EVALUATION OF FLUORESCENT PSEUDOMONADS FOR THE MANAGEMENT OF SHEATH BLIGHT AND BACTERIAL BLIGHT OF RICE (*Oryza sativa* L.)

BY

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THESIS
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DECLARATION

I hereby declare that this thesis entitled "**Evaluation of fluorescent pseudomonads for the management of sheath blight and bacterial blight of rice (*Oryza sativa* L.)**"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 of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

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Certified that this thesis entitled "**Evaluation of fluorescent pseudomonads for the management of sheath blight and bacterial blight of rice (*Oryza sativa* L.)**" is a record of research work done independently by Ms Heera.G, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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CONTENTS

		Page No.
1. INTRODUCTION	...	1
2. REVIEW OF LITERATURE	...	3
3. MATERIALS AND METHODS	...	14
4. RESULTS	...	29
5. DISCUSSION	...	46
6. SUMMARY	...	52
REFERENCES	...	I-XIV
APPENDIX	...	
ABSTRACT	...	

LIST OF TABLES

Sl. No.	Title	Page No
3.1	Characters of plant associated saprophytic fluorescent *Pseudomonas* species	28
4.1	*In vitro* inhibition of fluorescent *Pseudomonas* isolates against *R. solani* on King's medium B and Potato dextrose agar medium after 48 hr	32
4.2	*In vitro* inhibition of fluorescent *Pseudomonas* isolates against *X. oryzae* pv *oryzae* on Potato sucrose agar medium after 48 hr	33
4.3	Survival of fluorescent *Pseudomonas* sp. (P11) in talc based formulation	34
4.4	Survival of fluorescent *Pseudomonas* sp. (P33) in talc based formulation	34
4.5	Effect of seed treatment with P11 isolate of fluorescent *Pseudomonas* sp.on length and dry weight of root and shoot of cv. Jyothi	36
4.6	Effect of seed treatment with P33 isolate of fluorescent *Pseudomonas* sp. on length and dry weight of root and shoot of cv. TN-1	37
4.7	Estimation of IAA given as Spectrophotometer readings (Standard curve)	39
4.8	Effect of different methods of application of talc based formulation of fluorescent *Pseudomonas* isolate P11 on the management of sheath blight disease under green house conditions	41
4.9	Effect of different methods of application of talc based formulation of fluorescent *Pseudomonas* isolate P33 on the management of bacterial blight disease under green house conditions	43
4.10	Characters of isolate P11 and P33 of fluorescent *Pseudomonas* sp.	45

LIST OF FIGURES

Fig. No.	Title	Between Pages
4.1	Survival of fluorescent *Pseudomonas* sp. (P11) in talc based formulation	34-35
4.2	Percentage reduction of population of P11 in talc based formulation during storage	34-35
4.3	Survival of fluorescent *Pseudomonas* sp. (P33) in talc based formulation	34-35
4.4	Percentage reduction of population of P33 in talc based formulation during storage	34-35
4.5	Standard curve of IAA	39-40
4.6	IAA production by P11 and P33	39-40
4.7	Efficacy of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P11) on management of sheath blight disease intensity	41-42
4.8	Effect of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P11) on the biomass of plants	41-42
4.9	Efficacy of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P33) on management of bacterial blight disease intensity	43-44
4.10	Effect of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P33) on the biomass of plants	43-44

LIST OF PLATES

Plate No.	Title	Between Pages
4.1	Effect of fluorescent *Pseudomonas* sp. (P11) in inhibiting growth of *Rhizoctonia solani in vitro*	33-34
4.2	Effect of fluorescent *Pseudomonas* sp. (P33) in inhibiting growth of *Xanthomonas oryzae* pv *oryzae in vitro*	33-34
4.3	Effect of seed treatment with talc based formulation of P11 on increasing root and shoot length of rice seedlings (cv. Jyothi)	37-38
4.4	Effect of seed treatment with talc based formulation of P33 on increasing root and shoot length of rice seedlings (cv. TN-1)	37-38
4.5	Effect of application of talc based formulation of P11 by combination of seed treatment, seedling root dipping and foliar spray on development of sheath blight under green house condition	41-42
4.6	Leison development on the sheath of rice (cv. Jyothi)	41-42
4.7	Effect of application of talc based formulation of P33 by combination of seed treatment, seedling root dipping and foliar spray on development of bacterial blight under green house condition	43-44

LIST OF ABBREVIATIONS

%	Per cent
µ	Micro
^{0}C	Degree Celsius
ANOVA	Analysis of variance
CD	Critical difference
cfu	Colony forming units
cm	Centimeter
cv	Cultivar
DAS	Days after sowing
et al	And others
Fig	Figure
g	Gram
hr	Hours
IAA	Indole acetic acid
KMB	King's medium B
ml	Millilitre
nm	Nanometer
PDA	Potato dextrose agar
PSA	Potato sucrose agar

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple food crop of India which is cultivated over an area of 42.1mha with annual production of 82.3mT (1997-1998). Intensive cultivation of heavy tillering varieties which greatly respond to nitrogenous fertilizers has lead to the severe incidence of several diseases of this crop. Indiscriminate use of chemical pesticides to combat pests and diseases affecting rice has produced deleterious effects on the environment including pollution hazards. Due to lack of genetic diversity for disease resistance no single cultivar is suitable for the different rice growing tracts thus making biological control an additional option in disease management.

The use of microbial antagonists in management of crop diseases has now become a well established practice in view of the hazardous impact of pesticides in the ecosystem. Strains of fluorescent pseudomonads have long reigned to be the foremost among these biocontrol agents particularly in rice because of their capacity to survive under anaerobic soil conditions in which rice is normally grown and also on the phylloplane of the crop.

Sheath blight (*Rhizoctonia solani*) and bacterial blight (*Xanthomonas oryzae* pv *oryzae*) are the two most serious diseases which affects rice cultivation in Kerala. At present these diseases are managed only by the use of chemicals which are locally available. Efficacy of fluorescent pseudomonads for the control of sheath blight and bacterial blight diseases has been successfully demonstrated under field conditions (Muthamilan, 1994, Kamala, 1996). In

view of the urgent need to protect the ecosystem, especially because these pesticides are directly reaching the water ways, use of these efficient bacterial antagonists seems to be a suitable component in the biocontrol of these major diseases of rice.

The scope of utilizing ecofriendly and natural inhabitants of the rice ecosystem such as fluorescent pseudomonads has not been investigated so far in Kerala. Hence the present study was carried out with the following objectives :

- Evaluating fluorescent pseudomonads isolated from rhizosphere and phylloplane of rice plants against sheath blight and bacterial blight pathogens both *in vitro* and *in vivo*.
- Development of a suitable talc based formulation of the selected isolates for assessing their efficacy in the management of sheath blight and bacterial blight diseases.

Review of Literature

2. REVIEW OF LITERATURE

Fluorescent *Pseudomonas* spp. have emerged as the largest and potentially the most promising group of plant growth promoting rhizobacteria (PGPR) which can effectively control many soil borne plant pathogens (Kloepper and Schroth, 1978). 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).

2.1 Biocontrol of plant pathogens

Strains of fluorescent *Pseudomonas* have long reigned to be the foremost among biological control 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). Exhaustive studies were conducted for the control of the soil borne diseases using fluorescent *Pseudomonas* (Howell and Stipanovic, 1979; Sneh *et al.*, 1984; Gutterson *et al.*, 1986; Stutz *et al.*, 1986; Ganesan and Gnanamanickam, 1987; Lifshitz *et al.*, 1987; Walther and Gindart, 1988; Anuratha and Gnanamanickam, 1990; Callan *et al.*, 1990; Van Peer *et al.*, 1991; Lemanceau *et al.*, 1992; Gamliel and Katan, 1993; Capper and Higgins, 1995; Laha and Verma, 1998; Rangeshwaran and Prasad, 2000).

Recently some strains of fluorescent pseudomonads were found to suppress foliage diseases also. *Pseudomonas fluorescens* isolated from leaves of *Lolium perenne* were antagonistic to the pathogen *Drechslera dictyoides* (Austin *et al.*, 1977). *P. cepacia* 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). Mew and Rosales (1986) isolated fluorescent bacteria from the rice plants which inhibited the mycelial growth of *Rhizoctonia solani in vitro* and suppressed sheath blight disease of rice by seed treatment. Application of *P. maltophila* to mulberry leaves lead to the reduction in leaf spot caused by *Cercospora moricola* (Sukumar and Ramalingam, 1986). *P. cepacia* formulated as wettable powder effectively controlled peanut leaf spot (Knudsen and Spurr, 1987). Seed treatment with strains of *P. fluorescens* suppressed sheath rot of rice (*Sarocladium oryzae*) (Sakhtivel and Gnanamanickam, 1987). A fluorescent pseudomonad designated LECL suppressed the leaf pathogens *Septoria tritici* and *Puccinia recondita* (Levy *et al.*, 1988). Rice blast lesions per plant were lower when seeds were treated with *P. fluorescens* and *P. putida* than in soil incorporation treatments. Development of rice blast also decreased by 47 per cent in *P. fluorescens* treated rice seedlings of cv. UPLRi-5 (Lee *et al.*, 1990). Wei *et al.* (1991) demonstrated the ability of different PGPR strains to decrease the incidence of anthracnose *(Colletotrichum orbiculare*) of cucumber. Root bacterization with *Pseudomonas* spp. strain WCS 417r protected carnation from fusarial wilt (Van Peer *et al.*, 1991). *P. fluorescens* isolates from the surface of healthy cocoa pods were antagonistic to *Phytopthora palmivora in vitro* in the field and were more effective than

cupric oxide or chlorothalonil in controlling black pod (Galindo, 1992). Strains 4-15 and 7-14 of *P. fluorescens* afforded 59 and 47 per cent reduction in blast incidence in rice variety UPLRi-5 which received three sprays 500ml m^{-2} with bacteria (10^8cfu ml^{-1}) in addition to seed treatment (Gnanamanickam and Mew, 1992). Capper and Higgins (1993) proved the usefulness of *P. fluorescens* (2-79 and 13-79) in the biocontrol of take all disease of wheat (*Gaeumannomyces graminis*). Muthamilan (1994) observed the reduction in the incidence of rice blast by seed treatment with peat based formulation of *P. fluorescens* strain1. *P. fluorescens* significantly reduced rust infection in safflower when added both as soil drench and seed treatment (Tosi and Zazzerini, 1994). *P. fluorescens* and *P. lindbergii* isolated from the phylloplane of *Poa pratensis* inhibited growth of *Sclerotinia homoeocarpa* and *Bipolaris sorokinina* (Hodges et *al.*, 1994). *Pseudomonas aeruginosa* strain 7NSK2 inoculated on the roots produced salicylic acid which induced systemic resistance against *Botrytis cinerea* on beans (De Meyer and Hofte, 1997). Seed borne diseases caused by *Pyrenophora graminis* was suppressed by seed bacterization with *P. chloraphis* strain MA-342 (Johnson *et al.*, 1998; Gerhardson *et al.*, 1998). Infection of *Pyrenophora tritici* on wheat seeds was controlled by seed applied bioprotectant of *P. putida* (da Luz *et al.*, 1998). 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 Pf-1. They also observed increase in the phenolic content and activity of lytic enzymes.

2.1.1Control of *Rhizoctonia solani*

Although *Rhizoctonia solani* is primarily a soil borne pathogen it also affects the aerial plant parts and cause foliage blight disease in many crops. Among several antagonistic bacteria screened against *R. solani*, fluorescent pseudomonads were found as promising biocontrol agents.

Spraying of bacterial suspension on rice plants reduced the incidence and lesion size of sheath blight (*R. solani*) (Mew and Rosales, 1986). *Pseudomonas fluorescens* a rhizobacterium colonised roots, under ground portions of the stem and reduced severity and incidence of Rhizoctonia rot of potato (Bahme and Schroth, 1987). In field plots, IR-50 and TKM-9 rice plants raised from fluorescent *Pseudomonas* spp. treated seeds had 65-72 per cent less sheath blight than those plants from untreated seeds (Devi *et al.*, 1989). Seed treatment with *Pseudomonas* spp. reduced the incidence of *R. solani* on cotton (Qui *et al.*, 1990). Sarker *et al.* (1992) observed that seed bacterization with *P. fluorescens* IS-241 reduced the incidence of sheath blight of rice. *Pseudomonas* spp. isolated from the rhizosphere of rice inhibited the mycelial growth of *R. solani* and promoted the growth of seedlings (Lin *et al.*, 1992). *P. fluorescens* strain 1 reduced sheath blight incidence when seeds were treated with peat based formulation at 10g kg^{-1} (Muthamilan, 1994). Seed treatment combined with foliar spray of *P. fluorescens* suppressed rice sheath blight effectively and also reduced the germination capability of sclerotia of *R. solani* (Muthamilan, 1994). There was reduction in the number and size of lesions in sheath blight disease (*R. solani*) when seeds were treated with peat based inoculum of PfALR-2 (Rabindran, 1994). Seed bacterization with fluorescent *Pseudomonas* sp. reduced

the incidence of *R. solani* in cowpea (Barbosa *et al.*, 1995). Rabindran and Vidhyasekaran (1996) observed that combining foliar spray of *P. fluorescens* PfALR-2 with other methods of application like seed treatment, root treatment and soil application gave the best control against rice sheath blight (*R. solani*) and increased the yield. Strains of *P. fluorescens* Pf-1 isolated from rice plants were antagonistic to *R. solani* (Kamala, 1996). Kamala and Vidhyasekaran (1997) developed a talc based formulation of *P. fluorescens* strain P1 effective against *R. solani*. Combined application of the formulation by seed treatment, seedling root dip and foliar spray was effective in controlling the disease. Murakami *et al.* (1997) demonstrated the effectiveness of *P. fluorescens* HP-72 for the control of *R. solani* in bent grass. Brannen (1997) reported the suppression of *R. solani* infection in cotton by *Pseudomonas* spp. mainly due to the production of pyrrolnitrin. Mageswari and Gnanamanickam (1997) observed 61-62 per cent reduction in sheath blight of rice by the application of *P. putida* V14 strain. Seed bacterization of cotton by fluorescent pseudomonad suppressed the root rot and damping off of cotton incited by *R. solani* (Laha and Verma, 1998). Seed bacterization with strains of *P. fluorescens* Pf-1 controlled rice sheath blight (*R. solani*) (Sivakumar and Narayanaswami, 1998). Application of powder formulation of P1 on seeds, roots, and soil reduced the incidence of rice sheath blight by 45.30 per cent (Vidhyasekaran and Muthamilan, 1999). Velazhahan *et al.* (1999) observed the inhibition of mycelial growth of *R. solani* by *P. fluorescens* isolated from the rhizosphere of rice plants. Several pathogens of tomatoes including *R. solani* were inhibited by fluorescent *Pseudomonas* spp. (Varshney and Chaube, 1999). Control of *R. solani* in cucumber was achieved

by using strain of *P. fluorescens* BL-915 (Ligon *et al.*, 2000). Bacterized rice seedlings inoculated with *R. solani* at four leaf stage showed low disease index (Yong *et al.*, 2000). Sprayings with the liquid cultures of *P. fluorescens* strain S-18 at $3x10^9$cfu ml^{-1} suppressed rice sheath blight disease (*R. solani*). Seed treatment with peat based formulation of *P. fluorescens* Pf-1 suppressed the banded leaf and sheath blight of maize (*R. solani.* f.sp *sasaki*) (Sivakumar *et al.*, 2000). An isolate of fluorescent *Pseudomonas* P1 was identified as the best bacterial antagonist against *R. solani* inciting foliar blight disease of amaranthus (Smitha, 2000). Seed treatment with *Pseudomonas aeruginosa* strain CR-54 reduced *R. solani* infection on cucumber and tomato (Gan *et al.*, 2001). Nandakumar *et al.* (2001) observed that the application of *P. fluorescens* as bacterial suspension or talc based formulation reduced sheath blight disease in rice.

