MANAGEMENT OF FOLIAR BLIGHT OF AMARANTHUS USING RHIZOBACTERIA AND CHEMICAL ACTIVATOR-ACIBENZOLAR-*S*-METHYL

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DECLARATION

I hereby declare that this thesis entitled "**Management of foliar blight of amaranthus using rhizobacteria and chemical activator-*acibenzolar-S-methyl***" 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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CERTIFICATE

Certified that this thesis entitled "**Management of foliar blight of amaranthus using rhizobacteria and chemical activator-acibenzolar-*S*-methyl**" is a record of research work done independently by Mrs. Chitra B. Nair (2003-11-46) 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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Dedicated to
My Husband

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LIST OF ABBREVIATIONS

%	Per cent
µg	Microgram
µl	Microlitre
°C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
CRD	Completely Randomised Design
DAI	Days After Inoculation
et al.	And others
Fig.	Figure
g	Gram
kg	Kilogram
l	Litre
m	Metre
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
M	Molar
N	Normal
nm	Nanometre
ppm	Parts per Million
rpm	Rotations per minute
sec	Second
spp.	Species
var	Variety
viz.	Namely
w/v	Weight/volume

Introduction

1. INTRODUCTION

Green leafy vegetables constitute an important component of diet in tropical countries making it richer in minerals, vitamins and proteins. Nutritional experts recommend an average consumption of 50-100 g of green leafy vegetables per day for an adult. Owing to its low production cost and high yield, amaranthus is considered to be the cheapest leafy vegetable in the market and it could be rightly described as 'poor man's spinach'. It is a rich source of vitamins and minerals especially iron, calcium and phosphorous. As amaranthus is a short duration crop with a large yield of edible matter per unit area, it fits well in the crop rotation schedules in Kerala.

Foliar blight of amaranthus caused by *Rhizoctonia solani* is a serious disease of amaranthus (Kamala *et al.,* 1996). Susceptibility of popular cultivars and the humid conditions in Kerala make the disease a serious problem in amaranthus cultivation. The presently recommended measure for managing the disease is foliar spraying of mancozeb 0.4% in cowdung supernatant at fortnightly intervals (Gokulapalan *et al.*, 1999). Though disease management using fungicides is quite effective, their consistent use on a regular basis poses serious health hazards.

At present there is increased focus on organic farming practices, which are ecologically safe. In organic farming, at most importance is given for integrated pest management (IPM). Biological control is an important and inevitable part of IPM. Biological control practices reduce the risk of pesticide residues and enables maintenance of ecological balance. Biocontrol using microbial agents especially that with PGPR has been found to be effective under field conditions.

Plant defense system against several diseases can also be induced using many (PGPR) and certain novel chemicals. Other benefits of rhizobacterial application include plant growth promotion and maintenance of soil health.

In the present investigation an attempt has been made to use PGPR and a chemical activator, Acibenzolar-S-methyl (ASM), both individually and as combinations for managing the foliar blight disease of amaranthus. The study made use of two Pseudomonad strains, two *Bacillus* strains and the chemical activator. The main objectives of the study are:

- To find out the efficacy of Pseudomonads, *Bacillus* and ASM in inhibiting the mycelial growth of *R. solani* under *in vitro* conditions.
- To screen PGPR, ASM and their combination for Induced Systemic Resistance (ISR) / Systemic Acquired Resistance (SAR) activities.
- To study disease suppression and plant-growth promotion activity by the biocontrol agents.

Review of Literature

2. REVIEW OF LITERATURE

Foliar blight of Amaranthus (*Amaranthus tricolor* L.) was first reported in Kerala by Kamala *et al.* (1996). It was reported that disease is characterized by light cream coloured spots on the foliage which rapidly spread causing extensive damage. Gokulapalan *et al.* (2000) reported that the disease severity is more during high humid conditions. Aerial blights caused by *R. solani* have been previously reported on vegetables like radish, cabbage, spinach and sugarbeet (Baker, 1970; Galindo *et al.*, 1983; Shew and Main, 1985).

Though chemical control of the disease through the use of fungicides reduces the severity of the aerial blight disease (Gokulapalan *et al.*, 1999) the continuous application of them may cause serious environmental problems.

2.1 BIOLOGICAL CONTROL OF *R. solani*

Biological control has emerged as an important alternative in plant disease management (Whipps, 1997; Bowen and Rovira, 1999). Elad *et al.* (1982) reported that several fungal and bacterial antagonists like *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* have been found to be effective in checking the diseases caused by *R. solani* in rice, pea and cotton. *Aspergillus niger* isolated from the rhizosphere of coffee seedlings was antagonistic to the collar rot pathogen (*R. solani*) *in vitro* and hyperparasitised the pathogen completely in dual culture (Venkatasubbiah and Safeeulla, 1984). Hyakumachi (1999) reported that the mechanisms involved in *R. solani* disease decline are rapid decline in inoculum potential and suppressiveness of the soil. It was observed that soil suppressiveness was due to enhanced competitive pressure of soil microbes against *Rhizoctonia*. The possibility of biological control of foliar blight pathogen of amaranthus with microbial antagonists was

studied by Smitha (2000). *Trichoderma longibrachiatum* and a fluorescent Pseudomonad strain P1 were found to be the most effective bioagents in inhibiting *R. solani in vitro*. Priyadarsini (2003) reported that *T. harzianum, P. fluorescens* isolate B3, rice husk ash (1000g/m^2 top soil) and *Piriformospora indica* were effective in managing *R. solani* induced foliar blight of amaranthus.

2.1.1 Biocontrol Potential of Pseudomonads

Fluorescent pseudomonads have been reported to be one of the most effective rhizobacteria involved in reducing soil borne diseases in disease suppressive soils (Weller, 1988). Strains of *P. fluorescens* have been reported to suppress a variety of plant diseases caused by microbial pathogens including foliar diseases caused by fungal pathogens such as *Gaeumannomyces graminis*, *R. solani* and *Pythium* spp. in green house and field trials (De Freitas and Germida, 1991). Five Pseudomonad strains isolated from cotton rhizosphere were antagonistic to *R. solani* and *Sclerotium rolfsii* (Bhowmik *et al.*, 2003). Dantre *et al.* (2003) reported that 15 isolates of Pseudomonads inhibited the growth of *R. solani* both *in vivo* and *in vitro*. *P. aeruginosa* strain IE-6 inhibited root-infecting fungi, *Macrophomina phaseolina* and *R. solani* (Siddiqui and Ehteshamul-Haque, 2001). Suppression of brown patch disease on bent grass and the production of several secondary metabolites like 2,4-diacetylphloroglucinol (2,4-DAPG), Hydrogen cyanide (HCN), siderophore and Indole acetic acid (IAA) by *Pseudomonas fluorescens* strain HP72 has been reported by Suzuki *et al.* (2003).

2.1.2 Biocontrol Potential of *Bacillus*

Spraying of pepper seedlings in green house with a suspension of *Pseudomonas* strain J3, *Bacillus* strain BB11 and FH17 and the admixture of these strains have been reported to reduce the incidence of *Ralstonia*

solanacearum (Jinhua *et al.*, 2002). PGPR strain INR7 has been reported to control *R. solani* on soyabean in both green house and field conditions (Hui *et al.*, 2003). *Bacillus subtilis* and *Pseudomonas chororaphis* were proved to be effective against *Pythium aphanidermatum* under *in vitro* conditions (Kavitha *et al.*, 2003).

2.2 PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Rhizosphere bacteria are present in large numbers on the root surface where nutrients are abundant in the form of plant exudates and lysates (Lynch, 1991). Certain strains of rhizosphere bacteria are called Plant Growth Promoting Rhizobacteria (PGPR) as their application can stimulate growth and improve plant stand under stressful conditions (Kloepper *et al.*, 1980). Most strains of the PGPR like *Serratia*, *Bacillus* and *Pseudomonas* can effectively colonize plant roots and protect plants from diseases caused by a variety of root pathogens. They also promote growth of plants through direct induction of growth hormones (Veena Kumari and Srivastava, 1999).

2.2.1 Plant Growth Promotion by Rhizobacteria

Enhancement in the productivity of *Bacillus* treated Geranium by 88% over untreated control was reported by Abdul *et al.* (2003). *Bacillus* isolates were found to promote growth in chickpea, brinjal, okra and chilli among which the isolate SE34 enhanced maximum germination and seedling vigour (Amruthesh *et al.*, 2003). Also the assessment of growth promotion under green house conditions identified enhancement in emergence rate, germination percentage, height and fresh and dry weight of plants raised from seed treated with PGPR isolates. Anandaraj *et al.* (2003) found that application of two strains of *P. fluorescens*, a strain of *Bacillus* and *Trichoderma harzianum* resulted in significantly enhanced growth of black pepper. The increase was 44.7% over control for *P. fluorescens* and 38.9% for treatment with a consortium of *P. fluorescens* and *T. harzianum*. Biopriming pearl millet seeds with *P. fluorescens*

isolates resulted in enhancement of germination percentage, seedling vigour, plant height, leaf area, tillering capacity, seed weight and yield (Raj *et al.*, 2004).

2.2.2 Biocontrol Potential of PGPR

Most bacterial biocontrol agents of plant pathogens function partially or completely through antagonism (Brian, 1957). PGPR were used as biocontrol agents for the suppression of several pathogens (Broadbent *et al.*, 1971; Dunleavy, 1995 and Schippers *et al.*, 1987). Antibiotic production has been recognized as an important factor in the suppression of a variety of soil-borne plant pathogens by PGPR (Thomashow and Weller, 1988). It has been reported by Wei *et al.* (1991) that antagonism relies on direct function of the biological control agents such as production of antibiotics, siderophores and hydrogen cyanide (HCN). Krauss and Loper (1995) reported that all the siderophores-pyoveridine, salicylate and pyochelin were produced by fluorescent Pseudomonads when these bacteria were grown under iron limited conditions.

Gupta *et al.* (1999) tested the antifungal activity of *P. aeruginosa* by a dual culture technique. The growth of inhibition of *Macrophomina phaseolina* and *Fusarium oxysporum* was 74.1% and 70.5% respectively and the production of HCN was also recorded. Chitinase producing *Paenibacillus illinoisensis* KJA-424 suppressed the symptom of damping off of cucumber seedlings caused by *R. solani* (Jung *et al.*, 2003). Wang *et al.* (2003) evaluated five strains of *Bacillus* spp. in green house experiments and found that their application reduced the severity of *Rhizoctonia solani*.

2.2.3 Induction of Systemic Resistance by PGPR

Many PGPR strains can induce physiological changes throughout the plant, making it more resistant to pathogens. This phenomenon termed as Induced Systemic Resistance (ISR) has been demonstrated in several

plants (van Peer *et al.*, 1991; van Peer and Schippers, 1992; Maurhofer *et al.*, 1994; Liu *et al.*, 1995; De Meyer and Höfte, 1997; Müller *et al.*, 1998). Besides suppression of plant diseases by many mechanisms, fluorescent Pseudomonads induce systemic resistance in plants against attack by a wide range of pathogens (Whipps, 1997; Raupach and Kloepper, 2000; Ramamoorthy *et al.*, 2001; Viswanathan and Samiyappan, 2002).

Rhizobacteria mediated ISR has been demonstrated in many plants like bean, carnation, cucumber, radish, tobacco, tomato and *Arabidopsis thaliana* and reported to be effective against a broad spectrum of plant pathogens including fungi, bacteria and viruses (van Loon *et al.*, 1998). Specific recognition between the plant and the ISR inducing rhizobacterium is required for the induction of ISR (Pieterse *et al.*, 2001). *P. fluorescens* strain CHAO, EP1 and Pf1 significantly reduced incidence of *Colletotrichum falcatum* in sugarcane through induction of systemic resistance (Viswanathan and Samiyappan, 2002). These strains significantly improved vegetative sett germination and crop growth in the field also. Two strains of PGPR, *Bacillus pumilus* SE34 and *P. fluorescens* 89B61 elicited systemic resistance against late blight pathogen (*Phytophthora infestans*) in tomato (Yan *et al.*, 2002).

