorescens* maintained the highest population level of 18.3×10^7 after 40 days of storage in talc compared to storage in other carrier materials. Heera (2002) reported the good survival of fluorescent pseudomonad isolates P11 and P33 upto 75 days, maintained as talc based formulation under room temperature. Moreover, talc based formulation can be transported to a long distance without any difficulty and compatible with seed treatment fungicides like thiram and carbendazim (Vidhyasekaran and Muthamilan, 1995).

By and large the present study revealed the usefulness of talc based formulation of the pseudomonad isolates which could improve the crop

growth besides disease suppression. This gives immense scope for the utilization of this formulation using other antagonists in various diseases of other crops as well.

Various cultural, physiological and biochemical studies were conducted for the isolates Pf16, Pf19 and Pf8 which showed good diseases suppression at *in vitro* condition. A comparison was made with the taxonomic grouping of plant associated saprophytic fluorescent pseudomonas sp. (Schaad, 1992) showed Pf16, Pf19 and Pf8 as *Pseudomonas fluorescence* biovar II, *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* biovar I respectively. Stolp and Gadkari (1981) used the biochemical tests like gelatin liquefaction, levan production along with other tests like oxidase test, catalase test etc for the species identification of fluorescent pseudomonads. Heera (2002) identified P_{11} and P_{33} as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar I among 33 different isolates of fluorescent pseudomonads using the same set of biochemical, physiological and cultural tests used in the present study.

Culture filtrates (metabolite) of the promising isolate Pf16 was extracted and good suppression of sheath rot pathogen was obtained under *in vitro* condition. Similar extraction of cell free culture filtrates of *Pseudomonas fluorescens* suppressed the growth of *Septoria tritici* (Mehdizadegan and Gough, 1987). Extract of *Pseudomonas aeruginosa* reduced the mortality rate of plants affected by *Sclerotium rolfsii* up to 53.3 per cent (Singh and Dwivedi, 1987). Culture filtrates of *Pseudomonas fluorescens* completely inhibited the germination and reduced the virulence of sclerotia of *Rhizoctonia solani* (Rabindran, 1994) and inhibited the growth of *Pythium ultimum, Rhizoctonia solani* and *Erwinia carotovora* sub sp. (Nowak-Thomson *et al*., 1994) against *S. vesicarium* (Montesinos *et al*., 1996). The effectiveness of culture filtrates in biocontrol poses immense potentiality for development of a commercially viable product, which can be formulated as an eco-friendly fungicide (Jubina, 1997).

From the present investigation it is evident that isolate Pf16 is effective in suppressing sheath rot incidence as cell free culture filtrate under *in vitro* condition and as talc based formulated product under green house and field conditions. Moreover, the results showed the dual role of the isolate Pf16 as biocontrol agent and plant growth promoter. Several other workers have highlighted the role of PGPR in reducing the plant disease and improve the crop growth (Harris *et al*., 1994; Carruthers *et al*., 1995; Wei *et al*., 1996).

Further work on elucidation of the exact mechanism of biocontrol, interaction effects of different antagonists, their spectrum of activity, development of fungicide resistant/tolerant strain and effective delivery systems would also be necessary. Even though the potent bacterial antagonist evolved through the present study cannot be taken as a sole means of protecting the rice crop against sheath rot disease, it can be taken as an important component in the integrated disease management strategy.

Summary

6. SUMMARY

Sheath rot disease of rice caused by *Sarocladium oryzae* is one of the major diseases of rice in Kerala. It affects the crop mainly after tillering leading to heavy economic loss. Even though chemical control methods are partially successful, high cost of chemicals and hazards of environment pollution, forced the farming community to adopt an alternate strategy. One of the most important of all the options is biological control, which can combat the disease effectively, and eco friendly too. So the present study was undertaken with a view to evaluate the fluorescent pseudomonads for the management of sheath rot disease of rice. The salient features of various aspects of the study are as follows.

Virulent culture of the pathogen *Sarocladium oryzae* was isolated from the panicles of naturally infected rice plants from the fields of CSRC, Karamana and the pathogenicity was proved by following Koch's postulates. Carrot agar medium was the best medium for its growth in which the radial growth of mycelium was 8.78 cm and the maximum dry weight of biomass was obtained in Richard's broth.