2.1.2 Control of *Xanthomonas*

P. fluorescens biotype C and G isolated from the rhizosphere were inhibitory to *Xanthomonas oryzae* pv *oryzae* (Sakthivel *et al.*, 1986). Sivamani *et al.* (1987) obtained strains of *P. fluorescens* antagonistic to the bacterial blight pathogen (*Xanthomonas oryzae* pv *oryzae*). Bacterized rice plants showed a substantial reduction (40-60 per cent) in bacterial blight severity (Anuratha and Gnanamanickam, 1987). The antagonistic effect of fluorescent pseudomonads on related species of the pathogen *Xanthomonas* infecting other graminaceous fodder crops, citrus, cotton and cassava have been reported (Unnamalai and Gnanamanickam, 1984; Verma *et al.*, 1986; Carlos, 1987; Schmidt, 1988). Ohno *et al.* (1992) reported the control of bacterial seedling blight of rice by fluorescent *Pseudomonas* sp. *P. acidovorus* has

the ability for controlling bacterial blight disease in rice by foliar spray (Sindhan *et al.*, 1997). Combined application of seed treatment, seedling root dip, and foliar spray of *P. fluorescens* P1 was efficient in controlling *X. oryzae* pv *oryzae* (Kamala and Vidhyasekaran, 1997). The application of *P. putida* strain V14 I as foliar spray gave maximum suppression of bacterial blight of rice (Gnanamanickam *et al.*, 1999). Strains of fluorescent *Pseudomonas* antagonistic to *Xanthomonas axonopodis* pv *phaseoli* was isolated by Magabala (1999). Seed bacterization with *Pseudomonas fluorescens* reduced the disease intensity of bacterial blight in cotton (Mondal *et al.*, 2000). Seed treatment along with the foliar spray of talc based formulation of Pf-1 reduced the disease intensity of bacterial blight (*X. oryzae* pv *oryzae*) and increased the yield of the crop (Vidhyasekaran *et al.*, 2001).

2.2 Plant growth promoting activity

Some plant growth promoting rhizobacteria such as *P. fluorescens* and *P. aeruginosa* may promote plant growth by secreting plant hormones like gibberlic acid substances (A_9–like compounds) (Katznelson and Cole, 1965; Suslow, 1982; Lifshitz *et al.*, 1987; Schippers *et al.*, 1987; Weller, 1988; Cook, 1990). Barea *et al.* (1976) examined 50 phosphate dissolving bacteria for IAA, gibberellins and cytokinins and obtained 17 isolates of *Pseudomonas* which produced them. Enhancement of plant growth has been attributed to the yellow green, fluorescent siderophores produced by fluorescent pseudomonad (Kloepper *et al.*, 1980). Seed and root inoculation of rhizobacteria promoted plant growth by producing phytohormones like auxins and gibberellins (Loper and Schroth, 1986).

A number of strains of fluorescent *Pseudomonas* which can increase the growth of wheat primarily by suppression of *Pythium* have been isolated (Weller and Cook, 1986). Hofte *et al.* (1991) reported that plant growth promoting strains of *P. aeruginosa* 7NSK 2 and *P. fluorescens* ANP 15 significantly increased the germination of maize seeds by 60-300 per cent. *P. 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). Defreitas and Germida (1992) observed that the growth promotion of winter wheat was achieved by treating with several strains of *Pseudomonas* spp. in green house and field trials. Gnanamanickam and Mew (1992) obtained slight increase in grain yield due to seed treatment with strains of *P. fluorescens*. Seed treatment of rice seeds with fluorescent *Pseudomonas* spp. reduced the intensity of sheath blight and promoted seedling growth (Lin *et al.*, 1992). Dubeikovsky *et al.* (1993) reported the influence of bacterial indole acetic acid production by *P. fluorescens* on the development of black current cuttings. Seed bacterization of rice cv. IR-58, IR-42, IR-36 resulted in increased root and shoot length and dry weight of roots and shoots (Rosales *et al.*, 1993). Root weight of corn seedlings from seeds treated with *Pseudomonas lindbergii* was 44 per cent greater than from untreated seeds (Hodges *et al.*, 1994). Muthamilan (1994) observed increased growth rate of rice plants by seed treatment with *P. fluorescens*. Tosi and Zazzerini (1994) recorded increase in the length of safflower seedling by seeds treatment with *P. fluorescens* strain 14. Gupta *et al.* (1995) reported growth promotion of tomato plants by rhizobacteria especially *P. fluorescens*. Inoculation of *P. fluorescens* significantly increased seedling emergence rate, increased total dry weight, and length of root and shoot.

Epiphytic bacteria (*P. viridiflava*, *P. fluorescens*, *P. putida*) produced high amount of IAA on leaf surface of pear and reduced severity of fruit russet (Lindow *et al.*,

1998). da Luz *et al.* (1998) reported increased seedling emergence and grain yield of wheat by seed treatment with *Pseudomonas putida.* Izhar *et al.* (1999) observed that seed treatment with *P. fluorescens* enhanced the growth of cotton and reduced the infection by *R. solani.* Seed treatment with Pf1 reduced the infection of sheath blight and increased crop yield in field trials (Vidhyasekaran and Muthamilan, 1999). *P. fluorescens* inoculated nitrogen rich organic residues enhanced nitrogen uptake of wheat (Brimecombe *et al.,* 1999). Rangeshwaran and Prasad, (2000) reported increased growth in chick pea seeds treated with *P. fluorescens.* Mishra and Sinha (2000) observed plant growth promoting activity of bacterial agents on rice seed germination and growth.

2.3 Development of formulation of bacterial antagonist

Plant growth promoting rhizobacterium have been reported to survive in different formulations. 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). 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°C. Capper and Higgins, (1993) developed a peat based formulation of *P. fluorescens* for the control of take-all disease of wheat. The efficacy of various carriers sustaining the population of antagonist was studied by Vidhyasekaran and Muthamilan (1995). They demonstrated the survival of bacteria in talc based formulation even up to 240 days of storage even though the population declined from 30 days. Satisfactory survival of *P. fluorescens* isolate in peat soil has been reported by Hagedron *et al.,* 1993; Vidhyasekaran and Muthamilan, 1995; Rabindran and Vidhyasekaran, 1996.

A talc based formulation of *P. fluorescens* was prepared by mixing 48 hr old growth of bacteria in King's B broth with sterilized talc @ 400 ml kg^{-1} talc along with five g of sticker carboxy methyl cellulose (Vidhyasekaran and Muthamilan, 1995; Kamala, 1996). Lazzaertti and Bettiol (1997) prepared a biological product containing *Bacillus subtilis* cells (60g) and metabolites (60g) and transformed it into WP formulation with clay (480g), surfactant (129g) and water (2400 ml). The best disease control was observed in experiments with *R. solani* in rice.

Seed potatoes treated with *B. subtilis* formulated as water soluble granules reduced black scurf up to 67 per cent (Schmiedeknecht *et al.*, 1997). *Bacillus cereus* and *P. fluorescens* survived in peat or vermiculite/clay formulation for at least 150 days and effectively controlled *Rhizoctonia* damping off in green house trials (Gasoni *et al.*, 1998). Krishnamurthy and Gnanamanickam (1998) observed increased shelf life and enhanced viability of *P. putida* in a formulation containing methyl cellulose and talc in 1:4 proportions for up to 10 months. Kanjanamaneesathian *et al.* (1998) developed a granulated formulation of the bacterial isolates which inhibited the sclerotial germination and mycelial growth of *R. solani*. A powder formulation of the bacteria (*P. fluorescens)* with shelf life of more than 8 months has been developed by Vidhyasekaran and Muthamilan (1999). Kurdish *et al.* (1999) observed the best survival of *P. aureofaciens* UKM BIII was observed in clay materials.

Sivakumar *et al.* (2000) observed that the survival of *P. fluorescens* in peat and talc maintained the highest population level of 19.5 x 10^7 and

18.3 x 10^7cfu g^{-1} after 40 days of storage. After 160 days of storage the population was reduced to 3 x 10^7cfu g^{-1} and 6 x 10^7cfu g^{-1}.

2.4 Biochemical characterisation

First work on the taxonomy of *P. fluorescens* and *P. putida* were based on phenotypic characters such as metabolic tests, fatty acid composition and protein profiles. The saprophytic pseudomonads associated with plants were assigned generally to one of the three species viz., *P. fluorescens, P. putida* and *P. aeruginosa*. Of these only *P. aeruginosa* formed a tight cluster and is relatively easy to identify. Most of the plant associated strain belonged to *P. fluorescens–P. putida* complex (Schaad, 1992; Rosales *et al.*, 1993).

These studies led to the subdivision of *P. fluorescens* and *P. putida* into 5 and 2 biovars, respectively (Bossis *et al.*, 2000).

Materials and Methods

3. MATERIALS AND METHODS

3.1 Isolation of foliar pathogens of rice

Plant parts showing typical symptoms of sheath blight were collected from rice plants cultivated at the Cropping System Research Centre, Karamana. Diseased lesions along with some green healthy portions were cut into 0.5 cm bits, surface sterilized with 0.01 per cent mercuric chloride followed by three washings with sterile distilled water and plated on potato dextrose agar (PDA). Growth obtained from diseased lesions were purified by single hyphal tip method and maintained on PDA slants at laboratory temperature (27 ± 3°C).

Leaves showing typical bacterial blight symptoms were collected from Regional Agricultural Research Station, Pattambi. Infected leaf tissues with the initial symptoms of the disease were cut into bits of approximately 0.3 cm size and sterilized with 70 per cent ethyl alcohol followed by 0.01 per cent mercuric chloride and washed in a series of three petri plates containing sterile distilled water. Surface sterilized bits were then transferred to a drop of sterile distilled water taken on a sterilized glass slide. The bits were then teased apart using sterilized blade and forceps and kept for one minute to allow the bacterial ooze to mix with the water. The bacterial suspension thus obtained was streaked on potato sucrose agar medium (PSA) (Wakimoto, 1954) to get isolated colonies of the bacterium. Typical bacterial growth from the medium was subcultured on to PSA slants and purified by dilution plate technique. Pure culture of the bacterium was maintained on PSA slants at 4°C.

3.2 Pathogenicity test

Sclerotia of the pathogen of sheath blight of rice, *Rhizoctonia solani*, multiplied on PDA was used as inoculum for pathogenicity tests. One sclerotium was placed in between the stem and sheath, three to four centimetres above the water line of seven-week old rice plants (cv. Jyothi) grown in green house. From the artificially infected rice plant, *R. solani*, was reisolated as described under 3.1.

Inoculum of *Xanthomonas oryzae* pv *oryzae* was prepared by suspending 72 hr old culture in sterile distilled water, O. D value of 1 (10^9 cells ml^{-1}) at 600nm as determined by UV-VIS model Spectrophotometer 118. Plants of susceptible cultivar TN-1 was clip inoculated with the inoculum. The pathogen was reisolated from the artificially infected plants as described under 3.1.

3.3 Source of antagonists

Fluorescent pseudomonads antagonistic to sheath blight and bacterial blight were isolated from disease free rice plants from rice fields of southern Kerala viz Vellayani, Karamana, Moncompu and Vytilla.

3.3.1 Isolation of fluorescent pseudomonads from rhizosphere

Isolates of fluorescent pseudomonads were obtained from the rhizosphere by dilution plate technique (Johnson and Curl, 1972). One gram sample of the roots with adhering soil were washed with sterile distilled water and shaken in 250 ml Erlen Meyer flask containing 100ml of sterile distilled water, at 150 rpm for 30 minutes. Serial dilutions were prepared and one ml

of the aliquot from the dilutions 10^6 and 10^7 were transferred to sterilized petri dishes and plated with King's medium B agar (KMB) (King *et al.*, 1954), rotated gently and incubated for 48hr (27 ± 2°C). Typical colonies of fluorescent *Pseudomonas* spp. were transferred and maintained on KMB slants at 4°C.

3.3.2 Isolation of fluorescent pseudomonads from phylloplane

Leaf samples collected from disease free plants were used for isolation of the bacteria by dilution plate technique as mentioned under 3.3.1. Dilutions (10^3 and 10^4) were used for plating with KMB slants.

To obtain endophytic isolates of the bacterium, 10 gm of leaf samples were surface sterilized with 0.01 per cent mercuric chloride for one minute, rinsed in sterile distilled water and ground with sterilized pestle and mortar. From the extract, serial dilutions were prepared and one ml aliquots from the dilutions 10^3 and 10^4 were transferred to sterilized petri dishes over which 15 ml molten KMB was poured, rotated gently and incubated for 48 hr. Isolates of fluorescent *Pseudomonas* spp. were maintained on KMB slants.