The mechanisms for plant growth promotion and induced systemic resistance (ISR) by PGPR have been extensively studied in the past decade. The elicitor activity of lipopolysaccharide or more specifically of their O-antigenic side chain was identified for *P. fluorescens* strains WCS417r and WCS374 on carnation (Van Peer and Schippers, 1992). Salicylic acid production by most of the rhizobacteria that induce systemic resistance under iron limited conditions and its role in the ISR process have been demonstrated in *P. aeruginosa* KMPCH by De Meyer and Höfte (1997). Previous works demonstrated that several bacterial determinants such as siderophores, salicylic acid and lipopolysaccharides contributed to

ISR (van Loon *et al.*, 1998). PGPR that colonize root systems through seed applications and protect plants against foliar diseases include *P. fluorescens*, *P. putida*, *Bacillus pumilus* and *Serratia marcescens* (Kloepper *et al.*, 1999; Pieterse *et al.*, 2002). Liu *et al.* (1995) tested PGPR strains 89B-27 (*P. putida*) and 90-166 (*Serratia marcescens*) for their ability to induce systemic resistance against the pathogen, *F. oxysporum* fsp. *cucumerianum*. Both PGPR strains induced systemic resistance against the pathogen.

Direct evidence supporting the conclusion that PGPR which remain on plant roots can induce resistance in plants to foliar or systemic pathogens has been reported against anthracnose of cucumber (Wei *et al.*, 1991), Fusarium wilt of carnation (Van Peer *et al.*, 1991) and halo blight of beans (Alström, 1991).

2.2.4 Biochemical Changes

Investigations on mechanisms of biological control by plant growth promoting fluorescent Pseudomonads revealed that several strains protect the plants from various pathogens in many crops, by activating defense genes encoding chitinase, β-1,3-glucanase, peroxidase (PO), Phenyl alanine ammonia lyase (PAL) and other enzymes which are involved in synthesis of phytoalexins (Maurhofer *et al.*, 1994). Induced systemic resistance once expressed activates multiple potential defense mechanisms that induce increased activity of chitinases and peroxidases which showed resistance to various plant pathogens (Xue *et al.*, 1998). Expression of PAL regulating the biosynthesis of antifungal phytoalexin medicarpin in groundnut cultivars susceptible to early and late leaf spot diseases have been reported by Kale and Choudhary (2001). *P. fluorescens* when applied through seed, seedling dip, soil and on foliage reduced the tomato spotted wilt virus disease (Kandan *et al.*, 2002). The treated plants also showed an increase in PO and PAL activity and an increase in the accumulation of phenolic compounds. Sarma *et al.* (2002) reported that seed bacterisation

with PGPR in chickpea resulted in accumulation of phenolic compounds like gallic acid, chlorogenic acid and cinnamic acid which resulted in suppression of collar rot disease caused by *Sclerotium rolfsii*.

Reduced susceptibility to infection with the fungal pathogen *Cercospora nicotianae* have been reported by Shadle *et al.* (2003) in tobacco plants over-expressing L-PAL. It was noticed that these plants produced high levels of chlorogenic acid. Sivakumar and Sharma (2003) observed that there was an increase in phenolic content in leaf sheaths of maize plants raised from *P. fluorescens* treated seeds and inoculated with *R. solani*. PO, PPO and PAL activities were also found to be increased. Activation of PO and PAL in cotton by chemical and biological inducers have been reported by Padmaja *et al.* (2004). A total of nine isozymes of PO were observed when different inducers were inoculated.

2.3 CHEMICAL ACTIVATORS

Systemic acquired resistance (SAR)' is the phenomenon by which defense mechanisms in plants are activated by a pathogen or their metabolites or by a diverse group of structurally unrelated organic and inorganic compounds (Kuc, 2001). The development of SAR is associated with the accumulation of salicylic acid, derived from enhanced phenyl propanoid biosynthesis (Gaffney *et al.*, 1993; Mauch-Mani and Slusarenko, 1996 and Vernooji *et al.*, 1994). Several chemicals can induce the same resistance spectrum and biological changes as in biological SAR induction on cucumber and tobacco (Metraux *et al.*, 1991; Kessman *et al.*, 1994). The mechanisms through which salicylic acid (SA) mediated SAR was reported by Dempsey *et al.* (1999). They include alterations in the activity or synthesis of certain enzymes, increased defense responses and the generation of free radicals.

Functional analogues of SA, such as 2,6-dichloro isonicotinic acid or benzo (1,2,3) thiadiazole-7-carbothionic acid S-methyl ester (BTH or ASM) were developed which activate the resistance mechanisms

(Oostendorp *et al.*, 2001; Sticher *et al.*, 1997). Benzo(1,2,3) thiadiazole-7-carboxylic acid derivatives have been developed as a novel class of crop protection agents that increase crop resistance to disease (Görlach *et al.*, 1996). BTH was introduced by Novartis, Switzerland as a Plant activator (Leadbeater *et al.*, 1997).

Benhamou *et al.* (1994) reported induction of systemic resistance to *Fusarium* crown and root rot in tomato plants by seed treatment with chitosan. Reduction in the root rot disease caused by *R. solani* was reported in cowpea plants that received a foliar treatment of SA (Amaresh *et al.*, 2001). It was reported by Pajot *et al.* (2001) that Phytogard (K_2HPO_3) and DL-β-amino butyric acid (BABA) at specific concentrations had a curative effect and the resistance induced against *Bremia lactucae* lasted for atleast 15 days. The efficacy of plant products and chemicals like aspirin and potassium chloride on the mycelial growth and sclerotial production of *R. solani* has been studied by Reddy and Sudhakar (2002). Aspirin was highly effective in inhibiting mycelial growth and sclerotial production. Chun *et al.* (2003) found that treatment of tobacco leaves with salicylic acid or ethylene induced resistance to black shank and delayed the death of infected plants.

Akinwunmi *et al.*, (2001) reported induction of defense responses in cowpea against *Colletotrichum destructivum* after seed treatment with ASM. Oostendorp *et al.* (2001) reported that the best-studied resistance activator ASM (Bion), activates resistance in many crops against a broad spectrum of diseases caused by fungi, bacteria and viruses at low concentrations. Romero *et al.* (2001) reported that bell pepper sprayed with ASM (Actigard 50WG) showed resistance to subsequent infections with the bacterial spot pathogen *Xanthomonas axonopodis* pv. *vesicatoria.* ASM have been reported to induce resistance against sunflower rust and resulted in an increase in the amount of accumulated and excreted coumarins and other phenolic compounds (Prats *et al.*, 2002). Rashmi *et al.* (2002)

investigated the mode of action of ASM against *R. solani* in rice. The degree of protection was reported to be increased with increase in duration between Benzothiadiazole (BTH) application and inoculation. Roberto *et al.* (2002) reported the ability of ASM to induce resistance in pepper plants against *Xanthomonas campestris* pv. *vesicatoria*. The highest efficacy was obtained by spraying the plants six to seven times at an interval of every eight to twelve days with a mixture of ASM and copper hydroxide. Induction of systemic acquired resistance by benzothiadiazole has been reported against sheath blight of rice by Zhang *et al.* (2003).

Attempts have been made to combine chemical activators and rhizobacteria for management of diseases. Combination of biological and chemical tactics may provide remarkable advantages over each method alone, resulting in a series of benefits, including control efficacy, consistency, increase in the range of modes of action of pathogen control (da Luz, 2003). Srivastava and Arora (2003) reported that soil drenches or seed treatments of β-amino butyric acid combined with *P. fluorescens* isolate 12-92 enhanced effectiveness and consistency of the biological control agents against charcoal rot in chickpea. Anith *et al.* (2004) studied the effect of PGPR (*Bacillus pumilus* strain SE34, *Pseudomonas putida* strain 89B61 and two bacterial bioformulations *viz.*, Bioyield and Equity), ASM and a soil amendment on bacterial wilt caused by *Ralstonia solanacearum* in susceptible tomato plants. Combination of Actigard with *P. putida* strain 89B61 or Bioyield and Equity caused significant reduction in disease incidence. Significant reduction of disease caused by *Colletotrichum orbiculare* in cucumber plants induced by PGPR strains *Serratia marcescens* 90-166 and *P. fluorescens* 89B61 and the chemical activators aminosalicylic acid or DL-3-amino butyric acid has been reported by Jeun *et al.* (2004).

Materials and Methods

3. MATERIALS AND METHODS

3.1 PATHOGEN

3.1.1 Isolation of the Pathogen

Amaranthus leaves showing typical leaf blight symptoms caused by *Rhizoctonia solani* were collected from the Instructional Farm of College of Agriculture, Vellayani. They were cut into small bits along with some healthy portions. The bits were surface sterilized with 0.1 per cent mercuric chloride, followed by two changes of washings with sterile water. The bits were then placed on potato dextrose agar (PDA) (Appendix IA) in sterile petridishes and incubated at room temperature. The fungal growth was noticed 24 hrs after inoculation. The growth was purified by hyphal tip method and transferred to PDA slants. The isolate was maintained by periodic subculturing on PDA and stored at 4°C.

3.1.2 Pathogenicity Test

Koch's postulates were proved for confirming the pathogenicity of the different isolates of *R. solani*. The upper two leaves of thirty days old amaranthus seedlings raised in pots were inoculated on the upper surface with mycelial bits of one week old culture of the fungus grown on PDA. A thin layer of moistened cotton was placed over the inoculated portion in order to provide humidity. Inoculated plants were covered using polythene bags to maintain humid condition. Re-isolation of the pathogen was done from the leaves showing typical blight symptoms and the identity of the pathogen was established.

3.2 *IN VITRO* STUDIES ON INHIBITION OF FUNGAL GROWTH

3.2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Four plant-growth promoting rhizobacteria were used for the study. *Pseudomonas fluorescens* strain PN026R was procured from the Department of Plant Pathology, College of Agriculture, Vellayani. *Pseudomonas putida* strain 89B61, *Bacillus pumilus* strain SE34 and *Bacillus subtilis* strain GB03 were kindly given by J.W. Kloepper, Auburn University, Alabama, USA (Plate 1).

In vitro antagonism of the four rhizobacterial strains against *Rhizoctonia solani* were tested by dual culture plate assay on four different culture media. The culture media used for the study were Potato Dextrose Agar (PDA), King's medium B (KB) (Appendix IB), Carrot Agar (CA) (Appendix IC) and Nutrient Agar (NA) (Appendix ID). *P. fluorescens* strain PN026R and *P. putida* strain 89B61 were streaked and grown on King's medium B and *Bacillus pumilus* strain SE34 and *Bacillus subtilis* strain GB03 on nutrient agar medium for obtaining single colonies. Sterile Petri plates were poured with molten media and allowed to solidify. A heavy inoculum of the individual rhizobacterial strain was applied as a band of 1.5 cm length equidistantly on four opposite edges of the agar medium in the Petri plate using an inoculation loop. Mycelial discs of five mm diameter from seven days old culture of *R. solani* grown on PDA were cut out with a cork borer and placed at the center of the Petri plate. Four replications were maintained for each rhizobacteria. Plates containing the pathogen alone served as control. The inoculated plates were incubated at 28°C and observation on the mycelial growth of *R. solani* was taken after a period of five days.

Zone of inhibition by the rhizobacteria was measured in mm as the distance between the tip of the mycelial growth of the pathogen and the edge of the bacterial growth.

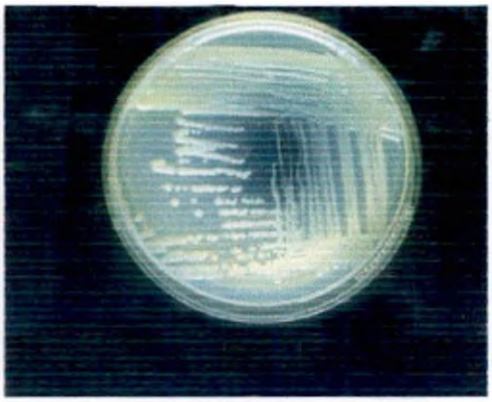
Plate 1 Colony morphology of rhizobacterial isolates on agar medium

A: *Pseudomonas putida* strain 89B61 on King's medium B

B: *Pseudomonas fluorescens* strain PN026R on King's medium B

C: *Bacillus pumilus* strain SE34 on Nutrient agar medium

D: *Bacillus subtilis* strain GB03 on Nutrient agar medium



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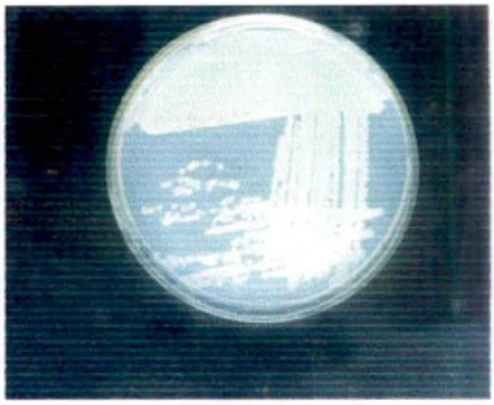

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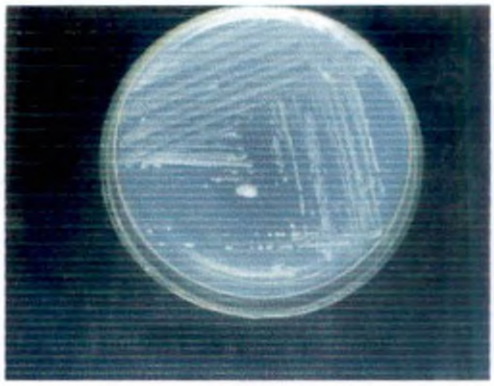
C



D

Plate 1.