Twenty different isolates of the fluorescent pseudomonads were isolated from rhizosphere and phyllosphere of healthy rice plants collected from various locations of southern Kerala following the standard procedure and pure culture was maintained in KMB slants. All the isolates were tested for their antagonism against sheath rot pathogen by dual plate method using kings B medium agar. A maximum inhibition zone of 11.3 mm was obtained in plates streaked with isolate Pf16. Based on the inhibition zone formation, six best isolates Pf16, Pf19, Pf8, Pf7, Pf13 and Pf20 were selected for further studies under green house condition.

The fluorescent pseudomonad isolates selected from *in vitro* studies were made into talc based formulation following the standard procedure to

enable different methods of application *viz.*, seed treatment, seedling root dip and foliar application at green house and field condition.

The green house experiment was conducted at College of Agriculture, Vellayani. Artificial inoculation of the pathogen was given to incite the disease at boot leaf stage. Various biometric observations like number of tillers, number of panicles and plant height were recorded at appropriate growth stages of the crop. Based on the efficiency of the isolates to suppress the disease and to enhance the growth characters one promising isolate Pf16 was selected for further evaluation under field condition.

Separate field experiment was conducted at CSRC, Karamana, to assess the efficacy of different methods of application of the talc based formulation of the selected isolate Pf16 against the sheath rot disease. Application of selected isolate was done as per the technical programme i.e., three different methods of application in nine combinations and the disease intensity were recorded. The lowest disease intensity was recorded for those plots, which received the combination of all the three methods of application of pseudomonad isolate i.e. seed treatment +seedling root dip + foliar application (ST + SRD + FS). Other biometric observations were recorded at appropriate stages.

Best three isolates i.e., Pf16, Pf19, Pf8 were subjected to various cultural, physiological and biochemical tests. They were Gram negative, slightly curved, rod shaped and formed white to cream to yellow coloured colonies with several polar flagella. Based on the preliminary biochemical tests conducted, the isolates Pf16, Pf19, Pf8 were tentatively identified as *Pseudomonas fluorescens* biovar II, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar I respectively.

The shelf life of the best isolate Pf16 was studied for over a period of 90 days and it was found that it retained nearly 45 per cent of the initial

population at the 90th day. This indicates that we can store the formulated product under room condition for the whole duration of the rice crop.

The metabolite of the best isolate Pf16 was tested against the *Sarocladium oryzae* showed a clear zone of inhibition around the pathogen at the centre of the dish whereas the control plate showed unrestricted growth of the fungal pathogen.

This study forms the first report of application of fluorescent pseudomonads as biocontrol agent for the management of sheath rot disease of rice in Kerala .The result of the study indicates that formulation of *Pseudomonas fluorescens* can be used for large scale field application against sheath rot disease There is immense scope for controlling other rice diseases using this type of biocontrol agents and thereby use of chemical fungicides can be minimized.

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*Original not seen

Appendices

APPENDIX I

Composition of Solid media used

1. Potato Dextrose Agar Medium

Peeled and sliced potato	– 200 g
Dextrose	– 20 g
Agar Agar	– 20 g
Distilled water	– 1000 ml
pH	- 7

2. Czapek's Agar Medium

$MgSO_4.7H_2O$	– 0.50 g
KH_2PO_4	– 1.00 g
KCl	– 0.50 g
$FeSO_4$	– 0.01 g
$NaNO_3$	– 2.00 g
Sucrose	– 30.00 g
Agar agar	– 20.00 g
Distilled water	– 1000 ml
pH	- 7

3. King's Medium B Agar

Peptone	– 20 g
Dihydrogen phosphate	– 1.5 g
Magnesium sulphate	– 1.5 g
Glycerol	– 10 ml
Distilled water	– 1000 ml
Agar agar	– 20 g
pH	- 7

4. Carrot Agar Medium

Peeled and sliced carrot	– 200 g
Agar agar	– 20 g
Distilled water	– 1000 ml
pH	- 7

5. Oat Meal Agar Medium

Oats	– 200 g
Agar agar	– 20 g
Distilled water	– 1000 ml
pH	- 7

Composition of liquid media used

1. Potato Dextrose Broth

Peeled and sliced potato	– 200 g
Dextrose	– 20 g
Distilled water	– 1000 ml
pH	- 7

2. Czapek's Broth

$MgSO_4.7H_2O$	– 0.50 g
KH_2PO_4	– 1.00 g
KCl	– 0.50 g
$FeSO_4$	– 0.01 g
$NaNO_3$	– 2.00 g
Sucrose	– 30.00 g
Distilled water	– 1000 ml
pH	- 7