3.4.1 *In vitro* screening of antagonists against fungal pathogen

Isolates of fluorescent *Pseudomonas* spp. were tested for antagonistic effect against fungal pathogen (*R. solani*) by dual plate method (Utkhede and Rahe, 1983) as described. KMB was allowed to settle for one hour in sterilized petri dishes. Then each isolate of fluorescent *Pseudomonas* sp. was streaked on the medium two cm from the edge of the petri dish prior to pathogen inoculation. Just opposite to bacterial streak, 3.5 cm away, a 3mm disc of the pathogen was placed. Three replications were maintained for each

isolate. Petri plates without inoculating the antagonist and containing only the pathogen served as control. Inhibition zone was measured using the formula

$$I = (C - T)$$

C = Growth of the pathogen in control plates (mm)

T = Growth of the pathogen in dual culture (mm).

I = Inhibition of mycelial growth (mm)

The inhibition zone was measured after 48 hr. The experiment was repeated on PDA also.

3.4.2 *In vitro* screening of antagonist against bacterial pathogen

Bacterial pathogen *X. oryzae* pv *oryzae* was grown on PSA slants. One ml of sterilized distilled water was poured into each 24 hr old PSA slants and properly shaken to get a uniform suspension (O.D value-1). One ml of this suspension was added to 10 ml of the melted and, cooled PSA medium contained in test tubes, swirled and poured into each sterile petri dishes. Sterilized filter paper discs, (1 cm diameter) dipped in a standardised and uniform the suspension of each isolate of fluorescent *Pseudomonas* sp. was placed at the center of the seeded PSA medium. Three replications were maintained for each isolate. Petri plates without inoculating the antagonist served as control. The inoculated plates were incubated at room temperature (27 ± 2°C). The inhibition zone was measured after 48 hr.

3.5 Preparation of talc based formulation of *Pseudomonas* spp.

Talc based formulation of the effective isolates of fluorescent *Pseudomonas* sp. obtained from *in vitro* screening test against fungal pathogen (*R. solani*) and bacterial pathogen (*X. oryzae* pv *oryzae*) respectively were prepared separately by following the method of Vidhyasekaran and Muthamilan (1995). Each isolate was multiplied in KMB. A loopful of cells of each isolate was inoculated into the broth and incubated at room temperature (29 ± 3°C). The bacterial population was adjusted to $4x10^9$cfu ml^{-1} after 48 hr of incubation.

To one hundred grams of talc powder in polypropylene bags four grams of calcium carbonate and one gram of carboxymethylcellulose was added, sealed and autoclaved at 1.5 kg cm^{-2} pressure for one hour on two successive days. After sterilization 40 ml of two day old inoculum was mixed with talc powder, and under aseptic conditions. The polypropylene bag containing the talc based formulation of the bacterial isolate was resealed and stored at room temperature. Population of the antagonist was periodically estimated by dilution plate method.

3.6 Field emergence potential

The selected bacterial isolates antagonistic to the rice pathogens *R. solani* and *X. oryzae* pv *oryzae* were tested for their effects on the growth of rice seedling of the varieties Jyothi and TN-1 susceptible to sheath blight and bacterial blight diseases respectively, in separate *in vitro* experiments conducted with the following treatments.

T1 = Bacterial cell suspension (15 ml broth culture adjusted to $4x10^9$ cells ml^{-1})

T2 = Talc based formulation (10 g kg^{-1} seed) +15ml distilled water

T3 = Talc alone (10 g kg^{-1} seeds) +15 ml distilled water

T4 = Control (15 ml distilled water)

Seeds were soaked for the above-mentioned treatments for 12 hours. Excess water was drained off and treated seeds were incubated in the dark for 24 hr after which they were sown in uniform plastic cups (diameter of 8cm and height of 15cm). For each treatment, 25 g of seeds were sown in five replicated cups. The number of normal seedlings emerged were counted on the fifth day and mean value expressed as germination percentage. Length and dry weight of root and shoot of individual seedlings in all the treatments were measured 14 days after emergence.

3.7 Estimation of Indole Acetic Acid (IAA) synthesis of the antagonistic bacterial isolates

Extraction and estimation of IAA was done following the method of Chandramohan and Mahadevan (1968). Filter sterilized 0.1 ml of tryptophan (10 g l^{-1}) was added to 100 ml sterilized KMB broth taken in 250 ml Erlen Meyer flask. Twenty four hour old culture of fluorescent *Pseudomonas* spp. isolate was inoculated into the medium and incubated for seven days in the dark. After seven days of incubation the broth was centrifuged to remove the bacterial cells and supernatant was added with 1N HCl to adjust the pH to 3. IAA was extracted using diethyl ether in a separating funnel cooled to 9°C.

The extraction was repeated using 100ml diethyl ether. Ether extract was flash evaporated at 35-40°C. IAA in methanol was quantified using Salper's reagent (1ml of 0.5 per cent FeCl in 50 ml of 35 per cent perchloric acid) as follows. The residue dissolved in 2 ml methanol was transferred to test tube and mixed thoroughly with 1.5 ml distilled water and 0.5 ml methanol. To this mixture 1 ml of Salper's reagent was added rapidly, incubated in the dark for 1hr for maximum colour development. Intensity of colour developed was measured at 535nm in UV-VIS Spectrophotometer 118. The quantity of IAA in the culture filtrate was calculated from the standard curve.

3.7.2 Preparation of Standard Curve

A standard curve was prepared by using an aqueous solution of IAA of different concentration such as 10, 20, 30, 40, 50 μg ml^{-1}. To 5 ml of each of this dilution 1.6ml of methanol and 13.4ml of Salper's reagent were added to get a final volume of 20 ml. These tubes were incubated in the dark for one hour at room temperature. The colour intensity was measured at 535 nm in spectrophotometer. The standard curve was prepared by plotting the spectrophotometer reading on Y axis and concentration of IAA (μg ml^{-1}) on X-axis. The quantity of IAA produced by the two isolates of fluorescent *Pseudomonas* sp. was determined with the help of this standard curve. The final value was expressed as μg of IAA per millilitre of culture broth.

3.8 Assessing the efficacy of talc based formulation of bacterial isolates applied by various methods for the control of rice disease

The most effective isolates of fluorescent *Pseudomonas* spp. antagonistic to the pathogen *R. solani* and *X. oryzae* pv *oryzae* obtained from *in vitro* screening experiments, were formulated in talc and tested for their efficacy in suppressing sheath blight and bacterial blight diseases in rice plants which were artificially inoculated with the respective pathogens. The formulations were tested by different methods of applications.

3.8.1 Seed treatment

One kilogram of rice seeds was soaked for 12hr in 400 ml water containing the talc-based formulation (10g kg^{-1} seed) of the respective bacterial isolates. Excess water was drained off and treated seeds were incubated in the dark for 24 hr after which they were sown.

3.8.2 Seedling root dipping

Aqueous suspensions of talc-based formulation of the respective bacterial isolates were prepared at a concentration of one per cent. Rice seedlings in bundles (300 plants $bundle^{-1}$) were dipped in the prepared suspension for two hours, before transplanting them in earthen pots, ensuring that the roots alone were completely immersed in the inoculum.

3.8.3 Foliar spray

One per cent suspension of the talc based formulation of the respective bacterial isolates were prepared, allowed to settle for one hour, filtered through muslin cloth and filtrate was sprayed on the plants.

3.9 Effect of talc based formulation of fluorescent *Pseudomonas* isolate antagonistic to *R. solani* on sheath blight disease

The experiment was conducted under green house condition using talc-based formulation of the bacterial isolate antagonistic to *R. solani* and consisted of the following treatments.

T1 = Seed treatment with formulated product at dose 10g kg^{-1} seed

T2 = Seedling root dip with one per cent solution of formulated product

T3 = Foliar spray with formulated product one percent concentration (three sprayings-

- First spraying -two days prior to pathogen inoculation
- Second spraying-two days after pathogen inoculation
- Third spraying-seven days after pathogen inoculation

T4 = Seed treatment + seedling root dip (T1 + T2)

T5 = Seed treatment + foliar spray (T1 + T3)

T6 = Seedling root dip + foliar spray (T2 + T3)

T7 = Seed treatment + seedling root dip+ foliar spray (T1 + T2 + T3)

T8 = Carbendazim (0.1 per cent)

T9 = Untreated check

The susceptible variety Jyothi was used in this experiment. The pathogen *Rhizoctonia solani* was inoculated 45 days after sowing. Plants were scored for sheath blight disease intensity based on 0-9 scale of the Standard Evaluation System IRRI for rice, (1980) 14 days after inoculating *R. solani.*

3.10 Effect of talc based formulation of fluorescent *Pseudomonas* isolate antagonistic to *Xanthomonas oryzae* pv *oryzae* on bacterial blight disease

The treatments mentioned in 3.9 were tested using bacterial isolate effective against *X. oryzae* pv *oryzae*. Streptocycline (15g 300 l^{-1} ha^{-1}/0.005 %) was used as treated check instead of carbendazim. The variety TN-1 susceptible to bacterial blight diseases was used in this experiment. *Xanthomonas oryzae* pv *oryzae* was clip inoculated on the plants three weeks after sowing. Plants were scored for bacterial blight disease intensity based on 0-9 scale of the Standard Evaluation System for rice, IRRI (1980) 14 days after inoculating the pathogen.

The above experiments were conducted in Completely Randomised Design (CRD) maintaining three replications for each treatment.

3.11 Characterisation of the bacterial isolates antagonistic to the rice pathogens *R. solani and X. oryzae* pv *oryzae*

A combination of physiological and biochemical tests (Schaad, 1992) was conducted to identify the isolates of fluorescent *Pseudomonas* spp. antagonistic to the rice pathogens *R. solani, X. oryzae* pv *oryzae*. Based on their properties each isolate was assigned a sub group of plant associated saprophytic fluorescent *Pseudomonas* spp. The following tests were conducted (Schaad, 1992) (Table 3.1).

3.11.1 Levan formation

Presence of large white dome and mucoid colonies on peptone beef extract medium containing 5% sucrose characterized the production of levan from sucrose. The composition of the medium is given below.

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

Dilute suspension of the bacterial isolates was streaked over the medium and growth character observed after 48 hr.

3.11.2 Gelatin liquefaction

Nutrient gelatin medium with the following composition was used in this test.

Peptone	-	10.0g
Beef extract	-	5.0g
Gelatin	-	120.0g
Agar-agar	-	20.0g
Distilled water	-	1000ml
pH	-	7.0

The medium was dispensed in test tubes to a depth of 4cm and sterilized at 1.5 kg cm^{-2} pressure for 20 minutes. The medium was checked for

its sterile condition for two days. Forty eight hour old culture of each isolate was stab inoculated in the properly sterilized gelatin columns. The tubes were incubated and observed for liquefaction of the gelatin column at regular intervals for one month.

3.11.3 Growth at 4°C and 41°C

The test was done in King's medium B broth with the following composition.

Peptone	-	20.0g
K_2HPO_4	-	1.5g
$MgSO_4.7H_2O$	-	1.5g
Glycerol	-	10.0 ml
Distilled water	-	1000 ml
pH	-	7.2

Five ml of King's medium B broth was dispensed into test tubes and autoclaved at 1.5 kg cm^{-2} pressure for 20 minutes. The test isolates were inoculated into the broth and kept at 4°C, 41°C along with control. The absorbance of the inoculated broth was measured at regular intervals for four days.

3.11.4 Utilization of sugars

The following compounds were employed in this test.

Sugars	-	Arabinose, Galactose
Sugar alcohols	-	Sorbitol, m-Inositol

The basal medium used in this test was Hayward's medium (Hayward, 1964).

Composition

Peptone	-	1.0 g
$NH_4H_2PO_4$	-	1.0 g
KCl	-	0.2 g
$MgSO_4.7H_2O$	-	0.2 g
Bromothymol blue	-	0.03 g
Agar-agar	-	3.0 g
Distilled water	-	1000 ml
pH	-	7.2

An aliquot of 90 ml each of the basal medium was dispensed in 250 ml conical flask and autoclaved at 1.5 kg cm^{-2} pressure for 20 minutes. Ten per cent solution of sugars and sugar alcohols were prepared in sterile distilled water and sterilized by filtration. Ten ml each of the filtered solution was added to 90 ml of aliquot of the melted medium and dispensed in sterilized test tubes to a depth of 4cm.In one set of the tubes containing the carbon compounds the agar surface was covered with sterile liquid paraffin to depth of 1 cm .The medium was inoculated with the test isolates. The inoculated tubes were incubated and observation recorded at regular intervals up to a period of one month. Change in colour of the medium from blue to yellow indicated positive utilization of the carbon compounds with the production of acids.

3.11.5 Utilization of alcohol

Hayward's medium was used in this test also. Ten per cent ethanol was added to at Hayward's medium to get 10 per cent concentration and it was inoculated with the bacteria as described under 3.11.4.

3.11.6 Utilization of propylene glycol

Hayward's medium was used in this test also with the addition of propylene glycol to obtain a concentration of one per cent.

3.11.7 Utilization of n-butyl amine

n-butyl amine was added to Hayward's medium so as to get a concentration of one per cent.

3.11.8 Utilization of testosterone, phenyl acetate and nicotinate

Utilization of testosterone, phenyl acetate and nicotinate was tested as described above in separate experiments using Hayward's medium containing one per cent each of the chemicals.

3.12 Statistical Analysis

The data generated from the experiment were subjected to Analysis of Variance (ANOVA) technique after appropriate transformation wherever needed.