3.2.2 Chemical Activator

Actigard ™ 50WG containing the active ingredient Acibenzolar-*S*-methyl (ASM) (manufactured by Novartis Crop Protection Inc., Greensboro, NC, USA) was kindly provided by T.M. Momol, University of Florida, USA. Effect of ASM on the mycelial growth of *R. solani* was tested using poison food technique. Two hundred mg Actigard 50WG was dissolved in one litre of sterile water to get a concentration of 100 ppm of ASM. To 100 ml each of sterile molten PDA medium in 250 ml conical flasks, appropriate quantity of the stock solution was added aseptically to get varying concentrations of ASM. These media with different concentrations of ASM was poured into sterile Petridishes. Fungal mycelial discs of five mm diameter were cut out from seven days old culture of *R. solani* grown on PDA medium using a cork borer and placed at the centre of each dish. Inoculated plates were incubated at 28°C.

For each combination, three replications were maintained. Plates without ASM inoculated with *R. solani* at the center served as control. Observations on the mycelial growth of the pathogen were taken after a period of seven days.

Inhibition of mycelial growth of the pathogen by the chemical activator was measured using the formula:

$$I = \frac{100\ (C - T)}{C}$$

I = Inhibition of mycelial growth of pathogen

C = Radial growth of pathogen in control plates (cm)

T = Radial growth of pathogen in treated plates (cm)

3.2.3 Interaction of PGPR with ASM

One hundred ppm ASM amended molten media was poured into sterile Petriplates. Isolated colonies of the four bacteria were streaked in four

quadrants of the petriplate. Control plates in which no ASM was added were also maintained. The plates were kept for incubation at 28°C. Bacterial growth in these plates was visually assessed and compared with that of the control plates after 48 hours.

3.3 *IN VIVO* STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

3.3.1 Screening the effect of Plant Growth Promoting Rhizobacteria and the Chemical Activator on Plant Growth and Disease Incidence

3.3.1.1 Treatments

T_1 – Soil and root application of *P. fluorescens* strain PN026R

T_2 – Soil and root application of *P. fluorescens* strain 89B61

T_3 – Soil and root application of *Bacillus pumilus* strain SE34

T_4 – Soil and root application of *Bacillus subtilis* strain GB03

T_5 – Soil application of Acibenzolar-*S*-methyl (25 ppm)

T_6 – Soil and root application of all the above bacterial strains

T_7 – Soil and root application of *P. fluorescens* strain PN026R + ASM

T_8 – Soil and root application of *P. fluorescens* strain 89B61 + ASM

T_9 – Soil and root application of *Bacillus pumilus* strain SE34 + ASM

T_{10} – Soil and root application of *Bacillus subtilis* strain GB03 + ASM

T_{11} – Soil and root application of all the above bacterial strains + ASM

T_{12} – Pathogen Inoculated control

T_{13} – Uninoculated control

Design : CRD

Replications : 4

Variety : Arun

3.3.1.2 Preparation of Rhizobacterial Inoculum

Pseudomonas strains 89B61 and PN026R and *Bacillus* strains GB03 and SE34 were grown on KB and NA respectively. Plates were heavily cross-streaked with the bacterial culture and incubated at 28°C for 24 hours. Bacterial cells were harvested from the plates after drenching with 10 ml of sterile water and scrapping with a glass spreader. The cells were finally suspended in 100 ml sterile water to get a bacterial cell density of approximately 10^8 cfu / ml and used for root dipping and soil application.

3.3.1.3 Preparation of ASM Solution

A 25 ppm ASM solution was prepared by dissolving 25 mg Actigard 50WG in 500 ml sterile water.

3.3.1.4 Application of Plant Growth Promoting Rhizobacteria and ASM

Seeds of Amaranthus variety Arun were sown in pots of 10 inch diameter containing sterile soil, sand and cowdung in the ratio of 2:1:1. Fifteen days old seedlings were used for transplanting. Plastic cups of diameter 7.5 cm and height of 9cm were filled with sterile potting mixture of same content as above. Before transplanting, the roots of the seedlings were dipped in respective bacterial suspension for 20 minutes. Each plastic cup contained a single plant. Soil application with rhizobacteria was done by drenching the base of the plants with the bacterial suspension at the rate of 5ml/plant after fifteen days of transplanting. The ASM solution (5 ml/plant) was drenched twice, on one day after transplanting and 12 days after transplanting.

3.3.1.5 Challenge Inoculation with the Pathogen

Challenge inoculation with the pathogen was done seven days after rhizobacterial drenching which corresponded to ten days after ASM drenching. The lower three leaves were selected for inoculating the pathogen. *R. solani* was grown on PDA for seven days. Small mycelial

bits of the pathogen were cut from the plates and placed on upper surface of leaves and a thin layer of moist cotton was placed over the inoculated portion. The whole plant was then covered using a polythene bag to provide humidity. Inoculation was done in all plants except the control.

3.3.1.6 Observations

Observations of amaranthus plants were taken 21 days after transplanting.

3.3.1.6.1 Disease Intensity

Disease severity was graded using a 0 – 9 scale (KAU, 1996) (Plate 2).

Grade	Description
0	No infection
1	1 – 10 per cent of leaf area infected
3	11 – 25 per cent of leaf area infected
5	26 – 50 per cent of leaf area infected
7	51 – 75 per cent of leaf area infected
9	76 – 100 per cent of leaf area infected

Percent disease index was calculated using the formula:

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

(Mayee and Dattar, 1986)

3.3.1.6.2 Shoot Length (cm)

The length of the shoot from the ground level to the growing tip of each plant was measured after depotting.

Plate 2 0-9 scale for the scoring of foliar blight of amaranthus

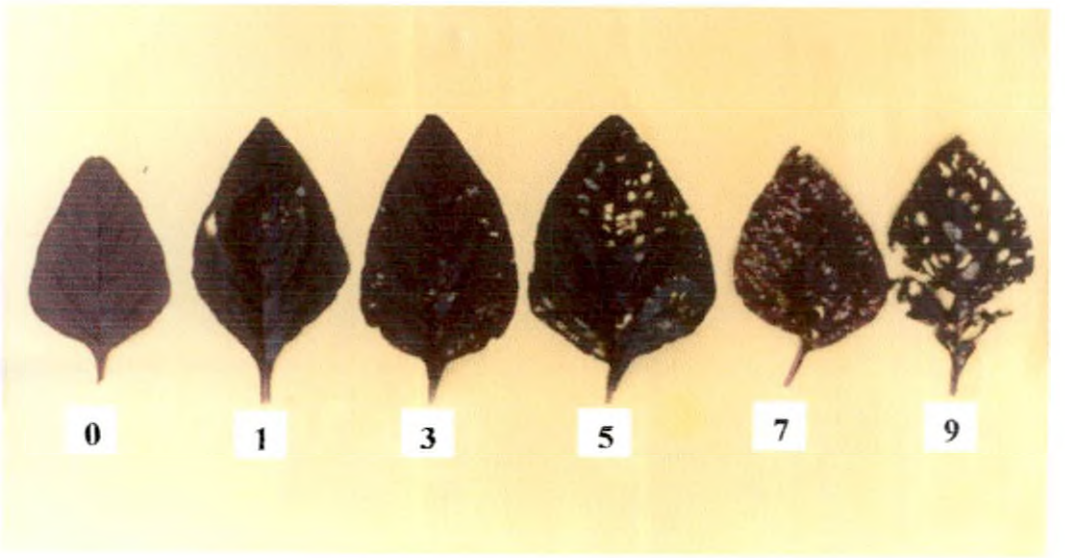


Plate 2

3.3.1.6.3 Root Length (cm)

The length of the longest root of each plant was measured after depotting.

3.3.1.6.4 Dry Weight of Shoot and Root (g)

Dry weight was taken after drying the samples to a constant weight in a drying oven at 60°C.

3.3.2 Effects of Plant Growth Promoting Rhizobacterial Strains on Plant Growth

3.3.2.1 Treatments

T_1 – Soil and root application of *P. fluorescens* strain PN026R

T_2 – Soil and root application of *P. fluorescens* strain 89B61

T_3 – Soil and root application of *Bacillus pumilus* strain SE34

T_4 – Soil and root application of *Bacillus subtilis* strain GB03

T_5 – Soil application of Acibenzolar-*S*-methyl (25 ppm)

T_6 – Soil and root application of all the above bacterial strains

T_7 – Soil and root application of *P. fluorescens* strain PN026R + ASM

T_8 – Soil and root application of *P. fluorescens* strain 89B61 + ASM

T_9 – Soil and root application of *Bacillus pumilus* strain SE34 + ASM

T_{10} – Soil and root application of *Bacillus subtilis* strain GB03 + ASM

T_{11} – Soil and root application of all the above bacterial strains + ASM

T_{12} – Control

Design : CRD

Replications : 4

Variety : Arun

Seeds of Amaranthus variety Arun were sown in pots of 10 inch diameter containing soil, sand and cowdung in the ratio of 2:1:1. Fifteen days old seedlings were used for transplanting. Plastic pots of diameter 7.5 cm and height of 9 cm were filled with potting mixture of same content as above. Before transplanting, the roots of the seedlings were dipped in respective bacterial suspension for 20 minutes. Each plastic pot contained a single plant.

Preparation of bacterial suspension and ASM solution and their application was done in the same way as in the screening *in vivo* experiment.

3.3.2.2 Observations

Shoot length (cm), root length (cm), fresh weight (g) and dry weight (g) of shoot and root were measured one month after transplanting as in the *in vivo* screening experiment.

3.3.3 Studies on Disease Suppression by Biocontrol Agents and their Plant Growth Promotion Activity Under Pot Culture

Treatments with the best ISR responses and plant-growth promotion in the previous *in vivo* experiments were selected and used for further disease suppression studies.

3.3.3.1 Treatments

T_1 – Soil and root application of *P. fluorescens* strain PN026R

T_2 – Soil and root application of *P. fluorescens* strain PN026R + ASM

T_3 – Soil and root application of *B. pumilus* strain GB03

T_4 – Soil and root application of *B. pumilus* strain GB03 + ASM

T_5 – Soil application of ASM

T_6 – Uninoculated control

T_7 – Pathogen Inoculated control

T_8 – Chemical control (Mancozeb 0.4% in cowdung supernatent)

Design : CRD

Replications : 4

Variety : Arun

Preparation and application of bacterial inoculum and ASM solution was done in the same way as described in section 3.3.1.2, 3.3.1.3 and 3.3.1.4. Inoculation with the pathogen was also done as described in section 3.4.1.5. Seeds of Amaranthus variety Arun were sown in pots of size 22 cm x 21 cm containing soil, sand and cowdung in 2:1:1 proportion. After sowing the seeds, the soil was drenched with respective rhizobacterial suspension. ASM was drenched in the pots after complete emergence of the plants. The treated seedlings were transplanted after 15 days into pots of size 22cm x 21cm containing a mixture of soil, sand and cowdung in a ratio of 2:1:1. Before transplanting, roots of the seedlings were dipped in respective bacterial suspension for 20 minutes. Three plants were planted in each pot. Soil application with rhizobacterial suspension was done twice by drenching the base of the plants with the bacterial suspension at the rate of 5 ml/plant at 15 and 22 days after transplanting. ASM solution was drenched thrice, at one day, 12 and 19 days after transplanting.

Challenge inoculation with the pathogen was done seven days after last rhizobacterial drenching, which corresponded to ten days after last ASM drenching. Inoculation was done on 5^{th} and 6^{th} leaves from the top of the plant. The plants were uprooted two months after transplanting.