3. Richard's Broth

KNO_3	– 10.00 g
KH_2PO_4	– 5.00 g
$MgSO_4$	– 0.50 g
NaCl	– 100.00 mg
$CaCl_2$	– 130.00 mg
Sucrose	– 30.00 g
pH	– 7

4. Paddy Leaf Extract Medium

Paddy leaves	- 200.00 g
Distilled water	- 1000.00 ml
pH	- 7

5. King's B Broth

Peptone	– 20.00 g
Dihydrogen phosphate	– 1.5 g
Magnesium sulphate	– 1.5 g
Glycerol	– 10 ml
Distilled water	– 1000 ml
PH	– 7.2

APPENDIX II

Lactophenol-Cotton blue

Anhydrous lactophenol	-67.0Ml
Distilled water	-20.0Ml
Cotton blue	-0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 31 ml glycerol.

Crystal violet

One volume of saturated alcohol solution of crystal violet in four volume of one per cent ammonium oxalate.

Gram's Iodine

Iodine crystals	-1.0 g
Potassium iodide	-2.0 g
Distilled water	-300 ml

Safranin

Ten ml saturated solution os safranin in 100 ml distilled water.

Malachite green

Malachite green	-5 g
Distilled water	-100 ml

Semi Solid Nutrient Agar Medium

Beef extract	-3.0 g
Peptone	-5.0 g
Sodium chloride	-5.0 g
Agar agar	-7.0 g
Distilled water	-1000 ml

APPENDIX III

Composition of basal medium used for biochemical tests

1. Peptone Beef Extract Agar Medium

Peptone	– 10.0 g
Beef extract	– 5.0 g
Sucrose	– 50.0 g
Agar agar	– 20.0 g
Distilled water	– 1000 ml
PH	– 7.0

2. Nutrient Gelatin Medium

Peptone	– 10.0 g
Beef extract	– 5.0 g
Gelatin	– 120.0 g
Agar agar	– 20.0
Distilled water	– 1000 g
PH	– 7.0

3. King's B Broth

Peptone	– 20 g
KHPO4	– 1.5 g
MgSO4.7H2O	– 1.5
Glycerol	– 10.0 ml
Distilled water	– 1000 ml
PH	– 7.2

4. Hayward's Medium

Peptone	- 1.0 g
Ammonium dihydrogen phosphate	- 1.0 g
Potassium chloride	- 0.2 g
Magnesium sulphate	- 0.2 g
Bromothymol blue	- 0.03 g
Agar agar	- 3.0 g
Distilled water	- 1000 ml
pH	- 7.2

EVALUATION OF FLUORESCENT PSEUDOMONADS FOR THE MANAGEMENT OF SHEATH ROT OF RICE

SUNDARAMOORTHY.M

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Kerala Agricultural University, Thrissur

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Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

ABSTRACT

The study entitled 'Evaluation of fluorescent pseudomonads for the management of sheath rot of rice' was carried out at three stages *i.e.,* under *in vitro* condition, green house condition, and field conditions. First two were conducted at the college of Agriculture, Vellayani, and the third one was conducted at CSRC, Karamana.

The pathogen *Sarocladium oryzae* was isolated from the sheath portion of naturally infected rice plants and its identity was confirmed based on the cultural and morphological studies.

Twenty different isolates of fluorescent pseudomonads were isolated from, rhizosphere and phyllosphere of healthy rice plants collected from various locations of southern Kerala. They were screened against *Sarocladium oryzae* under laboratory condition following duel culture technique. Based on the inhibition zone formation six best isolates were selected.

In green house studies, six selected isolates were made into a talc based formulation and applied as seed treatment, seedling root dip and foliar spray. Here, the isolate Pf 16 performed well over other isolates and proceeded further for study under field condition.

In the field, powder based formulation of selected isolate Pf16 in three different methods of application *viz*., seed treatment (ST), seedling root dip (SRD) and foliar spray (FS) in nine combinations were applied. Among the different combinations tried combined application of seed treatment + seedling root dip + foliar spray (ST+SRD+FS) gave considerable reduction in disease incidence and improved biomass yield.

Based on the preliminary biochemical studies conducted the isolates Pf16, Pf19 and Pf8 were tentatively identified as *Pseudomonas fluorescens* biovar II, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* biovar I respectively.

The metabolite of promising isolate Pf16 extracted and it formed a clear inhibition zone around the pathogen when antagonism was done.