Table 3.1 Characters of plant associated saprophytic fluorescent *Pseudomonas* species (Schaad,1992)

	P. aeruginosa	*P. fluorescens biovar*					*P. chlororaphis*	*P. aureofaciens*	*P. putida biovar*	
		I	II	III	IV	V			A	B
Levan formation	-	+	+	-	+	-	+	+	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	-	-
Growth at 4°C	-	+	+	+	+	v	+	+	v	+
Growth at 41°C	+	-	-	-	-	-	-	-	-	-
Utilization of Arabinose	-	+	+	v	+	-	-	+	v	+
Galactose	-	+	+	v	+	v	v	+	-	+
Sorbitol	-	+	+	v	+	v	-	-	-	v
Inositol	-	v	+	v	+	v	+	+	-	-
Propylene glycol	+	-	+	v	-	v	-	-	v	+
Ethanol	+	-	+	v	-	v	v	-	v	v
n-Butyl amine	-	-	-	-	-	v	-	v	+	+
Phenyl acetate	-	-	-	-	-	v	v	+	+	+
Testosterone	-	-	-	-	-	-	-	-	-	+
Nicotinate	-	-	-	-	-	v	-	-	v	+

Results

4. RESULTS

4.1 Isolation of foliar pathogens of rice

The foliar pathogen *Rhizoctonia solani* was isolated from rice cultivar Aishwarya showing symptoms of sheath blight. Young hyphae were colourless and measured 8-12 µm diameter. The culture showed brown pigmentation with age. Sclerotial initials were minute, pale white in colour, fluffy and reaching a maximum size after 30 hr. Mature sclerotia were subglobose, rough surfaced, and mustard shaped. More sclerotia were observed nearer to the periphery of culture plates.

Bacterial blight pathogen (*Xanthomonas oryzae* pv *oryzae)* was isolated from the rice cultivar, Jyothi showing typical symptom of the disease. The bacterium gave rise to yellow circular slimy and convex colonies with entire margin on potato sucrose agar medium. The bacterium was gram negative and rod shaped.

4.2 Pathogenicity test

The isolates of *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae* induced typical and severe symptoms of sheath blight and bacterial blight when inoculated on susceptible rice cultivar Jyothi and TN-1 respectively. On reisolation, the pathogens produced growth characters similar to those of the original isolates as described in 4.1.

4.3 Source of antagonist

Thirty three isolates of fluorescent *Pseudomonas* spp. were obtained from rice samples collected from Vellayani, Karamana, Moncompu and Vytilla. Out of these isolates, 16 were obtained from the rhizosphere and 17 from the phylloplane of the plants.

4.3.1 Isolation of fluorescent pseudomonads from the rhizosphere.

Typical colonies of the fluorescent pseudomonads were obtained from 10^6 and 10^7dilutions on KMB agar. Colonies were circular to rhizoid, flat or convex, elevated with entire margin and produced fluorescent pigment on KMB agar.

4.3.2 Isolation of fluorescent pseudomonads from the phylloplane

Seventeen isolates of fluorescent *Pseudomonas* spp. were obtained from dilution of 10^3 and 10^4 on KMB agar. Colonies showed typical cultural characteristics of fluorescent *Pseudomonas* spp. as described in 4.3.1.

4.4 *In vitro* screening of antagonist against the foliar pathogens.

Thirty three isolates of fluorescent *Pseudomonas* spp. obtained from the serial dilution of rhizosphere and phylloplane of rice plants were numbered from P1 to P33. They were screened for their antagonistic property against *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae*.

4.4.1 Antagonism against *Rhizoctonia solani*

In KMB the maximum inhibition zone of 18.4mm was obtained when P11 was used in dual plate culture against *Rhizoctonia solani* (Plate 4.1). Next to P11 the isolate P13 was effective in inhibiting the pathogen and produced

an inhibition zone of 12mm. P8, P9, P10 produced inhibition zone which measured 7.6mm, 8.8mm, and 8.9mm respectively. Measurement of inhibition zone of all the remaining isolates ranged from 1.2 – 5.3 mm. Slight inhibition (0.27mm) of the pathogen was exhibited by P11 on PDA medium (Table 4.1).

4.4.2 Antagonism against *Xanthomonas oryzae* pv *oryzae*

Maximum inhibition zone of 22 mm was obtained when filter paper disc dipped in culture filtrate of P33 was placed on potato sucrose agar seeded with *Xanthomonas oryzae* pv *oryzae* (Plate 4.2). Next to P33 isolate P28 was effective in inhibiting the pathogen and produced an inhibition zone of 19 mm. P30 and P24 produced inhibition zones of 16 mm and 17 mm respectively. Inhibition zones of P4, P32, P5, and P29 were measured as 10.5 mm, 11.7 mm, 12 mm and 14.7 mm respectively. Inhibition zones of P2, P3, P6, P8, P9, P10, P11, P13, P14, P16, P17, P18, P19, P20, P21, P22, P23, P26, P27 and P31 ranged from 0.5-5.2 mm. Isolates P1, P7, P12, P15, and P25 did not produce any inhibition zone on the medium (Table 4.2).

4.5 Preparation of talc based formulation of the effective isolates of fluorescent *Pseudomonas* spp.

A talc based formulation of the isolate of fluorescent *Pseudomonas* spp. which produced maximum inhibition against *R. solani* was developed. One gram of the formulation contained 9.6×10^9cfu. Fifteen days later population declined to 8.4×10^9cfu g^{-1} when stored at room temperature (30±3°C) (Fig. 4.1). Population level was reduced by 70.8 per cent when stored for 60 days in talc based formulation (Table 4.3, Fig. 4.2).

Table 4.1 *In vitro* inhibition of fluorescent *Pseudomonas* against *Rhizoctonia solani* on King's medium B (KMB) and Potato dextrose agar medium (PDA) after 48 hr.

Isolates	Inhibition zone (mm) (KMB)	Inhibition zone (mm) (PDA)
P1	3.6	0.0
P2	1.5	0.0
P3	2.8	0.0
P4	3.2	0.0
P5	1.2	0.0
P6	4.2	0.0
P7	4.1	0.0
P8	7.6	0.0
P9	8.8	0.0
P10	8.9	0.0
P11	**18.4***	**0.27***
P12	2.5	0.0
P13	12.0	0.13
P14	1.2	0.0
P15	1.0	0.0
P16	1.2	0.0
P17	1.2	0.0
P18	3.5	0.0
P19	2.6	0.0
P20	4.5	0.0
P21	2.4	0.0
P22	1.8	0.0
P23	5.3	0.0
P24	4.5	0.0
P25	1.7	0.0
P26	2.3	0.0
P27	2.2	0.0
P28	3.2	0.05
P29	2.7	0.0
P30	3.2	0.0
P31	2.0	0.0
P32	1.5	0.0
P33	3.2	0.0
CD(5%)	0.1208	0.0172

*Maximum inhibition zone on KMB and PDA

Table 4.2 ***In vitro*** **inhibition of fluorescent** ***Pseudomonas*** **isolates against** ***X. oryzae*** **pv** ***oryzae*** **on Potato sucrose agar medium (PSA) after 48 hr.**

Isolates	Inhibition zone (mm)
P1	0.0
P2	1.0
P3	3.0
P4	10.5
P5	12
P6	0.5
P7	0.0
P8	0. 5
P9	1.5
P10	3.0
P11	1.0
P12	0.0
P13	1.5
P14	0.5
P15	0.0
P16	5.2
P17	2.0
P18	1.7
P19	2.0
P20	4.0
P21	5.0
P22	2.5
P23	0.5
P24	17.0
P25	0.0
P26	0..5
P27	4.7
P28	19.0
P29	14.7
P30	16.0
P31	4.0
P32	11.7
P33	**22.0***
CD (5%)	0.1015

*Maximum inhibition zone on PSA

Plate 4.1. Effect of fluorescent *Pseudomonas* sp. (P11) in inhibiting growth of *Rhizoctonia solani in vitro*

1. Inhibition of P11

2. Control

Plate 4.2. Effect of fluorescent *Pseudomonas* sp. (P33) in inhibiting growth of *Xanthomonas oryzae* pv *oryzae*

1. Control

2. Inhibition of P33

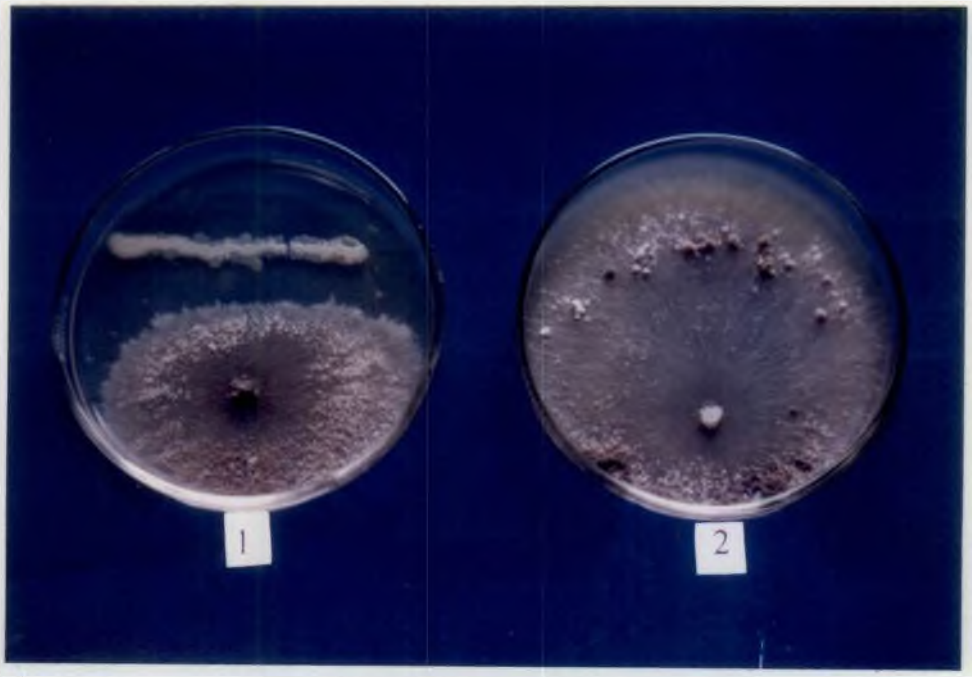


PLATE 4.1

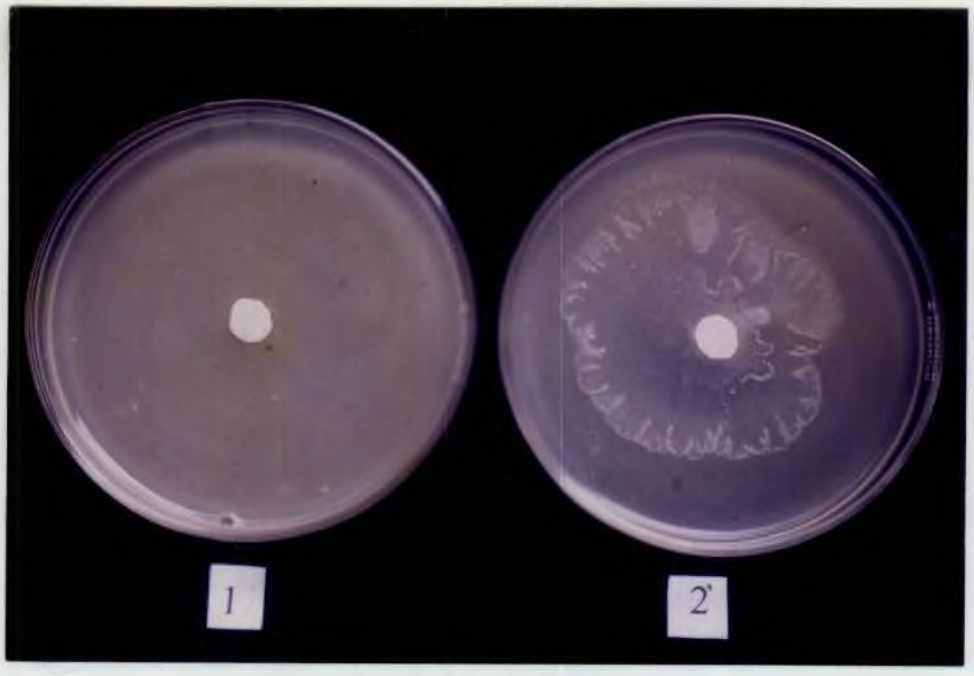


PLATE 4.2

Table 4.3 Survival of fluorescent *Pseudomonas* sp. (P11) in talc based formulation at room temperature (30 ± 3°C)

Days after preparation	cfu g^{-1} ($x10^9$)	Percentage reduction over initial population
0	9.6	-
15	8.4	12.5
30	6.6	31.2
45	5.0	47.9
60	2.8	70.8
75	1.5	84.3

Table 4.4 Survival of fluorescent *Pseudomonas* sp. (P33) in the talc based formulation at room temperature (29 ± 3°C)

Days after preparation	cfu g^{-1} ($x10^9$)	Percentage reduction over initial population
0	14.8	-
15	12.0	18.91
30	10.0	32.43
45	8.7	41.21
60	5.3	64.18
75	3.2	78.37

Fig. 4.1 Survival of fluorescent *Pseudomonas* sp. (P11) in talc based formulation

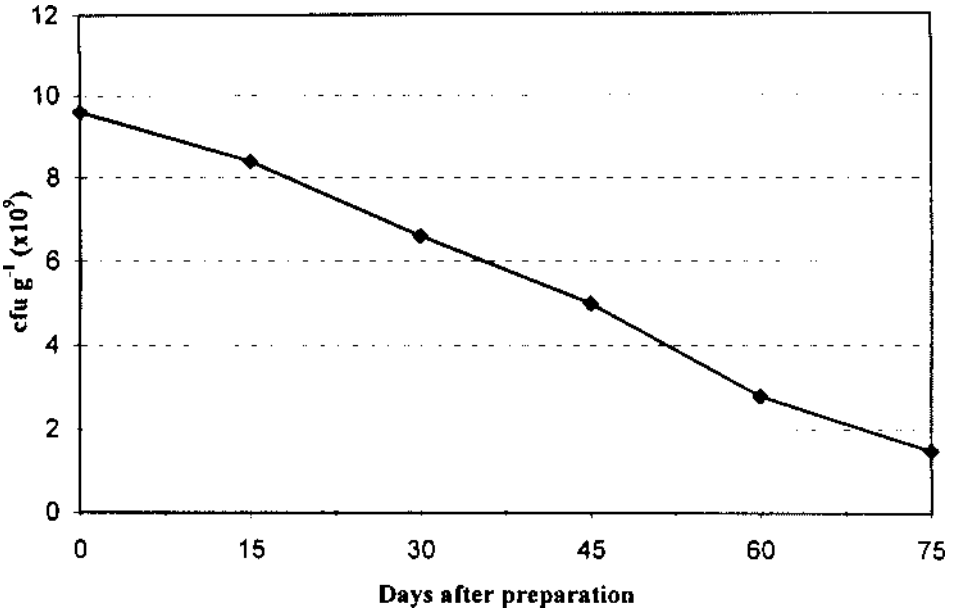


Fig 4.2 Percentage reduction of population of P11 in talc based formulation during storage