3.3.3.2 Observations

Shoot length (cm), root length (cm), fresh weight (g) and dry weight (g) of shoot and root were measured as in the above experiment. Disease intensity was also calculated using the same formulae.

3.3.3.2.1 Disease Incidence (%)

Disease incidence was calculated as:

$$\frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

3.3.3.3 Biochemical Studies

Leaf samples from the plants that received different treatments were collected for estimating changes in activity of phenols and defense related enzymes such as phenyl alanine ammonia lyase (PAL), peroxidase (PO) and polyphenol oxidase (PPO). Leaf samples were taken one day, five days and 10 days after inoculation with the pathogen. The 2nd and 3rd leaves from the top were taken for the analysis.

3.3.3.3.1 Total Phenols

Total phenol content was estimated following the procedure described by Bray and Thorpe (1954).

One gram of leaf sample was ground in 10 ml of 80 per cent ethanol using a chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min, and residue extracted with five times the volume of 80 per cent ethanol and centrifuged. The supernatant was evaporated to dryness and the residue dissolved in five ml of distilled water. An aliquot of 0.3 ml was pipetted out and made upto 3 ml with distilled water. Folin–Ciocalteau reagent (0.5 ml) was pipetted out and added followed by addition of 2 ml of 20 per cent sodium carbonate solution to each tube after three min. This was mixed thoroughly and kept in boiling water for one min. The reaction mixture was cooled and absorbance measured at 650 nm against reagent blank using a spectrophotometer. Standard curve was prepared using different concentrations of catechol and expressed as catechol equivalent in microgram per gram leaf tissue on fresh weight basis.

3.3.3.3.2 Phenylalanine Ammonialyase (PAL)

PAL activity was analysed using the procedure described by Dickerson *et al.* (1984).

The enzyme extract was prepared by homogenizing one g leaf sample in five ml of 0.1 M sodium borate buffer (pH 8.8) (Appendix IIA) containing 0.05g of polyvinyl pyrrolidone using a chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used for the assay. The reaction mixture contained three ml of 0.1 M sodium borate buffer pH 8.8, 0.2 ml enzyme extract and 12 mM L-phenylalanine prepared in the same buffer. The blank contained 3ml of buffer and 0.2ml enzyme extract. The reaction mixture and blank were incubated at 40°C for 30 min. The reaction was stopped by adding 0.2 ml of 3 N hydrochloric acid after which absorbance was read at 290 nm in a spectrophotometer.

PAL activity was expressed as micromoles of cinnamic acid produced per minute per gram on fresh weight basis.

3.3.3.3.3 Peroxidase (PO)

Peroxidase activity was determined according to procedure described by Srivastava (1987).

Leaf sample of 200 mg was homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) (Appendix IIB) to which 0.05 g of polyvinyl pyrrolidone was added. Homogenization was done at 4°C using a chilled pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000 rpm for 15 min at 4°C. The supernatant was used as enzyme extract. The reaction mixture consisting of one ml of 0.05 M pyrogallol and 50µl enzyme extract was taken in both reference and sample cuvettes, mixed and placed in the spectrophotometer with reading adjusted to zero at 420 nm. The reaction was started by adding one ml of one per cent hydrogen peroxide into sample cuvettes and

change in absorbance was measured at 30 sec. interval. PO activity was expressed as change in absorbance of reaction mixture per minute per gram on fresh weight basis.

3.3.3.3.4 Polyphenol Oxidase (PPO)

PPO was determined as per the procedure given by Mayer *et al.* (1965).

Leaf samples of 200 mg were homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone was added. Homogenization was done at 4°C using a chilled pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000 rpm for 15 min at 4°C. The supernatant was used as enzyme extract. The reaction mixture contained one ml of 0.1 M sodium phosphate buffer and 1 ml of 0.1 M catechol. Cuvettes filled with the reaction mixture were placed in spectrophotometer and absorbance was set at zero. The reaction was started after adding 50 µl of enzyme extract. The change in absorbance was recorded at 495 nm and PPO activity expressed as change in absorbance of reaction mixture per minute per gram on fresh weight basis.

3.3.3.3.5 Electrophoretic Analysis of Isozyme

Electrophoresis of protein extracts from plant tissues using different kinds of support media and buffer systems allows separation of the multiple forms of enzyme (isozymes) on the basis of charge and molecular weight.

The present work was undertaken to study isozyme pattern of peroxidase enzyme in uninoculated leaves of plants subject to different treatments after challenge inoculation.

3.3.3.3.5.1 Enzyme Extraction and Assay

Soluble and ionically bound enzymes were extracted by grinding the leaf sample under chilled condition in 50 mM tris-cl (pH 7.6) in the ratio of

1 : 2 w/v. The homogenate was centrifuged and the supernatant was used for isozyme analysis.

3.3.3.3.5.2 Isozyme Separation and Staining

Discontinuous anionic polyacrylamide gel electrophoresis was conducted under non-denaturing conditions as previously described by Wagih and Coutts (1982) with slight modification. Protein extracted by 50 mM tris (pH 7.6) was separated by gel electrophoresis in 7.5 per cent gel. The gel was prepared using the stock solution for protein gel electrophoresis without SDS (native gel).

3.3.3.3.5.3 Reagents

a) Separating gel (7.5 %)

Tris chloride buffer stock solution (pH 8.9)	–	5 ml
Resolving gel acrylamide solution	–	10 ml
Distilled water	–	25 ml
APS	–	300 µl

b) Staking gel (4 %)

Tris chloride buffer stock (pH 6.7)	–	2.5 ml
Resolving gel acrylamide solution	–	3.1 ml
Distilled water	–	14.1 ml
APS	–	300 µl

To 100 µl of the extract of each sample 10µl of glycerol and 4µl of bromophenol blue solution were added and mixed thoroughly. 30µl of samples were loaded in the wells using a micropipette. A low temperature of 18-20°C was maintained throughout the run.

Following electrophoresis, the gel was incubated in 0.6 M sodium acetate buffer (pH 5.4) (Appendix IIC) containing 0.5 per cent O-dianisidine HCl for 30 minutes at room temperature. The gel was transferred to 0.1 M hydrogen peroxide until visible bands of peroxidase activity were developed.

3.3.4 Effect of ASM on Plant Growth

3.3.4.1 Treatments

T_1 – 0 ppm ASM

T_2 – 5 ppm ASM

T_3 – 10 ppm ASM

T_4 – 20 ppm ASM

T_5 – 25 ppm ASM

The details of the experiment are as follows.

Design : CRD

Replications : 4

Variety : Arun

3.3.4.2 Preparation of Solution of ASM

A 100 ppm stock solution of ASM was prepared by dissolving 20 mg Actigard 50WG in 100 ml sterile water. ASM solutions with different concentrations were made from the stock solution.

Seeds of Amaranthus variety Arun were sown in pots of 10 inch diameter containing soil, sand and cowdung in the ratio of 2:1:1. Fifteen days old seedlings were used for transplanting. Plastic cups of diameter 7.5cm and height of 9cm were filled with potting mixture of same content as above. The ASM solutions were drenched in the cups one and 15 DAT (5 ml/plant). Each plastic cup contained a single plant.

3.3.4.3 Observations

Shoot length (cm), root length (cm), fresh weight (g) and dry weight (g) of shoot and root were measured one month after transplanting.

3.4 STATISTICAL ANALYSIS

Duncan's Multiple Range Test (DMRT) was used for comparing the means, using the statistical package SAS version 8.1 (SAS Institute Inc., Cary, NC, USA). Data were subjected to arc-Sine transformation wherever necessary.

Results

4. RESULTS

4.1 PATHOGEN

4.1.1 Isolation of the Pathogen

Foliar blight pathogen of amaranthus, *Rhizoctonia solani* was isolated from the diseased plants in the Instructional Farm of College of Agriculture, Vellayani. The mycelial growth of *R. solani* was creamish in colour initially. Later the colour of the culture turned brownish. The culture of the fungus had zonations and small sclerotia were produced after the fungus completed its growth in the Petri plate. The fungal culture was maintained on PDA slants with periodic subculturing (Plate 3).

4.1.2 Pathogenicity Test

Koch's postulates were proved for confirming the pathogenicity of the different isolates of *R. solani*. On artificial inoculation of the upper two leaves of amaranthus seedlings with the pathogen, pale water-soaked lesions appeared within 48 hours. Later these water-soaked lesions enlarged under humid conditions. Gradually the spots became translucent with irregular brown margins. Finally shot hole symptoms were noticed (Plate 4).

4.2 *IN VITRO* STUDIES ON INHIBITION OF FUNGAL GROWTH

4.2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Pseudomonas fluorescens strain PN026R *Pseudomonas putida* strain 89B61, *Bacillus pumilus* strain SE34 and *Bacillus subtilis* strain GB03 were dual cultured against *R. solani* on PDA, CA, NA and KB.

The presence or absence of a zone of inhibition in dual culture is shown in Table 1. PN026R showed no antagonism against the pathogen on PDA, KB and CA (Plates 5-7). However, it showed antagonism on NA medium (Plate 8). GB03 showed antagonism against *R. solani* in all the four media and SE34 showed antagonism in 3 media (Plates 9-12 and 13-15).

Table 1 Antagonism of PGPR against *R. solani* in dual culture

Bacterial isolate	Inhibition zone*			
	PDA	NA	KB	CA
PN026R	-	+	-	-
89B61	++	+	-	++
SE34	++	+	+++	-
GB03	+++	+++	+++	++

+++ Zone of inhibition >5 mm

++ Zone of inhibition <5 mm

+ Antagonism present but zone of inhibition not measurable

- No antagonism as pathogen overgrew the antagonist

*Observations from four replications

Plate 3 Growth of *Rhizoctonia solani* on PDA medium

Plate 4 Symptoms of foliar blight disease on amaranthus leaves

Plate 3.

Plate 4

Plate 5 Inhibition of mycelial growth of *R. solani* by *P. fluorescens* strain PN026R on PDA

A: *R. solani*+PN026R
B: *R. solani*

Plate 6 Inhibition of mycelial growth of *R. solani* by *P. fluorescens* strain PN026R on KB

A: *R. solani*+PN026R
B: *R. solani*

Plate 7 Inhibition of mycelial growth of *R. solani* by *P. fluorescens* strain PN026R on CA

A: *R. solani*+PN026R
B: *R. solani*

Plate 8 Inhibition of mycelial growth of *R. solani* by *P. fluorescens* strain PN026R on NA

A: *R. solani*+PN026R
B: *R. solani*

Plate 9 Inhibition of mycelial growth of *R. solani* by *Bacillus subtilis* strain GB03 on PDA

A: *R. solani*+GB03
B: *R. solani*

Plate 10 Inhibition of mycelial growth of *R. solani* by *Bacillus subtilis* strain GB03 on KB

A: *R. solani*+GB03
B: *R. solani*

Plate 11 Inhibition of mycelial growth of *R. solani* by *Bacillus subtilis* strain GB03 on CA

A: *R. solani*+GB03
B: *R. solani*

Plate 12 Inhibition of mycelial growth of *R. solani* by *Bacillus subtilis* strain GB03 on NA

A: *R. solani*+GB03
B: *R. solani*

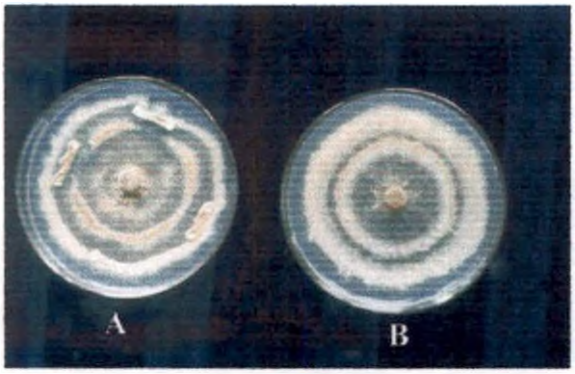
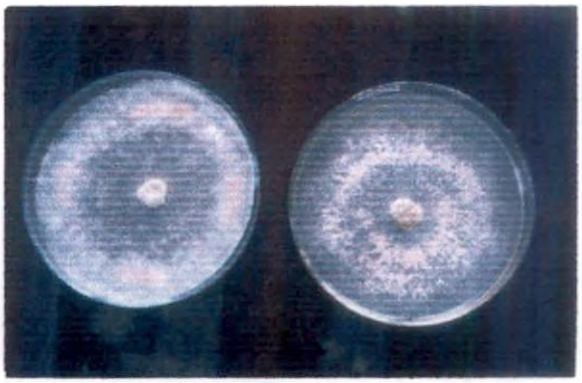


Plate 5.