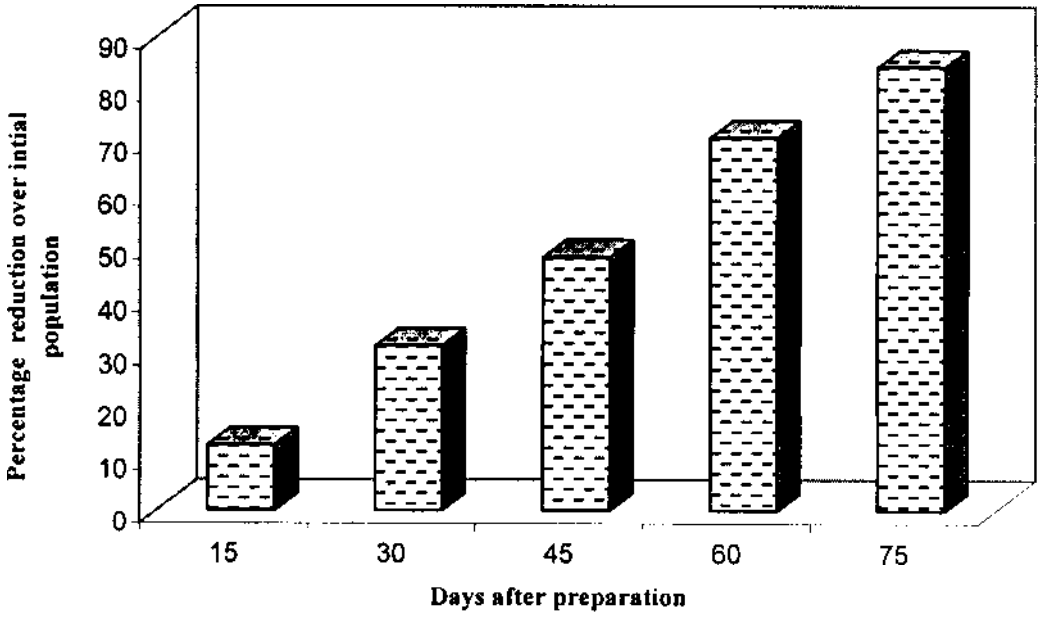


Fig. 4.3 Survival of fluorescent *Pseudomonas* sp. (P33) in talc based formulation

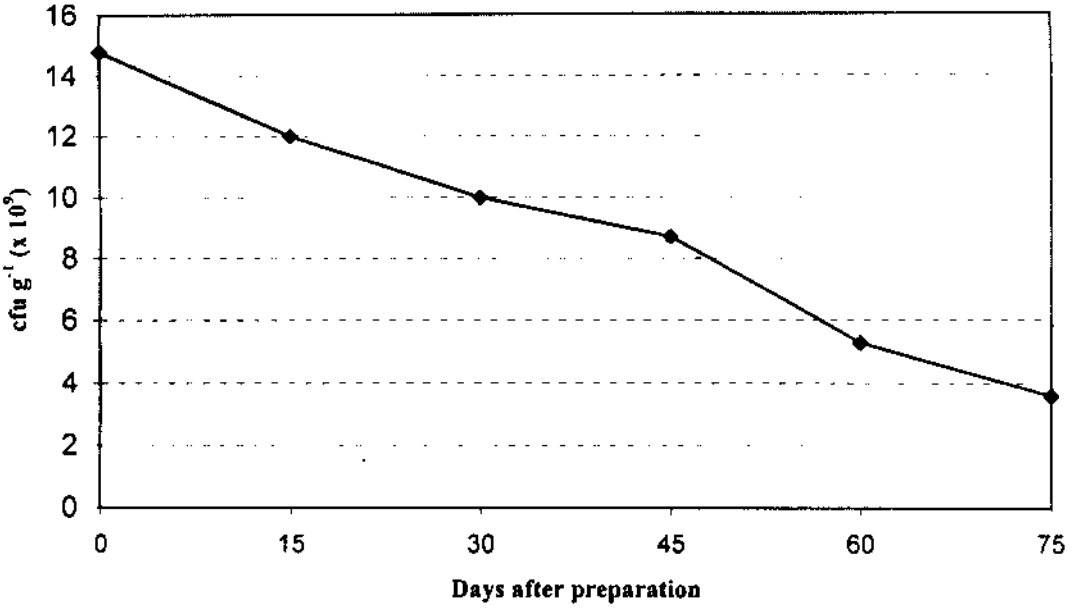
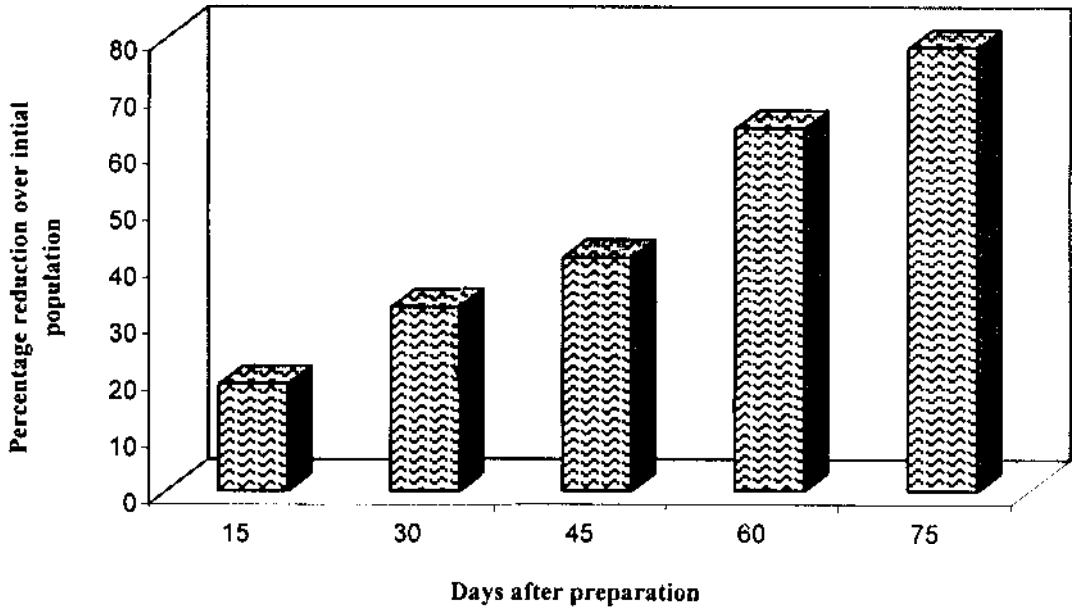


Fig 4.4. Percentage reduction of population of P33 in talc based formulation during storage



Similarly a talc based formulation was also developed with isolate P33 of fluorescent *Pseudomonas* sp. P33 which effectively inhibited the *Xanthomonas oryzae* pv *oryzae*. There was 64 per cent reduction over initial population level ($14.8x10^9$cfu g^{-1}) 60 days after storage in talc at room temperature (29±3°C) (Table 4.4,Fig. 4.3 and Fig. 4.4).

4.6 Effect of seed treatment with fluorescent *Pseudomonas* spp. on the growth of rice seedlings

There was 100 per cent germination of seeds in all the treatments tested using P11 and P33 on cultivar Jyothi and TN-1 respectively.

4.6.1 Length of root and shoot

There was no significant effect on root and shoot length of rice seedlings cv Jyothi due to seed treatment with talc based formulation of P11 and talc alone when compared to control (Table 4.5). Seed treatment with culture suspension of P11 significantly increased root length but not shoot length when compared to other treatments (Plate 4.3). There was elongation of roots and shoots due to seed treatment with culture suspension, talc based formulation of P33 when compared to control (Table 4.6).

4.6.2 Dry weight of root and shoot

Dry weight of root (0.195 g 25 plants^{-1}) and shoot (0.602g 25 plants^{-1}) was significantly higher in rice seedlings (cv. Jyothi) due to seed treatment with culture suspension of P11 (Table 4.5). The increase in dry weight of root and shoot were 168.1 per cent and 241.7 per cent respectively, over that of control which comprised of seedlings raised from untreated seeds. Talc based formulation of P11 also significantly increased the root and shoot weight by

Table 4.5 Effect of seed treatment with P11 isolate of fluorescent *Pseudomonas* sp. on length and dry weight of root and shoot of cv. Jyothi

Treatment	Root length (cm 25 plants^{-1}) 14 DAS	Shoot length (cm 25 plants^{-1}) 14 DAS	Root dry wt (g 25 plants^{-1}) 14 DAS	Percentage increase over control	Shoot dry wt (g 25 plants^{-1}) 14 DAS	Percentage increase over control
Bacterial suspension	5.37	13.08	0.195	168.08	0.602	241.72
Talc based formulation	5.26	13.02	0.147	101.09	0.562	202.05
Talc	4.86	12.37	0.086	18.90	0.227	22.18
Control	4.58	11.46	0.073	-	0.186	-
CD (5%)	0.78	0.85	0.043	60.11	0.088	49.25

DAS –Days after sowing

Table 4.6 Effect of seed treatment with P33 isolate of fluorescent *Pseudomonas* sp. on length and dry weight of root and shoot of cv. TN-1

Treatment	Root length (cm 25 plants^{-1}) 14 DAS	Shoot length (cm 25 plants^{-1}) 14 DAS	Root dry wt (g 25 plants^{-1}) 14 DAS	Percentage increase over control	Shoot dry wt (g 25 plants^{-1}) 14 DAS	Percentage increase over control
Bacterial suspension	9.28	17.62	0.165	54.58	0.207	32.95
Talc based formulation	8.97	17.87	0.164	53.08	0.205	31.54
Talc	7.84	17.08	0.109	1.86	0.161	2.95
Control	6.85	15.19	0.107	-	0.156	-
CD (5%)	1.12	2.07	0.055	35.99	0.039	24.51

Plate 4. 3. Effect of seed treatment with talc based formulation of P11 on increasing root and shoot length of rice seedlings (cv. Jyothi)

ST-Seed treatment

C-Control

Plate 4.4 Effect of seed treatment with talc based formulation of P33 on increasing root and shoot length of rice seedlings (cv. TN-1)

ST- Seed treatment

C-Control

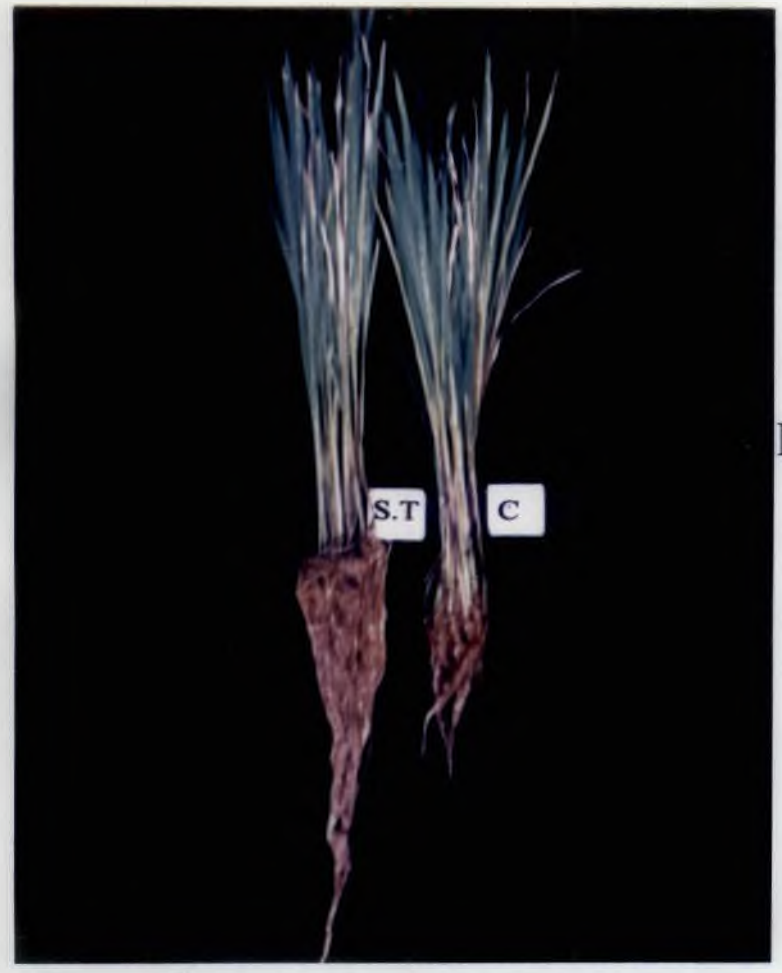


PLATE 4.3

PLATE 4.4

101.1 per cent and 202.0 per cent respectively but the effects were not on par with those produced by culture suspension of P11. Dry weight of root (0.086 g 25 plants^{-1}) and shoot (0.227 g 25 plants^{-1}) of seedlings raised from seeds treated with talc were on par with those raised from untreated seeds.

Rice seedlings (cv. TN-1) raised from seeds treated with culture suspension and talc based formulation of P33 recorded significantly higher dry weight of root and shoot and their effects were on par (Table 4.6, Plate 4.4). Dry weight of root and shoot of seedlings raised from seeds treated with talc were on par with dry weights of those raised from untreated seeds. The increase in dry weight of root (54.6 per cent) and shoot (32.9 per cent) of rice seedling (cv. TN-1) due to seed treatment with either culture suspension or talc based formulation of P33 was lower compared to the effect of culture suspension or talc based formulation of P11 on the growth of rice seedlings (cv. Jyothi).

4.7 Estimation of IAA

Estimation of IAA by isolates of fluorescent *Pseudomonas* sp. indicated that P11 and P33 produced 20 µg ml^{-1} and 23 µg ml^{-1} IAA under laboratory conditions (Table 4.7, Fig 4.5 and Fig 4.6).

4.8.1 Assessing the efficacy of talc based formulation of the bacterial isolate P11 applied by various methods for the control of sheath blight disease

In the green house experiment seed treatment with talc based formulation of P11 recorded disease intensity of 3.53 which was on par with that of untreated plants (4.13) and those plants treated with the formulation by

Table 4.7 Estimation of IAA given as spectrophotometer readings (Standard curve)

Concentration of IAA in ppm	O.D. Value
10	0.06
20	0.13
30	0.17
40	0.25
50	0.3
Isolate P11	0.14
Isolate P33	0.15

Fig 4.5 Standard curve of IAA

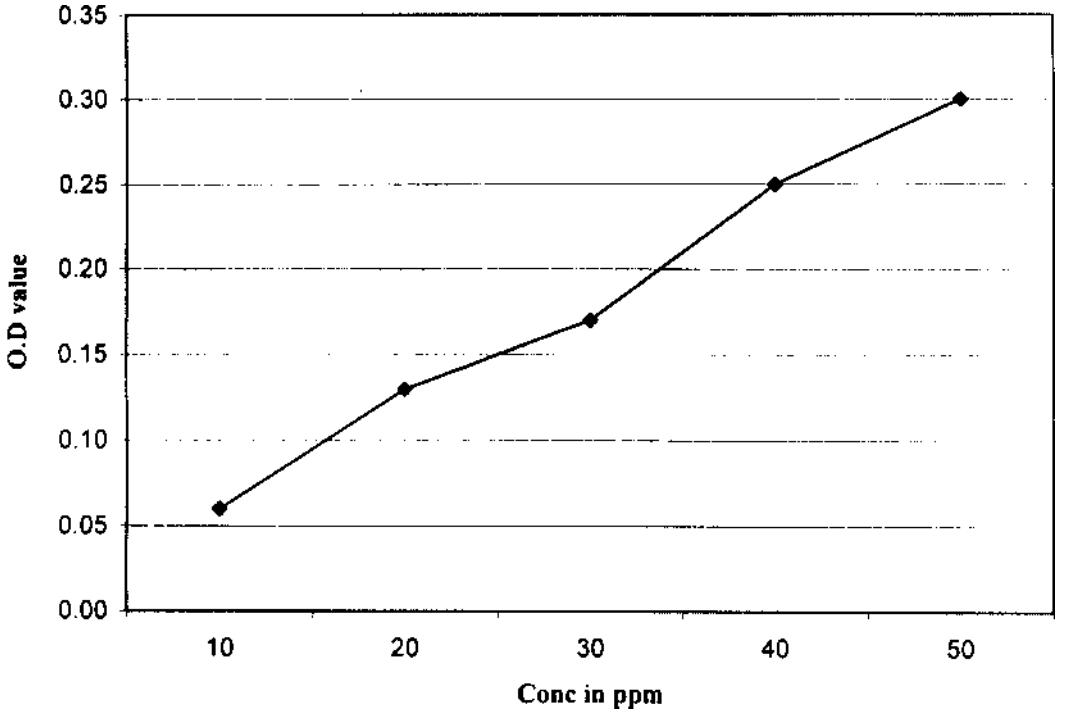
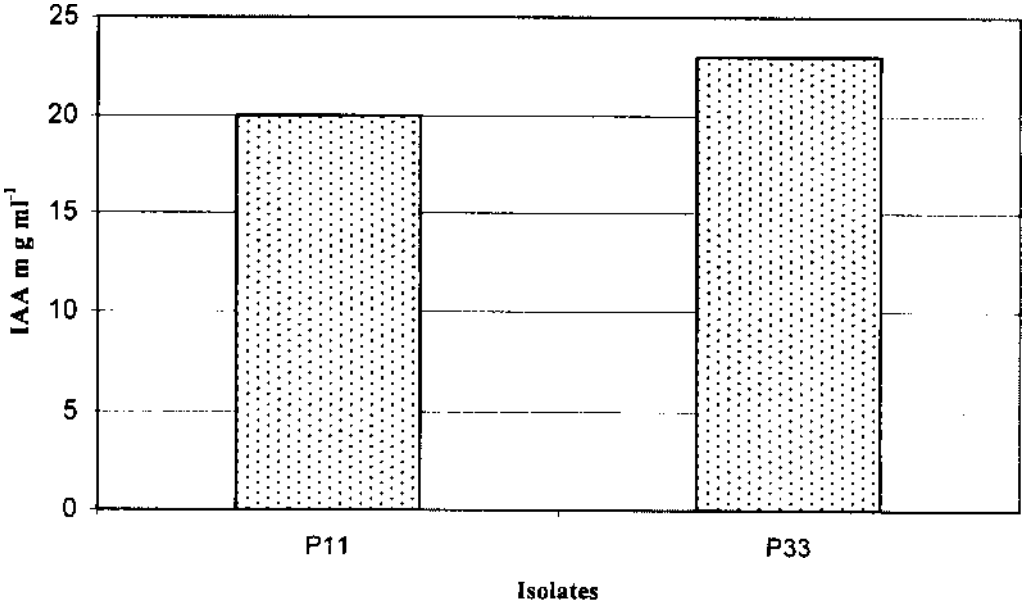


Fig. 4.6 IAA production by P11 and P33



seedling root dipping (3.13) (Table 4.8). The method of application of the formulation of P11 by seed treatment followed by seedling root dipping and seed treatment or seedling root dipping followed by foliar spray resulted in disease intensities of 2.93, 2.73 and 2.70 which were also on par with that of plants sprayed with carbendazim (2.96). A combination of all three methods of application (seed treatment + seedling root dipping + foliar spray) recorded lowest disease intensity of 1.76 which was on par with disease intensity of plants applied with the formulation by foliar spray (1.80) (Plate 4.5 and Plate 4.6). Disease intensity recorded on plants sprayed with the formulation was also on par with that of plants treated with the formulation by seedling root dipping followed by foliar spray. The percentage reduction in disease intensity over untreated plants were 34.66 and 34.09 due to the two methods of application of the formulation of P11 which resulted in significantly lower disease intensities (Fig 4.7). The biomass of plants treated with the formulation of P11 by combination of all 3 method of application was 44.94 g 20 plants^{-1} (Table 4.8) which recorded 109.5 per cent increase over the untreated plants (Fig 4.8). Seed treatment or seedling root dipping followed by foliar spray resulted in biomass yield of 39.6 g 20 plants^{-1} and 35.7 g 20 plants^{-1} which were on par with the biomass of plants treated with the formulation by all three methods of application.

4.8.2 Assessing the efficacy of talc based formulation of the bacterial isolate P33 applied by various methods for the control of bacterial blight disease

Lowest disease intensity of bacterial blight (2.30) was recorded when talc based formulation of the bacterial isolate P33 was treated on rice plants

Table 4.8 Effect of different methods of application of talc based formulation of fluorescent *Pseudomonas* isolate P11 on the management of sheath blight disease under green house conditions

Treatments	Disease intensity in grade value	Percentage reduction in disease over untreated check	Biomass (g 20 plants^{-1})	Percentage increase in biomass over untreated check
Seed treatment	3.53 (1.86)	8.16	33.97	58.36
Seedling root dip	3.13 (1.77)	12.67	33.79	57.56
Foliar spray	1.80 (1.34)	34.09	33.34	55.42
Seed treatment + seedling root dip	2.93 (1.70)	15.10	39.60	84.63
Seed treatment + Foliar spray	2.73 (1.65)	18.54	27.15	26.56
Seedling root dip + Foliar spray	2.70 (1.64)	18.85	35.75	66.68
Seed treatment + Seedling root dip + Foliar spray	1.76 (1.32)	34.66	44.94	109.5
Carbendazim (0.1 %) (Treated check)	2.96 (1.71)	14.75	25.92	20.93
Untreated check	4.13 (2.03)		21.45	
CD(5%)	0.30	1.82	10.62	52.46

Figures in brackets are $\sqrt{x}$ transformed values

Fig. 4.7 Efficacy of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P11) on management of sheath blight disease intensity

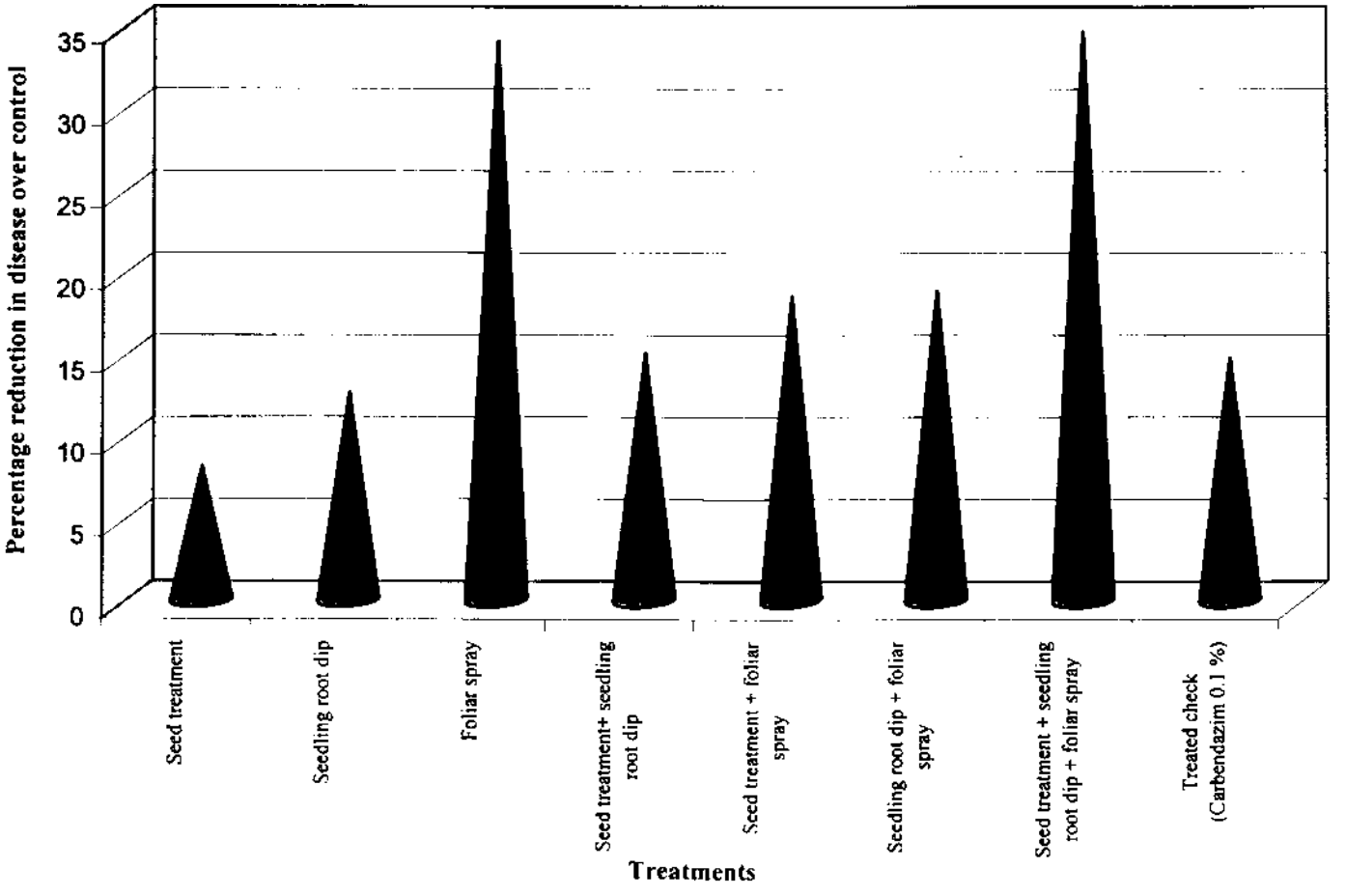


Fig. 4.8 Effect of methods of application of talc based formulation of fluorescent *Pseudomonas* sp. (P11) on the biomass of plants

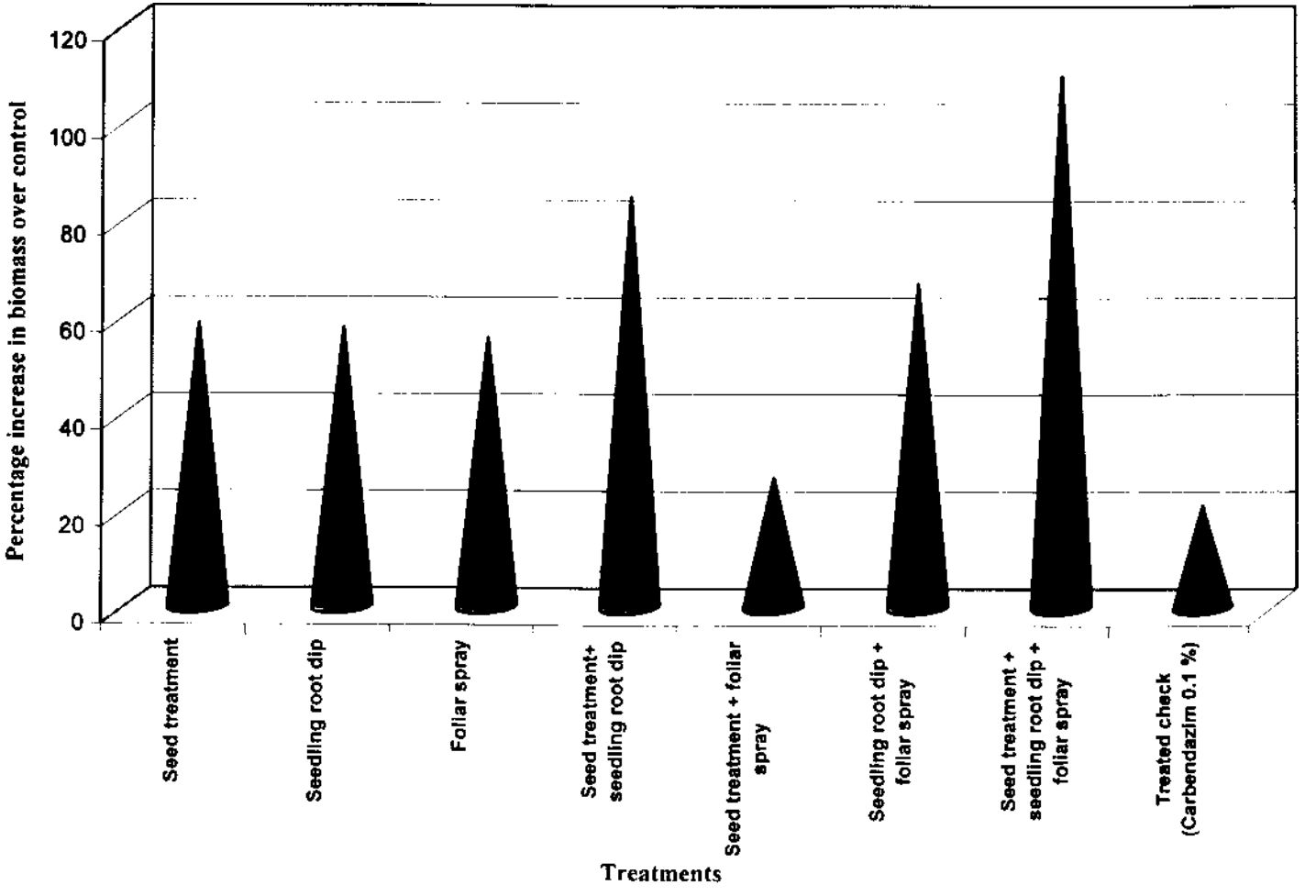


Plate 4.5. Effect of application of talc based formulation of P11 by combination of seed treatment, seedling root dipping and foliar spray on development of sheath blight under green house condition