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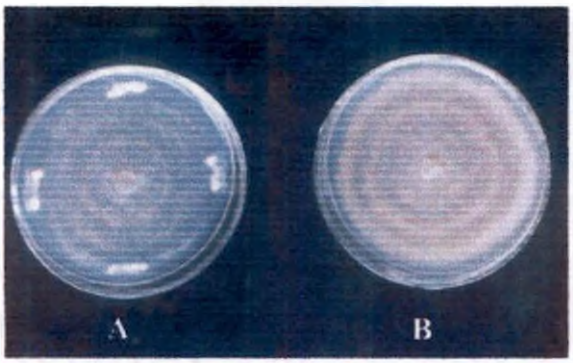

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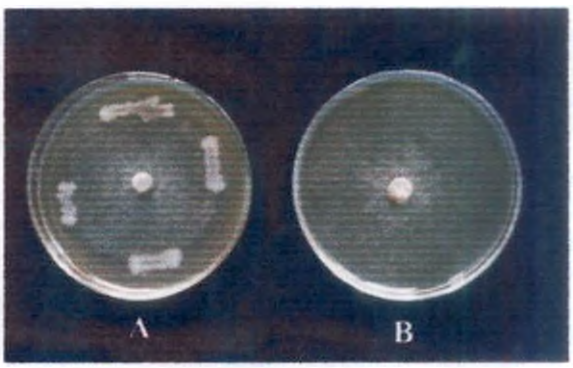

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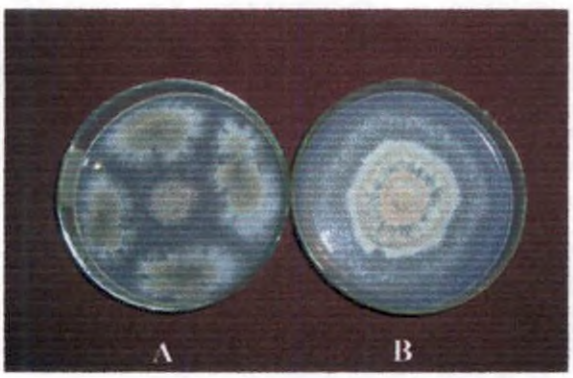

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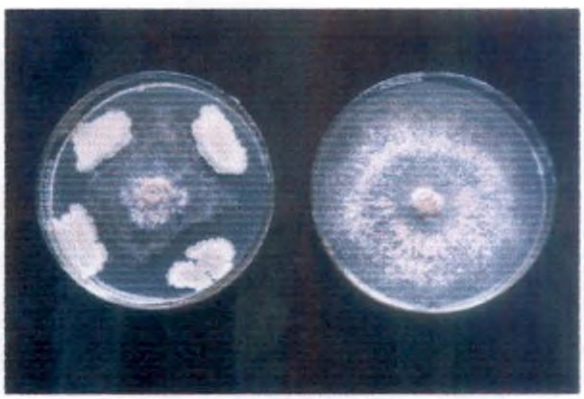

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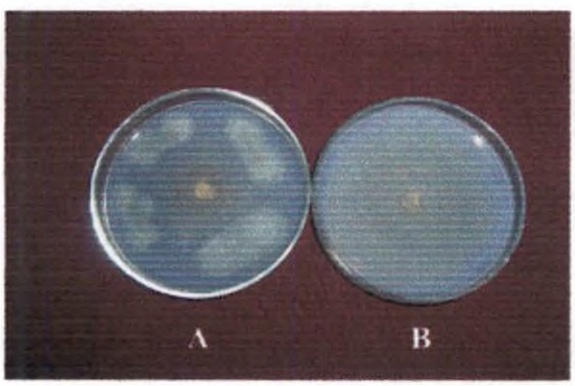

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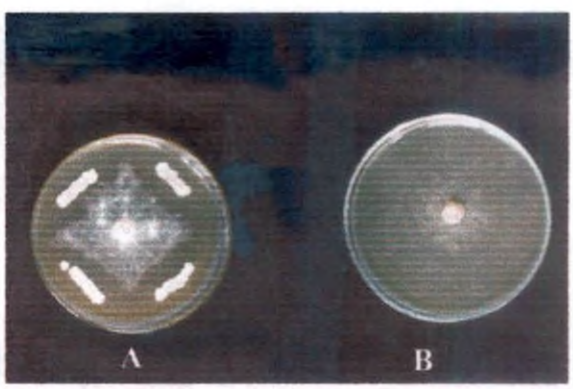


Plate 12.

Plate 13 Inhibition of mycelial growth of *R. solani* by *Bacillus pumilus* SE34 on PDA

A: *R. solani*+SE34

B: *R. solani*

Plate 14 Inhibition of mycelial growth of *R. solani* by *Bacillus pumilus* SE34 on KB

A: *R. solani*+SE34

B: *R. solani*

Plate 15 Inhibition of mycelial growth of *R. solani* by *Bacillus pumilus* SE34 on CA

B: *R. solani*+SE34

A: *R. solani*

Plate 16 Inhibition of mycelial growth of *R. solani* by *P. putida* strain 89B61 on PDA

A: *R. solani*+89B61

B: *R. solani*

Plate 17 Inhibition of mycelial growth of *R. solani* by *P. putida* strain 89B61 on KB

A: *R. solani*+89B61

B: *R. solani*

Plate 18 Inhibition of mycelial growth of *R. solani* by *P. putida* strain 89B61 on CA

A: *R. solani*+89B61

B: *R. solani*

Plate 19 Inhibition of mycelial growth of *R. solani* by *P. putida* strain 89B61 on NA

A: *R. solani*+89B61

B: *R. solani*

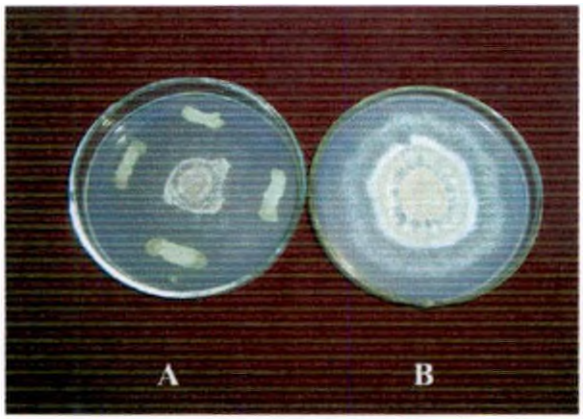
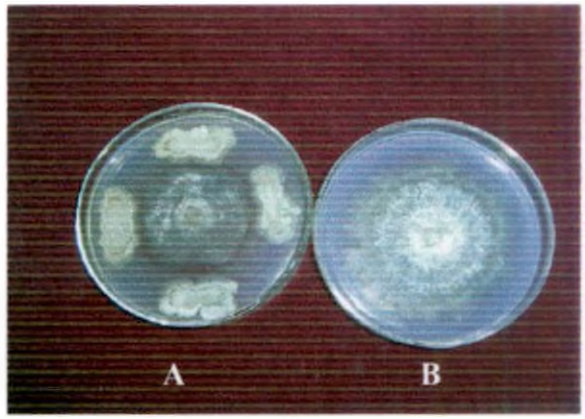


Plate 13.



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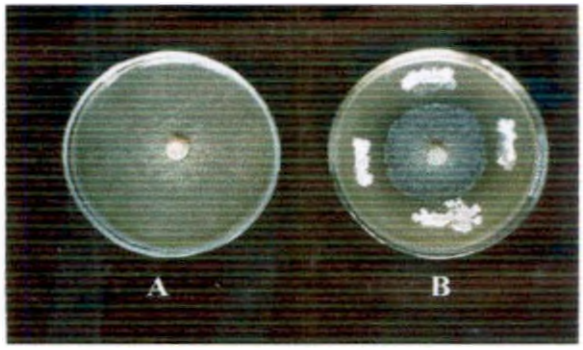

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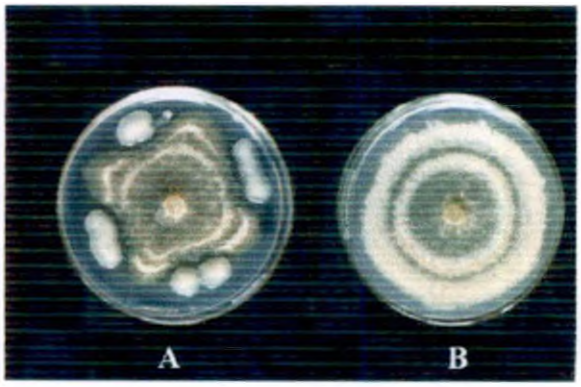


Plate 16.

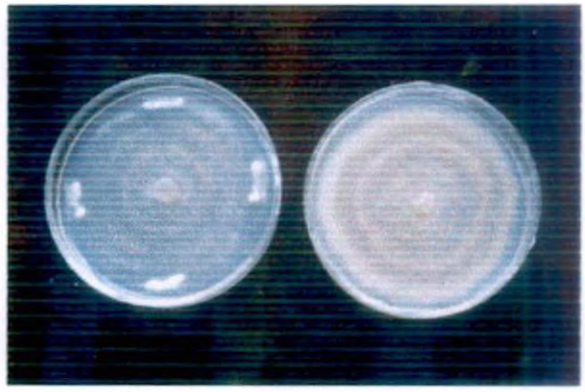


Plate 17.

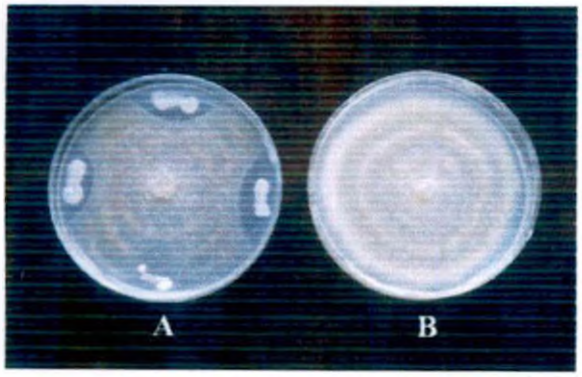


Plate 18.

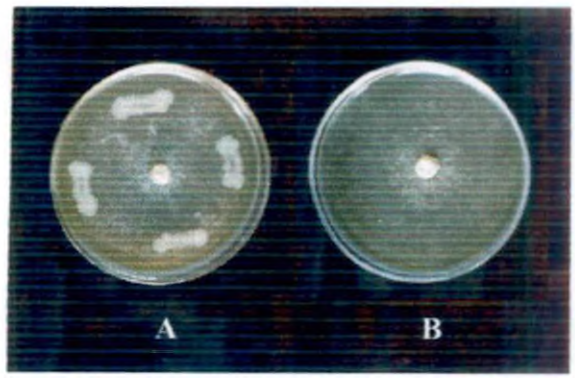


Plate 19.

The antagonism by 89B61 ranged from slight antagonism to an inhibition zone of less than 5mm in different media (Plates 16-19).

Not all the strains produced a measurable zone of inhibition in every medium. The zone of inhibition in those interactions that showed a measurable antagonism are presented in Table 2.

4.2.2 Chemical Activator

The mycelial growth inhibition of the pathogen by different concentration of ASM in the medium is shown in Table 3 (Plates 20 and 21). The maximum mycelial growth inhibition was recorded with 37.5-ppm concentration of ASM. There was reduction in mycelial growth of the fungus when the medium was amended with ASM.

4.2.3 Interaction of PGPR with ASM

All the four PGPR strains showed similar growth pattern and morphology on PDA medium amended with 100 ppm of ASM compared with that on control plates having no ASM amended in it (Plate 22).

4.3 *IN VIVO* STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

4.3.1 Screening the Effect of Plant Growth Promoting Rhizobacteria and the Chemical Activator on Plant Growth and Disease Incidence

4.3.1.1 Disease Severity

The disease severity after challenge inoculation with *R. solani* on amaranthus plants that received different treatments is shown in Table 4. Maximum disease severity of (36.11) was observed for inoculated control treatment. The minimum disease severity was observed in combined treatment with GB03 and ASM (Plate 23). Plants treated with PN026R, GB03 and the consortium of bacteria showed same disease severity of 8.33% (Plate 24-26). However, statistically there was no significant difference between the treatments.

Table 2 Inhibition of *R. solani* by antagonistic bacterial isolates in dual culture

Bacterial isolate	Medium	Inhibition zone (mm)*
89B61	PDA	3.78
89B61	CA	3.43
GB03	NA	9.54
GB03	KB	7.5
GB03	PDA	7.7
GB03	CA	3.75
SE34	NA	8.2
SE34	KB	7.68
SE34	PDA	2.55
CD (0.05)		1.092

*Mean of four replications of one plate each

Table 3 Effect of Acibenzolar-S- Methyl (ASM) on the growth of *R. solani*

Conc. of ASM in the medium	Diameter of mycelial growth (cm)*	Percentage mycelial inhibition*
5 ppm	7.93	11.24
12.5 ppm	7.72	13.62
25 ppm	7.60	14.91
37.5 ppm	6.41	28.27
50 ppm	6.68	25.28
75 ppm	6.63	27.24
100 ppm	6.48	27.51
No ASM	9.20	-

*Mean of four replications having one plate each

Plate 20 Effect of ASM on the *in vitro* growth of *R. solani*

1. 50 ppm ASM
2. 37.5 ppm ASM
3. 25 ppm ASM
4. 12.5 ppm ASM
5. 0 ppm ASM
6. 5 ppm ASM

Plate 21 Effect of ASM on the *in vitro* growth of *R. solani*

1. 75 ppm ASM
2. 100 ppm ASM
3. 0 ppm ASM

Plate 22 Growth of different PGPR strains in the presence of ASM

A. 100 ppm ASM
B. 0 ppm ASM

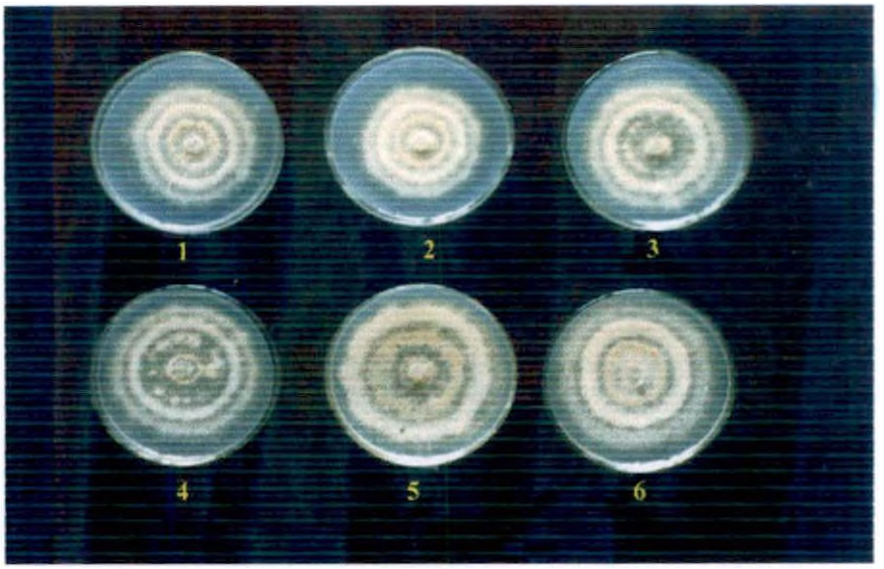


Plate 20.

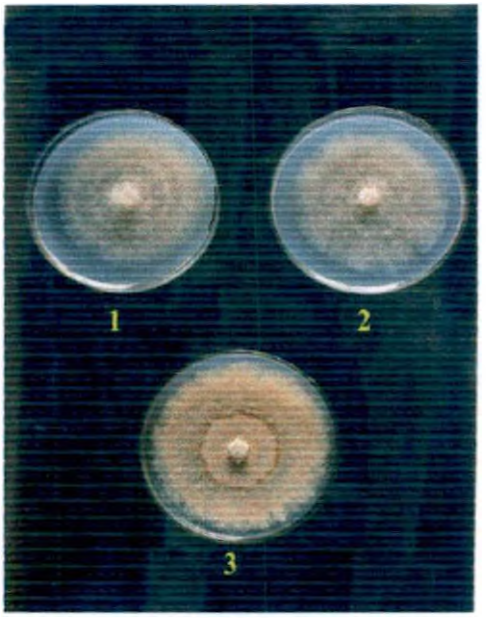


Plate 21.

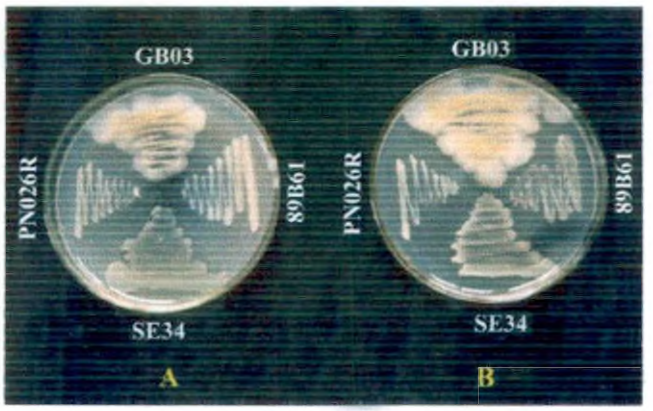


Plate 22.

Table 4 Effect of PGPR and chemical activator on disease incidence

Treatments	Disease severity*[x,y]
PN026R	8.33(11.46a)
89B61	16.67(16.29a)
SE34	16.67(19.42a)
GB03	8.33(11.46a)
ASM	16.67(19.42a)
Consortium of bacteria	8.33(11.46a)
ASM+PN026R	33.33(32.21a)
ASM+89B61	25.00(24.25a)
ASM+SE34	25.00(24.25a)
ASM+GB03	0.00(3.50a)
ASM + Consortium of bacteria	27.78(29a)
Uninoculated control	0.00(3.5a)
Inoculated control	36.11(33.83a)

*Mean of four replications having one plant each

[x]Values in paranthesis after arc sine transformation

[y]Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Plate 23 Incidence of foliar blight on amaranthus plants treated with *Bacillus subtilis* strain GB03 and ASM

T_{10}: GB03+ASM
T_{12}: Control

Plate 24 Incidence of foliar blight on amaranthus plants treated with *Pseudomonas fluorescens* strain PN026R

T_1: PN026R
T_{12}: Control

Plate 25 Incidence of foliar blight on amaranthus plants treated with *Bacillus subtilis* strain GB03

T_4: GB03
T_{12}: Control

Plate 26 Incidence of foliar blight on amaranthus plants treated with consortium of bacteria

T_6: Consortium of bacteria
T_{12}: Control

Plate 23

Plate 24

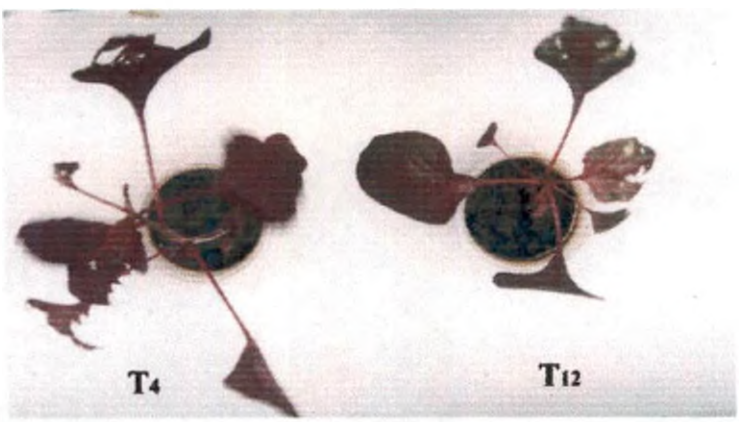


Plate 25

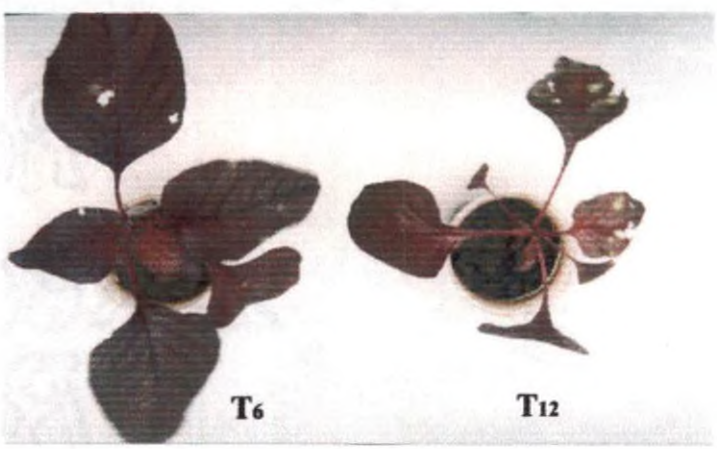


Plate 26

4.3.1.2 Shoot Length

Treatment with bacterial strain 89B61 resulted in maximum shoot length (22.65 cm), followed by bacterial strain PN026R (21.98 cm) and both were statistically on par. The minimum shoot length of 13.08 cm was observed for treatment with ASM+SE34. Length of shoots in treatments with PN026R and 89B61 were significantly different from that of uninoculated control (Table 5).

4.3.1.3 Root Length

The root length of the plants with different treatments is given in Table 6. The maximum root length was observed for treatment with the bacterial strain GB03 (16.5 cm). This was followed by treatment with the bacterial strain 89B61 that was statistically on par with that of treatments with GB03, PN026R, SE34, ASM, consortium of bacteria, ASM+PN026R and ASM+89B61. Combined treatment with ASM and SE34 produced the minimum root length.

4.3.1.4 Dry Weight of Shoot and Root

The maximum dry weight of shoot was observed for the treatment with the bacterial strain 89B61 (1.03g) that was statistically on par with that of the treatments with SE34, GB03, bacterial consortium and PN026R and control. The lowest dry weight of shoots was observed for treatment with ASM+SE34 (0.16 g). Treatments with 89B61 and SE34 showing shoot dry weight of 1.03g and 1.01g respectively were superior to the inoculated control, which recorded an average shoot dry weight of 0.4 g. (Table 5).

The dry weight of roots of the treated and the control plants is shown in Table 6. The maximum dry weight of roots was recorded for the treatment with 89B61 (0.36 g). It was followed by treatments with PN026R, GB03, SE34 and consortium of bacteria. Root weight in treatments with 89B61 and PN026R were found statistically significant

Table 5 Effect of PGPR and chemical activator on shoot length and dry weight of amaranthus plants*

Treatments	Shoot length (cm)**	%variation over inoculated control	Dry weight of shoots/plant (g)**	%variation over inoculated control
PN026R	21.98 a	21.24	0.62 abc	-24.39
89B61	22.65 a	24.93	1.03 a	25.6
SE34	20.1 abc	10.87	1.01 a	23.17
GB03	19.88 abc	9.65	0.81 a	-1.22
ASM	15.13 bcd	-16.5	0.23 cd	-71.95
Consortium of bacteria	20.8 ab	14.73	0.73 ab	-10.98
ASM+PN026R	20.73 ab	14.34	0.28 cd	-65.85
ASM+89B61	16.68 abcd	-8.00	0.25 cd	-69.51
ASM+SE34	13.08 d	-27.9	0.16 d	-80.49
ASM+GB03	15.47 bcd	-14.67	0.27 cd	-67.1
ASM+ Consortium of bacteria	14.63 bcd	-19.31	0.27 cd	-67.1
Uninoculated control	14.45 bcd	-20.30	0.82 a	-51.22
Inoculated control	18.13 abcd		0.40 bcd	

* Mean of four replications having one plant each

** Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

Table 6 Effect of PGPR and chemical activator on root length and dry weight of amaranthus plants*

Treatments	Root length (cm)**	% variation over inoculated control	Dry weight of roots/plant (g)**	%variation over inoculated control
PN026R	14.00 abc	-4.30	0.345a	115.63
89B61	15.88 ab	8.50	0.36 a	125.00
SE34	15.00 abc	2.50	0.25 abc	58.75
GB03	16.50 a	12.78	0.30 ab	86.88
ASM	15.38 abc	5.13	0.01 cde	-40.63
Consortium of bacteria	12.63 abcd	-13.67	0.25 abcd	55.00
ASM+PN026R	15.50 abc	5.95	0.16 bcde	0.00
ASM+89B61	12.50 abcde	-14.56	0.06 e	6.25
ASM+SE34	8.13 e	-44.43	0.14 bcde	-62.50
ASM+GB03	11.18 cde	-23.58	0.08 de	-12.50
ASM + Consortium of bacteria	9.00 de	-38.48	0.16 bcde	-50.00
Uninoculated control	14.63 abc	-20.20	0.09 cde	-43.80
Inoculated control	11.68 bcde		0.16 bcde	

* Mean of four replications having one plant each

** Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

from that of control treatments. The lowest dry weight of roots was recorded for the treatment with SE34+ASM.