1-Seed treatment + Seedling root dipping + Foliar spray

2- Control

Plate 4. 6. Leison development on the sheath of rice (cv. Jyothi)

1-Seed treatment + Seedling root dipping + Foliar spray

2-Control

PLATE 4.5

PLATE 4.6

(cv. TN-1) by a combination of all three methods of applications (seed treatment + seedling root dipping + foliar spray) and was significantly superior to all other methods of application of the formulation (Table 4.9). There was 40.28 per cent reduction in disease intensity over that of untreated plants (Fig 4.9, Plate 4.7). Effects of all other methods of application viz., foliar spray, seedling root dipping followed by foliar spray, seed treatment, seed treatment followed by foliar spray and seed treatment followed by seedling root dipping in lowering the intensity of bacterial blight were on par. Comparatively higher disease intensity was recorded when talc based formulation of P33 was applied by seedling root dipping alone, but it was on par with the formulation applied by seed treatment followed by seedling root dipping. Bacterial blight intensity of plants sprayed with streptocycline was 6.23 which was on par with that of untreated plants. There was 0.92 per cent reduction in disease intensity over untreated plants (control). Biomass of plants treated with the formulation of P33 by combination of all three methods (seed treatment + seedling root dipping + foliar spray) was 7.8g/20 plants which was 323.91 per cent greater than that of untreated plants (Fig 4.10). Application of the formulation by seedling root dipping followed by foliar spray recorded a biomass of 4.61g 20 plants^{-1} with an increase of 150.88 per cent. Per cent increase in biomass was 133.87 per cent when the formulation was applied by seed treatment followed by foliar spray. Seed treatment followed by seedling root dipping resulted in biomass yield of 4.04g 20 plants^{-1} with 119.38 per cent increase compared to untreated plants. Application of the formulation of P33 by foliar spray resulted in 73.19 per cent increase in biomass (3.18g 20 plants^{-1}). The biomass of plants treated with the

Table 4.9 Effect of different methods of application of talc based formulation of fluorescent *Pseudomonas* isolate P33 on the management of bacterial blight disease under green house conditions

Treatments	Disease intensity grade value	Percentage reduction in disease over untreated check	Biomass (g 20 plants^{-1})	Percentage increase in biomass over untreated check
Seed treatment	3.63 (1.90)	24.99	2.74	48.91
Seedling root dip	4.40 (2.09)	17.24	2.92	60.32
Foliar spray	3.23 (1.79)	29.13	3.18	73.19
Seed treatment + seedling root dip	3.83 (1.95)	22.84	4.04	119.38
Seed treatment + foliar spray	3.63 (1.91)	25.19	4.30	133.87
Seedling root dip + foliar spray	3.42 (1.85)	27.01	4.61	150.88
Seed treatment + seedling root dip + foliar spray	2.30 (1.52)	40.28	7.8	323.91
Streptocycline (0.005 %) (Treated check)	6.23 (2.49)	0.92	2.14	15.96
Untreated check	6.43 (2.54)		1.84	
CD	0.19	1.01	0.23	11.69

Figures in brackets are $\sqrt{x}$ transformed values

Fig. 4.9 Efficacy of methods of application of talc based formulation of P33 on management of bacterial blight disease intensity

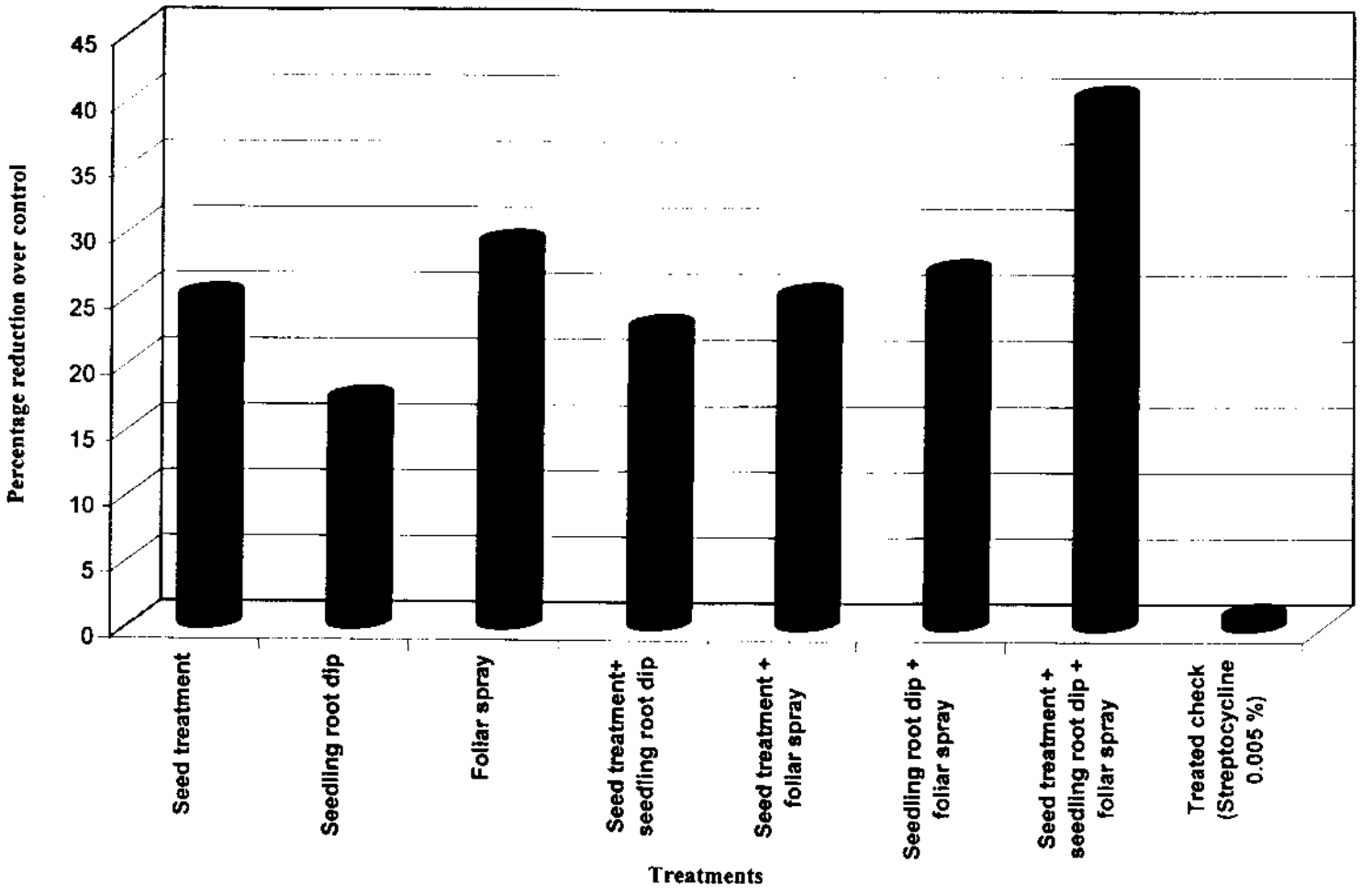


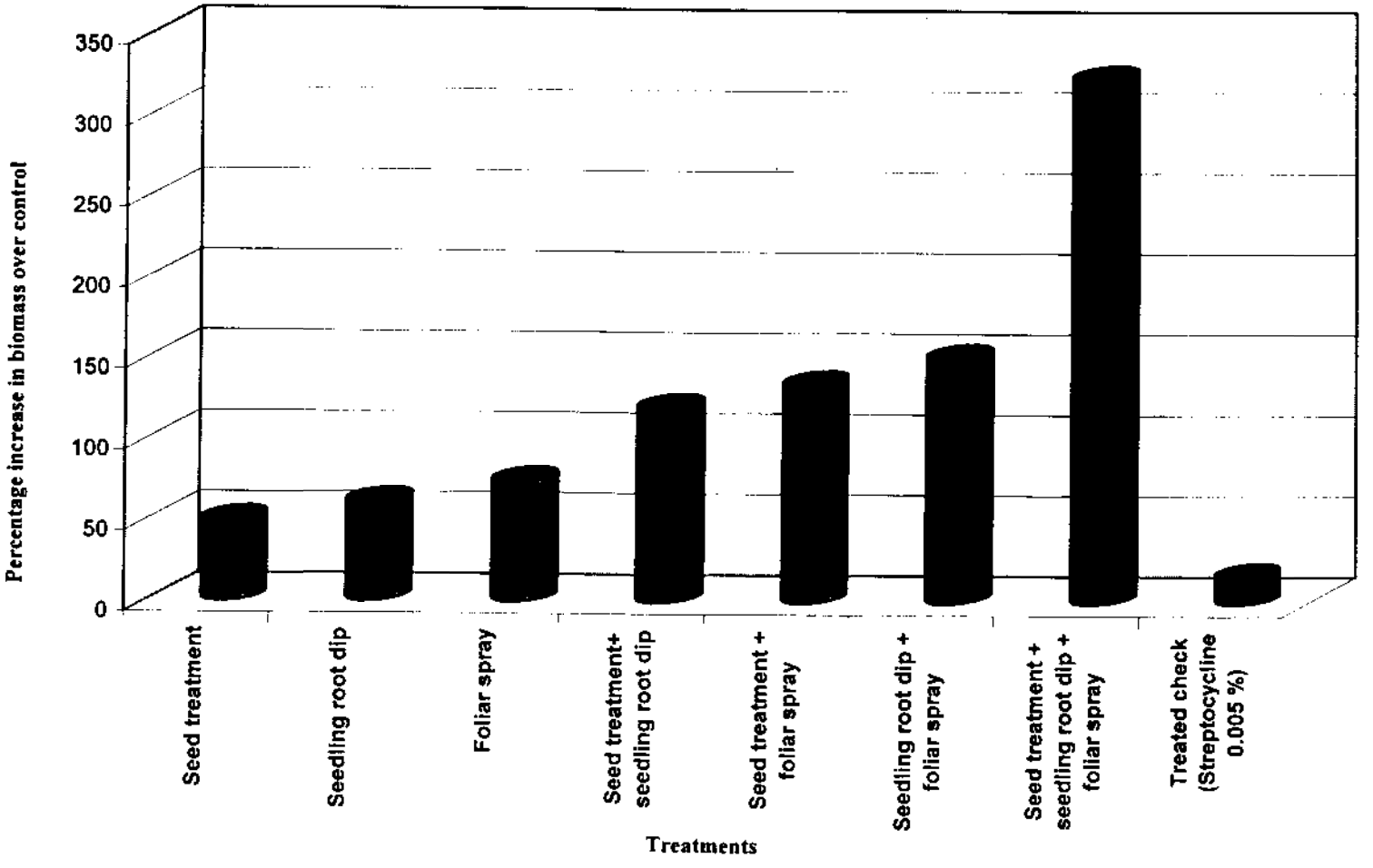
Plate 4.7. Effect of application of talc based formulation of P33 by combination of seed treatment, seedling root dipping and foliar spray on development of bacterial blight under green house condition

1- Control

2- Seed treatment + Seedling root dipping + Foliar spray

PLATE 4.7

Fig. 4.10 Effect of methods of application of talc based formulation of fluorescent Pseudomonas sp. (P33) on the biomass of plants



formulation by seedling root dipping or seed treatment were 2.92 g 20 plants^{-1} and 2.74g 20 plants^{-1} respectively and were on par. Plants sprayed with streptocycline recorded biomass of 2.14 g 20 plants^{-1} with 15.94 per cent increase over untreated plants.

4.9. Biochemical characterisation

The isolate P11 and P33 effective against *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae* respectively were characterized based on the tests prescribed by Schaad (1992).

Convex mucoid colonies were produced on peptone beef extract medium by the isolate P33 indicating levan formation but not by the isolate P 11. Both P11 and P33 liquefied gelatin seven days after incubation in gelatin broth.

There was positive growth of P11 at 41°C but not at 4°C. P33 recorded growth at 4°C but not at 41°C.

The sugars arabinose and galactose and sugar alcohol sorbitol were utilized only by P33. But inositol was not utilized by either of the isolates. Propylene glycol and ethanol were utilized by P11 only. The two isolates did not utilize testosterone. Phenyl acetate, n-butylamine and nicotinate were not utilized by the isolates P11 and P33 (Table 4.10).

The properties of the isolates P11 and P33 derived from the results of the critical physiological and biochemical tests conducted were compared with the characters of saprophytic fluorescent pseudomonads described by Schaad (1992) (Table 3.1). From results of the above tests isolates P11 and P33 have been tentatively identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar 1.

Table 4.10 Characters of isolates P11 and P33 of fluorescent *Pseudomonas* sp.

Biochemical tests	P11	P33
Levan formation	-	+
Gelatin liquefaction	+	+
Growth at 4°C	-	+
Growth at 41°C	+	-
Utilisation of Arabinose	-	+
Galactose	-	+
Sorbitol	-	+
Inositol	-	-
Propylene glycol	+	-
Ethanol	+	-
Testosterone	-	-
n-butylamine	-	-
Phenyl acetate	-	-
Nicotinate	-	-

Discussion

5. DISCUSSION

Strains of fluorescent pseudomonads indigenous to the soils of Kerala and having potential for controlling some major diseases of rice like sheath blight and bacterial blight were isolated and evaluated in the present investigation.