4.3.2 Effects of PGPR Strains on Plant Growth

4.3.2.1 Shoot Length 20 DAT

After 20 days of transplanting the maximum shoot length was recorded for the treatment with PN026R (18.33 cm) (Plate 27). It was significantly different from the control treatment. A minimum shoot length of 7.5 cm was recorded for GB03 (Table 7) (Plate 28).

4.3.2.2 Number of Leaves 20 DAT

None of the treatments differed significantly from each other with respect to the number of leaves. The maximum number of leaves was recorded for SE34. Lowest number of leaves was recorded for treatment with GB03 (Table 7).

4.3.2.3 Number of Leaves at Harvest

None of the treatments differed significantly from each other. The maximum number of leaves was recorded for SE34. The minimum number of leaves is recorded for GB03.(Table 7)

4.3.2.4 Shoot Length at Harvest

The shoot length of the treated and the control plants is given in Table 8. The maximum shoot length was recorded for the treatment with SE34 (36 cm) (Plate 29) followed by PN026R, consortium of bacteria + ASM and the control. These were found to be statistically on par and significantly different from the treatments with GB03 and ASM. The lowest shoot length was recorded for ASM (14.5 cm) (Plate 30).

4.3.2.5 Root Length

There was no significant difference between the treatments in case of root length (Table 9). The maximum root length was recorded for GB03+ASM (23 cm) (Plate 31). It was followed by control. All other

Plate 27 Shoot length of amaranthus plants treated with *Pseudomonas fluorescens* strain PN026R twenty days after transplanting

T_1: PN026R
T_{12}: Control

Plate 28 Shoot length of amaranthus plants treated with *Bacillus subtilis* strain GB03 twenty days after transplanting

T_4: GB03
T_{12}: Control

Plate 29 Shoot length of amaranthus plants treated with *Bacillus pumilus* strain SE34 at harvest

T_3: SE34
T_{12}: Control

Plate 30 Shoot length of amaranthus plants treated with ASM at harvest

T_5: ASM
T_{12}: Control

Plate 31 Root length of amaranthus plants treated with *Bacillus subtilis* strain GB03 and ASM at harvest

T_{10}: GB03+ASM
T_{12}: Control

Plate 32 Root length of amaranthus plants treated with *Bacillus pumilus* strain SE34 and ASM on the root length of amaranthus at harvest

T_9: SE34+ASM
T_{12}: Control

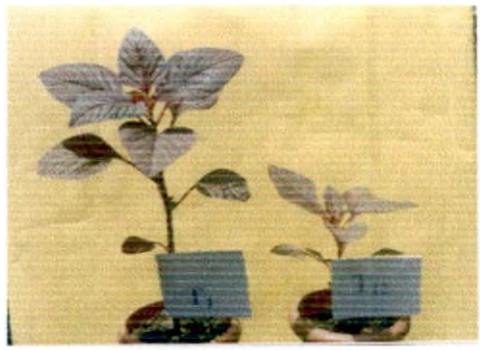


Plate 27.

Plate 28.

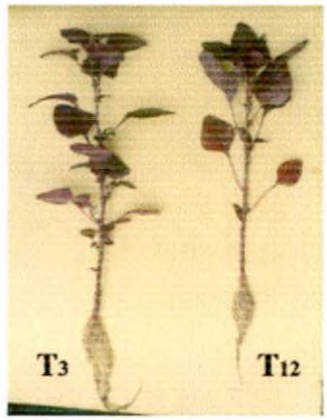


Plate 29.

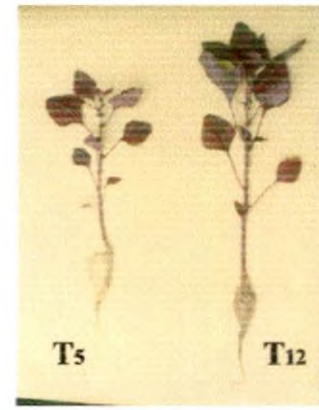


Plate 30.

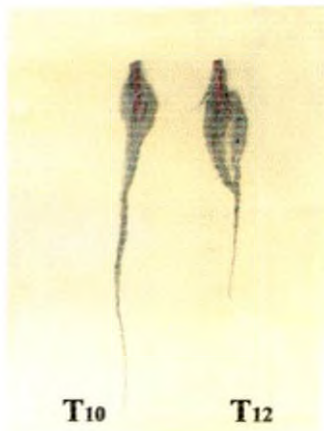


Plate 31.

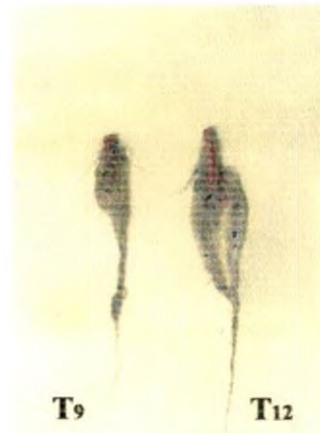


Plate 32.

treatments except GB03+ASM showed lesser root lengths when compared to the control. The lowest root length was recorded for treatment with ASM+SE34 (Plate 32). ASM+SE34 and ASM+89B61 showed lesser root length than ASM alone.

4.3.2.6 Shoot and Root Fresh Weight

Table 8 gives the shoot fresh weight of the treated and the control plants. The maximum shoot fresh weight was recorded for PN026R (17.22 g). It was followed by ASM+89B61. Treatment with PN026R resulted in significantly different shoot fresh weight compared with that received GB03+ASM, GB03 and ASM alone.

There were no significant differences between the treatments with respect to root fresh weight. The root fresh weights are given in Table 9. The maximum root fresh weight was recorded for SE34 (4.7 g). Treatments with ASM+89B61, consortium of bacteria, GB03+ASM, ASM+SE34, GB03 and ASM alone recorded lesser root fresh weight than the control treatment. The lowest root fresh weight was recorded when plants were treated with ASM alone.

4.3.2.7 Shoot and Root Dry Weight

Maximum shoot dry weight of 2.52 g was recorded for PN026R (Table 8). The treatments significantly different from PN026R were GB03+ASM, consortium of bacteria, ASM+PN026R, ASM+ SE34, ASM alone and GB03. The plants treated with 89B61+ ASM also showed lesser shoot dry weight than the control. The lowest shoot dry weight was recorded for GB03.

Table 9 shows the root dry weight of treated and control plants. Treatment with PN026R recorded the maximum root dry weight (0.94 g). Treatments such as GB03 and ASM alone were with lesser root dry weight when compared to PN026R. The lowest root dry weight was recorded for the treatment with ASM alone (0.12 g).

Table 7 Effect of PGPR and ASM on leaf number at 20 days after transplanting (DAT) and at harvest and shoot length at 20 DAT of amaranthus plants*

Treatments	Leaf no. (20 DAT)**	Leaf no. at harvest**	Shoot length (20 DAT)**
PN026R	8.30 a	12.67 a	18.33 a
89B61	7.00 a	9.00 a	11.83 abc
SE34	9.30 a	13.00 a	16.50 ab
GB03	5.30 a	7.67 a	7.50 c
ASM	7.70 a	8.00 a	7.73 c
Consortium of bacteria	7.70 a	11.00 a	11.83 abc
ASM+PN026R	7.00 a	9.00 a	12.00 abc
ASM+89B61	8.00 a	12.67 a	13.33 abc
ASM+SE34	8.00 a	10.00 a	12.50 abc
ASM+GB03	6.70 a	8.33 a	9.67 c
ASM+ Consortium of bacteria	7.70 a	11.33 a	13.5 abc
Control	8.30 a	9.67 a	11.00 bc

** Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

*Mean of four replications having one plant each

Table 8 Effect of PGPR and ASM on length, fresh and dry weight of shoots of amaranthus plants*

Treatments	Shoot length (cm)**	%variation over control	Shoot fresh weight (g)/ plant**	%variation over control	Shoot dry weight (g)/ plant**	%variation over control
PN026R	35.67 a	8.09	17.22 a	120.77	2.52 a	90.91
89B61	24.50 abc	-25.76	10.16 ab	30.26	1.08 ab	-18.18
SE34	36.00 a	9.09	11.37 ab	45.77	1.71 ab	29.55
GB03	25.00 abc	-24.24	3.78 b	-51.54	0.30 b	-77.27
ASM	14.50 c	-56.06	3.02 b	-61.28	0.31 b	-76.52
Consortium of bacteria	29.17 abc	-11.61	9.15 ab	17.31	1.01 b	-23.48
ASM+PN026R	24.33 abc	-26.27	7.13 ab	-8.59	0.97 b	-26.52
ASM+89B61	31.33 ab	-5.06	11.73 ab	50.38	1.53 ab	15.91
ASM+SE34	27.00 abc	-18.18	8.83 ab	13.21	0.90 b	-31.89
ASM+GB03	25.00 abc	-24.24	5.17 b	-33.72	1.02 b	-22.73
ASM+ Consortium of bacteria	35.50 a	7.58	9.37 ab	20.13	1.65 ab	25.00
Control	33.00 a		7.80 ab		1.32 ab	

*Mean of four replications having one plant each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

Table 9 Effect of PGPR and ASM on length, fresh weight and dry weight of roots of amaranthus plants *

Treatments	Root length(cm)**	%variation over control	Root fresh weight(g)**	%variation over control	Root dry weight (g)**	%variation over control
PN026R	22.17 a	-2.89	3.97 a	25.24	0.94 a	63.19
89B61	20.83 a	-8.76	4.32 a	57.73	0.57 ab	-1.04
SE34	21.33 a	-7.26	4.70 a	48.26	0.66 ab	14.58
GB03	19.83 a	-13.14	1.17 a	-63.09	0.16 b	-72.22
ASM	19.00 a	-16.78	0.798 a	-74.83	0.12 b	-79.69
Consortium of bacteria	20.17 a	-11.65	3.93 a	23.98	0.62 ab	7.64
ASM+PN026R	23.33 a	2.19	4.40 a	38.80	0.42 ab	-27.08
ASM+89B61	15.00 a	-34.30	2.92 a	-7.89	0.45 ab	-21.88
ASM+SE34	12.17 a	-46.69	1.79 a	-43.53	0.23 ab	-60.07
ASM+GB03	23.00 a	0.74	2.06 a	-35.02	0.29 ab	-49.65
ASM + Consortium of bacteria	22.67 a	-0.70	2.49 a	-21.45	0.40 ab	-31.08
Control	22.83 a		3.17 a		0.58 ab	

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

*Mean of four replications having one plant each

4.3.3 Studies on Disease Suppression by Biocontrol Agents and their Plant Growth Promotion Activity Under Pot Culture

4.3.3.1 Disease Incidence

The effect of selected biocontrol agents and chemical activator on disease incidence is given in Table 10. The maximum disease incidence was recorded in combined treatment with GB03+ASM (91.67%)(Plate 33). The minimum disease incidence was recorded in uninoculated control. It was followed by PN026R + ASM, GB03 and ASM (Plate 34-36).

4.3.3.2 Disease Severity

The disease severity in the treated and control plants is given in Table 10. The minimum disease severity was recorded for uninoculated control. GB03 and PN026R + ASM recorded same disease severity (20.84).

4.3.3.3 Shoot Length

The shoot length of the treated and non-treated plants is given in Table 11. Maximum shoot length of 36.43 cm was observed for the treatment with PN026R +ASM. It was followed by treatment with PN026R and GB03+ASM, which were statistically on par with that of PN026R+ ASM. The lowest shoot length was observed for chemical control. All other treatments produced comparable shoot length.

4.3.3.4 Root Length

Table 12 shows the root length of the treated and control plants. The maximum root length was recorded for inoculated control (24.8 cm). The lowest root length was recorded for chemical control (16.65 cm). Treatment with ASM alone recorded lowest root length among the other treatments. The treatments of PGPR alone, or their combinations with ASM showed reduced root length when compared to the inoculated and uninoculated controls, though all of them were statistically on par.