Exhaustive studies have been conducted on the control of soil borne diseases in a wide range of crops using this group of bacterium which are also popular for plant growth promoting activity (Cook and Rovira, 1976; Weller and Cook, 1980; Kloepper *et al.*, 1980; Rangeshwaran and Prasad, 2000).

However research on the management of foliar diseases using fluorescent pseudomonads has been initiated only recently (Mew and Rosales, 1986; Rabindran and Vidhyasekaran, 1996; Kamala and Vidhyasekaran, 1997; Vidhyasekaran *et al.*, 2001). Although intensive studies are being conducted in India for the control of plant pathogens using fluorescent pseudomonads practically no work has been reported so far from Kerala, on the management of crop diseases.

In this study among the 33 isolates of fluorescent pseudomonads which were obtained from the rhizosphere and phylloplane of rice plants two isolates viz., P11 and P33 showed highest *in vitro* antagonism against *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae* respectively. High degree of inhibition by fluorescent pseudomonads against *in vitro* growth of foliar pathogens affecting various crops including rice have been reported in earlier

studies conducted (Sakthivel *et al.*, 1986; Lee *et al.*, 1990; da Luz *et al.*, 1998; Sivakumar and Narayanaswami,1998; Rangeshwaran and Prasad,2000).

The isolates P11 and P33 which produced significantly greater inhibition zones in dual plate culture were used for subsequent *in vitro* experiments. Earlier studies with fluorescent pseudomonads were conducted using cell suspension of the bacteria to control the foliage pathogens of rice (Mew and Rosales, 1986; Lee *et al*., 1990; Krishnamurthy and Gnanamanickam, 1998). However use of such cell suspension is impractical for large scale field application (Capper and Higgins, 1993). The two isolates of fluorescent pseudomonads screened in the present study were formulated in talc. Although the percentage reduction over the initial population of the two isolates P11 and P33 over a period 75 days of storage were 70.8 per cent and 64 per cent respectively, these isolates survived fairly well in the formulation maintaining a population level of 1.5 x 10^9cfu g^{-1} and 3.2 x 10^9cfu g^{-1} respectively for that period when stored at room temperature. Vidhyasekaran and Muthamilan (1995) demonstrated that the bacterium survived better in talc and that talc based formulation was superior to any other formulation for controlling crop diseases. Muthamilan (1995) recorded the survival of the bacteria in talc based formulation even upto 240 days of storage although the population declined 30 days onwards. Sivakumar *et al.* (2000) observed that *P. fluorescens* maintained the highest population level of 18.3 x10^7cfu g^{-1} after 40 days of storage in talc compared to storage in other carrier materials. However the population level was reduced to 6 x 10^7cfu g^{-1}.

Rosales *et al.* (1993) indicated that the effect of bacterial isolates on rice seed germination radicle and hypocotyl development were important parameters for selecting bacterial isolates for seed treatment and biological control purposes in rice as some of the isolates may be deleterious to the growth of plants. In this study the effect of the bacterial isolates P11 and P33 on the germination and seedling development were tested on the rice cultivars Jyothi and TN-1 which were susceptible to sheath blight and bacterial blight pathogens against which the isolates were screened. The isolates did not produce any appreciable change on germination percentage. Although there was no significant effect due to the culture suspension and talc based formulation of the isolates on root and shoot elongation, increase in dry weight of root and shoot was remarkably high in both cultivars. Many strains of fluorescent *Pseudomonas* viz., *P. putida-P. fluorescens* isolated from various crops and applied on the plants were observed to promote their growth (Burr *et al.*, 1978; Kloepper, 1983; Weller and Cook, 1986; Mishra and Sinha, 2000). Seed bacterization of rice cv. IR-58, IR-42, IR-36 resulted in increased root and shoot length and dry weight of roots and shoots (Rosales *et al.*, 1993). Root weight of corn seedlings from seeds treated with *Pseudomonas lindbergii* was 44 per cent greater than from untreated seeds (Hodges *et al.*, 1994).

Production of indole-3-acetic acid (IAA) or auxin like substance has been attributed to the plant growth promoting effect of most of the fluorescent *Pseudomonas* (Suslow, 1982; Schippers *et al.*, 1987; Weller ,1988; Cook, 1990). Bacterial isolates P11 and P33 which were capable of increasing the dry weight of root and shoot when applied by seed treatment produced

detectable but not very high amounts of IAA in culture supernatant. Loper and Schroth (1986) and Lindow *et al.* (1998) observed that the isolates of plant growth promoting bacteria which stimulated growth of roots released small amounts of the auxin IAA whereas the isolates which were high IAA producers were inhibitory to root development. Further studies are required to correlate the amount of IAA produced by the bacterial isolates in culture supernatant, with the changes in root growth pattern.

Talc based formulation of fluorescent *Pseudomonas* for the control of foliage diseases of rice has been reported only recently (Muthamilan, 1994; Rabindran, 1994; Kamala and Vidhyasekaran, 1997). Treating seeds with culture suspension of fluorescent *Pseudomonas* sp. in the field is laborious and incompatible with the planting system (Vidhyasekaran and Muthamilan, 1995). Therefore talc based formulation of P11 and P33 were subsequently used to evaluate the efficacy of bacterial isolates under *in vivo* conditions although the plant growth promoting effect of culture suspension of P11 was greater than that of talc based formulation of the isolate P33. Results of the present study clearly indicated that talc based formulation of the effective strain P11 was capable of controlling rice sheath blight. Suppression of the disease was significantly greater when formulation of P11 was applied as foliar spray in combination with seed treatment and seedling root dipping or foliar spray alone when compared to the other treatments. The percentage reduction in disease intensities due to these two treatments over that of control was 34.66 and 34.09. There was 109.5 per cent increase in biomass over that of control, due to the application of the talc based formulation of P11 by a combination of all three methods (seed treatment, seedling root dip,

foliar spray). This formulation was more effective than carbendazim in decreasing the disease intensity. However mere seed treatment with the formulation of P11 did not produce significant reduction in disease intensity. Similar yield increases and effective control of rice sheath blight had been observed in studies conducted earlier. Seed treatment combined with foliar spray of *P. fluorescens* suppressed rice sheath blight (Muthamilan, 1994). Combining foliar spray of Pf ALR-2 with seed treatment, root treatment and soil application gave the best control of rice sheath blight (Rabindran and Vidhyasekaran, 1996). Application of talc based formulation of P-1as seed treatment, seedling root dip and foliar spray was effective in controlling sheath blight of rice (Kamala and Vidhyasekaran, 1997).

Unlike in sheath blight disease seed treatment with talc based formulation of isolate P33 was effective in controlling the disease intensity of bacterial blight. Bacterized rice plants showed a substantial reduction of 40-60 per cent in bacterial blight severity (Anuratha and Gnanamanickam, 1987). Lower disease intensity was recorded by spraying the formulation on the foliage which enhanced the effect of seed treatment or seedling root dip. Seed treatment along with the foliar spray of talc based formulation of Pf-1 reduced the intensity of bacterial blight (*X. oryzae* pv *oryzae*) (Vidhyasekaran *et al.*, 2001). The application of *P. putida* strainV14 I as foliar spray gave maximum suppression of bacterial blight disease (Gnanamanickam *et al.*, 1999). Foliar spray of *Pseudomonas acidovorus* was effective for controlling bacterial blight disease (Sindhan *et al.*, 1997). However, as in sheath blight management, maximum control of bacterial blight disease (40.28 per cent reduction) disease was obtained by combining all three methods of

application viz., seed treatment, seedling root dip and foliar spray. Biomass production was also highest due to this treatment (323.91 per cent). This formulation of P33 was highly effective for the management of bacterial blight disease of rice compared to the antibiotic streptocycline normally used in rice fields for controlling the disease.

The present studies on the management of sheath blight and bacterial blight diseases indicated that a powder formulation of an effective strain of fluorescent *Pseudomonas* controlled these diseases apart from increasing the biomass of plants, thereby suggesting the scope of utilising this bacterium for large scale field application. However detailed studies are required to investigate the exact mechanism by which diseases are controlled in plants by these bacterial strains. Vidhyasekaran *et al.* (2001) have indicated in their study that induced systemic resistance due to treatment with *Pseudomonas fluorescens* appeared to be transient as there was no induction of disease resistance 30 days after foliar application.

Based on preliminary studies on characterisation of P11 and P33, the isolates were tentatively identified as *P. aeruginosa* and *P. fluorescens* biovar 1 following the taxonomic grouping of plant associated saprophytic fluorescent *Pseudomonas* sp. (Schaad, 1992). Further research combining phenotypic and genotypic methods are required to clarify taxonomy of fluorescent pseudomonads.

Summary

6. SUMMARY

Sheath blight incited by the fungal pathogen *Rhizoctonia solani* and bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* are the two major diseases of rice in Kerala. The endemic nature of sheath blight disease which occurs throughout the growth phases of rice from tillering up to the flowering stage entails the application of chemical fungicides during the major cropping seasons of rice. Bacterial blight affects the crop only after tillering stage leading to the heavy loss of the crop at the time of panicle initiation. Chemical control of bacterial blight has not been very successful and the absence of a suitable resistant variety aggravates losses due to this systemic disease. The dense foliar canopy of modern high yielding varieties provides a favourable environment for the rapid development of these diseases. Therefore, the present investigation was undertaken to develop a suitable biocontrol strategy using the bacterial antagonist belonging to fluorescent pseudomonad group for managing these two serious diseases of rice.

Virulent isolates of the pathogen *R. solani* and *X. oryzae* pv *oryzae* were obtained from rice fields of Thiruvananthapuram and Palakkad districts respectively. Thirty three isolates of fluorescent pseudomonads obtained from rhizosphere and phylloplane, of healthy rice plants were screened for their efficacy in inhibiting the growth of sheath blight and bacterial blight pathogens. Isolates P11 and P33 obtained from phylloplane were observed to be superior in inhibiting the pathogens *R. solani* and *X. oryzae* pv *oryzae* respectively. The two isolates were mass multiplied and formulated in inert

material talc, for application under green house conditions. P11 and P33 survived well in talc and recorded a population of 1.5 x $10^9$and 3.2 x 10^9 respectively, 75 days after preparation of the formulation.

Effect of the talc based formulation of the bacterial isolates on the growth of rice seedlings indicated that the formulation did not have any adverse effect on germination of rice seeds of cultivar Jyothi and TN-1 used in this study. Root and shoot weight of seedlings arising from seeds treated with the formulation of P11 and P33 were significantly greater than those obtained from untreated seeds. However studies on the estimation of IAA by P11 and P33 did not indicate noticeably high production of the hormone by the isolates.

Separate pot culture experiments were conducted to assess the efficacy of different methods of application of the talc based formulation of P11 and P33 in controlling the sheath blight and bacterial blight respectively. In case of both isolates P11 and P33, a combination of all three methods of application viz., seed treatment (10g kg seed^{-1}), seedling root dipping (1 per cent), foliar spray (1 per cent) with the formulation, not only reduced the severity of the diseases but also significantly increased the biomass yield of the rice plants. Efficacy of seedling root dipping in checking the diseases was improved when it was followed by foliar spray with the formulation of fluorescent pseudomonad isolate, at the tillering stage of the crop. Seed treatment was effective in controlling bacterial blight disease. Formulations of the bacterial isolates were more effective than the fungicide carbendazim and the antibiotic streptocycline in controlling sheath blight and bacterial blight diseases respectively.

On the basis of preliminary biochemical tests conducted, isolates P11 and P33 were tentatively identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar1.

This investigation forms the first report of application of fluorescent pseudomonads as biocontrol agent for the management of rice diseases in Kerala. The promising results of this study indicate that the formulation of fluorescent pseudomonads may be highly useful for large scale field application in order to control sheath blight and bacterial blight diseases and thereby minimize the use of chemicals in the rice fields of Kerala.

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*Original not seen

Appendix

APPENDIX – I

COMPOSITION OF DIFFERENT MEDIA

(a) King's medium B (KMB)

Peptone	:	20 g
Dihydrogen phosphate	:	1.5 g
Magnesium sulphate	:	1.5 g
Glycerol	:	10 ml
Distilled water	:	1 l
Adjust pH to 7.2		

(b) Potato dextrose agar (PDA)

Potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1 litre

(c) Potato sucrose agar (PSA)

Potato	:	300 g
Disodium hydrogen phosphate	:	2.0 g
Calcium nitrate	:	0.5 g
Peptone	:	5.0 g
Sucrose	:	20.0 g
Agar	:	20.0 g
Distilled water	:	1 l
pH	:	6.8 – 7.0

EVALUATION OF FLUORESCENT PSEUDOMONADS FOR THE MANAGEMENT OF SHEATH BLIGHT AND BACTERIAL BLIGHT OF RICE (*Oryza sativa* L.)

BY

HEERA. G.

ABSTRACT OF THE THESIS
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ABSTRACT

Biological control of major foliar diseases of rice in Kerala viz., sheath blight and bacterial blight using fluorescent pseudomonads was studied. Isolates obtained from the phylloplane were observed to be effective in inhibiting the growth of foliar pathogens. Isolate P11 was most effective in inhibiting the growth of *Rhizoctonia solani* inciting sheath blight disease whereas growth of bacterial blight pathogen *Xanthomonas oryzae* pv *oryzae* was suppressed by the isolate P33, under *in vitro* conditions.

P11 and P33 survived well in the inert material talc used as carrier material in the formulation developed, using these isolates.

Seed treatment with talc based formulation of each isolate (P11 and P33) increased the dry weight of root and shoot of rice seedlings of cultivar Jyothi and TN-1 14 days after sowing.

The isolates produced almost equal and detectable amounts of IAA in culture supernatant.

Greenhouse studies conducted at the College of Agriculture, Vellayani, indicated that a combination of seed treatment (10g kg^{-1} seed), seedling root dipping (1 per cent solution), and foliar spray (1per cent) with the formulation of P11 and P33 effectively suppressed sheath blight and bacterial blight disease respectively and also appreciably increased the biomass yield of treated rice plants. Three foliar sprays starting two days prior to inoculation with the pathogen, during the tillering stage of the crop enhanced the efficacy of seedling root dipping with the biocontrol formulation.

Isolates P11 and P33 were tentatively identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar 1 respectively.