Table 10 Effect of biocontrol agents and chemical activator on disease severity*

Treatments	Disease incidence**, ^{x}	Disease severity**, ^{x}
PN026R	66.67 (57.79 a)	34.72 (33 ab)
PN026R+ASM	41.67 (40.17ab)	20.84 (24.27 bc)
GB03	41.67 (40.17 ab)	20.84 (24.27 bc)
GB03+ASM	91.67 (73.71a)	50.28 (45.13 ab)
ASM	41.67 (37.04 ab)	25 (27.03 b)
Uninoculated control	0.00 (78.54 a)	0.00 (3.5 c)
Chemical control	83.33 (3.5 b)	61.11 (51.54 a)
Inoculated control	91.67 (73.71 a)	52.22 (46.15 ab)

*Mean of four replications having three plants each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

^{x}Values in paranthesis after arc sine transformation

Plate 33 Incidence of foliar blight of amaranthus plants treated with *Bacillus subtilis* GB03 and ASM

T_4: GB03+ASM
T_6: Inoculated control

Plate 34 Incidence of foliar blight of amaranthus plants treated with *Pseudomonas fluorescens* PN026R and ASM

T_2: PN026R+ASM
T_6: Inoculated control

Plate 35 Incidence of foliar blight of amaranthus plants treated with *Bacillus subtilis* GB03

T_3: GB03
T_6: Inoculated control

Plate 36 Incidence of foliar blight of amaranthus plants treated with ASM

T_5: ASM
T_6: Inoculated control

Plate 33.

Plate 34.

Plate 35.

Plate 36.

4.3.3.5 Shoot and Root Fresh Weight

Table 11 shows the shoot fresh weight of the treated and the control plants. The maximum shoot fresh weight was recorded in the treatment with combinations of PN026R and ASM (13.35 g). It differed significantly from the inoculated control. The treatments with PN026R + ASM, GB03 and PN026R recorded shoot fresh weight more than that of ASM alone, uninoculated control, GB03 + ASM, chemical control and inoculated control.

Root fresh weight in all the treatments was statistically on par (Table 12). The maximum root fresh weight was recorded for the treatment with PN026R (4.23 g). The lowest root fresh weight was recorded for the chemical control (2.49 g). Treatment with PN026R + ASM was found to have lesser root fresh weight than the inoculated and uninoculated controls. All the other treatments were found to have higher root fresh weight than the inoculated control.

4.3.3.6 Shoot and Root Dry Weight

The observations on shoot dry weight of treated and control plants are shown in Table 11. The maximum shoot dry weight was recorded when GB03 and ASM were combinely used (2.18 g). It was followed by treatments with PN026R+ASM and PN026R which were found to be statistically on par with the former. These treatments significantly differed from the treatments such as inoculated control, uninoculated control, GB03, chemical control and ASM. The lowest shoot dry weight was recorded for plants treated with ASM alone.

The values of root dry weight of treated and non-treated plants are shown in Table 12. The maximum root dry weight of 1.1 g was recorded for PN026R+ASM. It was significantly different from that of chemical control. All other treatments except the PN026R+ASM showed lesser root

Table 11 Effect of biocontrol agents and chemical activator on length, fresh and dry weight of shoots of amaranthus plants*

Treatments	Shoot length (cm)**	%variation over inoculated control	Shoot fresh weight/ Plant (g)**	%variation over inoculated control	Shoot dry weight/Plant (g)**	%variation over inoculated control
PN026R	33.233 a	46.93	11.28 ab	64.00	2.13 a	36.73
PN026R+ASM	36.425 a	61.07	13.35 a	94.18	2.14 a	37.37
GB03	25.633 a	13.33	11.30 ab	64.36	1.46 b	-6.41
GB03+ASM	32.225 a	42.48	9.18 ab	33.57	2.18 a	40.00
ASM	23.408 b	3.49	11.18 ab	62.66	1.2 b	-23.33
Uninoculated control	20.588 b	-8.98	9.92 ab	44.36	1.51 b	-11.17
Chemical control	20.225 b	-10.58	8.98 a	30.55	1.36 b	-12.76
Inoculated control	22.618 b		6.88 b		1.56 b	

*Mean of four replications having three plants each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

Table 12 Effect of biocontrol agents and chemical activator on length, fresh and dry weight of roots*

Treatments	Root length (cm)**	% variation over inoculated control	Root fresh weight(g)/ plant**	% variation over inoculated control	Root dry weight(g)/ plant**	% variation over inoculated control
PN026R	19.53 ab	-21.27	4.23 a	59.43	0.80 ab	1.77
PN026R+ASM	18.65 ab	-24.82	2.61 a	-1.396	1.10 a	39.54
GB03	19.06 ab	-23.15	4.00 a	50.94	0.85 ab	7.48
GB03+ASM	18.89 ab	-23.83	4.19 a	58.04	0.82 ab	4.31
ASM	17.71 ab	-28.59	3.08 a	16.04	0.70 ab	-10.77
Uninoculated control	21.15 ab	-14.72	2.98 a	12.57	0.89 ab	12.55
Chemical control	16.65 b	-32.86	2.49 a	-6.23	0.56 b	-28.52
Inoculated control	24.8 a		2.65 a		0.79 ab	

*Mean of four replications having three plants each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test (P=0.05)

dry weight than the uninoculated control. Among the treatments other than the controls, ASM showed reduced root dry weight (0.704 g).

4.3.3.7 Biochemical Studies

4.3.3.7.1 Total Phenols

The phenol content of the plants in response to the different treatments and the pathogen was studied. Total phenol content expressed as µg catechol equivalents/g of leaf tissue at different days of inoculation is shown in Table 13.

Initially an increasing trend in the phenol content was noticed in the samples from the treated plants. Most of the treated plants showed significant differences in the phenol content. The maximum phenol content was recorded for GB03+ASM (920). The minimum phenol content was recorded for inoculated control (120). The phenol content of PN026R and ASM treated plants were statistically on par. There was no significant difference in samples taken between the treatments 10 DAI.

4.3.3.7.2 Phenylalanine Ammonialyase (PAL)

The studies on the changes in PAL activity were carried out to elucidate its alterations in amaranthus in response to inoculation with the pathogen and different treatments. The PAL activity expressed as µ moles of cinnamic acid equivalent /g fresh leaves / minute of the different treatments is shown in Table 14.

The observations showed that there was an increasing trend in the PAL activity in all the treated plants, one day after inoculation. The PAL activity was at its maximum in ASM treated plants (0.225) one day after inoculation when compared to other treatments. The enzyme activity showed a decreasing trend in samples taken subsequently. There was no significant change in samples analysed during five days and ten days after inoculation. On statistical analysis it was noticed that ASM treated plants showed significant differences from other treatments one day after

Table 13 Phenol content (µg / g leaf tissue) of amaranthus plants

Treatments	Five days after inoculation	Ten days after inoculation
PN026R	830	440
PN026R+ASM	270	140
GB03	770	350
GB03+ASM	920	200
ASM	830	350
Uninoculated control	530	430
Chemical control	600	560
Inoculated control	120	150
CD (0.05)	20.75	25.32

*Mean of three replications

Table 14 Phenylalanine ammonialyase activity (μ moles/g/min cinnamic acid equivalent) in amaranthus plants*

Treatments	One day after inoculation	Five days after inoculation	Ten days after inoculation
PN026R	0.195	0.140	0.113
PN026R+ASM	0.197	0.153	0.163
GB03	0.195	0.166	0.149
GB03+ASM	0.197	0.159	0.191
ASM	0.225	0.168	0.128
Uninoculated control	0.169	0.157	0.176
Chemical control	0.163	0.146	0.169
Inoculated control	0.158	0.151	0.168
CD (0.05)	0.009	0.008	0.009

*Mean of three replications

inoculation. GB03+ASM treated plants showed significant difference ten days after inoculation compared to other treatments.

4.3.3.7.3 Peroxidase (PO)

The studies on the alterations in peroxidase activity of different treatments at different days after inoculation measured as change in absorbance per min per gram of leaf is shown in Table 15. There was no significant change in the peroxidase activity of the treated plants initially. The maximum peroxidase activity was observed one day after inoculation in plants treated with combination of GB03 and ASM (0.6). Subsequent sampling showed that there was an increasing trend in all the treated plants five and ten days after inoculation. The PO activities of treated plants differed significantly. Five days after inoculation, plants treated with GB03+ASM and ASM, PN026R and chemical control showed that the PO activities were on par. Ten days after inoculation it was observed that plants treated with GB03 and uninoculated control showed PO activities on par with inoculated control. All the other treatments showed significant difference from the control.

4.3.3.7.4 Polyphenol Oxidase (PPO)

The polyphenol activity was measured as change in absorbance per min per gram of leaf at different days after inoculation and is shown in Table 16. The study was conducted to observe the alterations in the activities of the enzyme in response to the pathogen and the different treatments.

The treated plants showed an increasing trend in PPO activity initially. The maximum PPO activity was recorded one day after inoculation for PN026R (0.067). PPO activities of the plants treated with ASM, PN026R+ASM and GB03 were on par. All the other treatments showed significant differences in the activity. Subsequent sampling on five and ten days after inoculation did not show any significant increase

Table 15 Peroxidase activity (change in absorbance per minute per gram) in amaranthus plants*

Treatments	One day after inoculation	Five days after inoculation	Ten days after inoculation
PN026R	0.047	0.105	0.720
PN026R+ASM	0.100	0.120	0.260
GB03	0.020	0.057	0.120
GB03+ASM	0.600	0.075	1.120
ASM	0.093	0.082	1.390
Uninoculated control	0.133	0.155	0.215
Chemical control	0.020	0.130	0.043
Inoculated control	0.093	0.030	0.033
CD (0.05)	0.03	0.012	0.095

*Mean of three replications

Table 16 Polyphenol oxidase activity (change in absorbance per minute per gram) in amaranthus plants*

Treatments	One day after inoculation	Five days after inoculation	Ten days after inoculation
PN026R	0.067	0.242	0.023
PN026R+ASM	0.027	0.108	0.065
GB03	0.027	0.060	0.183
GB03+ASM	0.060	0.018	0.172
ASM	0.027	0.012	0.055
Uninoculated control	0.020	0.013	0.182
Chemical control	0.040	0.027	0.033
Inoculated control	0.006	0.015	0.067
CD (0.05)	0.004	0.006	0.008

*Mean of three replications

among treatments. The PPO activities of plants treated with ASM at five days and PPO activities of plants treated with PN026R+ ASM ten days after inoculation were on par with inoculated control. All the other treatments differed significantly.

4.3.3.7.5 Isozyme Separation and Staining

The isozyme analysis of peroxidase was performed to find out the expression of isoforms of PO in treated plants. Only one isoform (ISO PO1) was observed in three treatments *viz.*, PN026R, GB03+ASM and ASM when compared to other samples analysed (Plate 37).

4.3.4 Effect of ASM on Plant Growth

4.3.4.1 Shoot Length

The observations on shoot length are given in Table 17. Treatment with 25 ppm ASM recorded maximum shoot length of 14.63 cm. It was followed by treatments with 10 ppm ASM. The shoot length in these two treatments differed significantly from that in the control. The lowest shoot length was recorded for the control treatment (10.43 cm).

4.3.4.2 Root Length

Maximum root length of 14.9 cm was recorded for treatment with 25 ppm (Table 18). It differed significantly with the control and the treatment with 5 ppm ASM. The lowest root length was recorded for 5 ppm ASM (9.43 cm).

4.3.4.3 Shoot and Root Fresh Weight

Table 17 shows the shoot fresh weights of the treated and control plants. Treatment with 25 ppm ASM differed significantly from control treatment and 5 ppm ASM. The maximum shoot fresh weight was recorded for the treatment with 10 ppm ASM (12.5 g). The lowest shoot fresh weight was recorded for the treatment with 5 ppm ASM (9.43 g).

Table 18 gives the root fresh weights of the treated and control plants. The treatments showed no statistically significant differences in

Plate 37 Induction of peroxidase isoforms in response to treatments with PGPR and chemical activator

Lane1- PN026R
Lane2- PN026R+ASM
Lane3- GB03
Lane4- GB03+ASM
Lane5- ASM
Lane6- Uninoculated control
Lane7- Inoculated control

Isozyme analysis (Peroxidase)

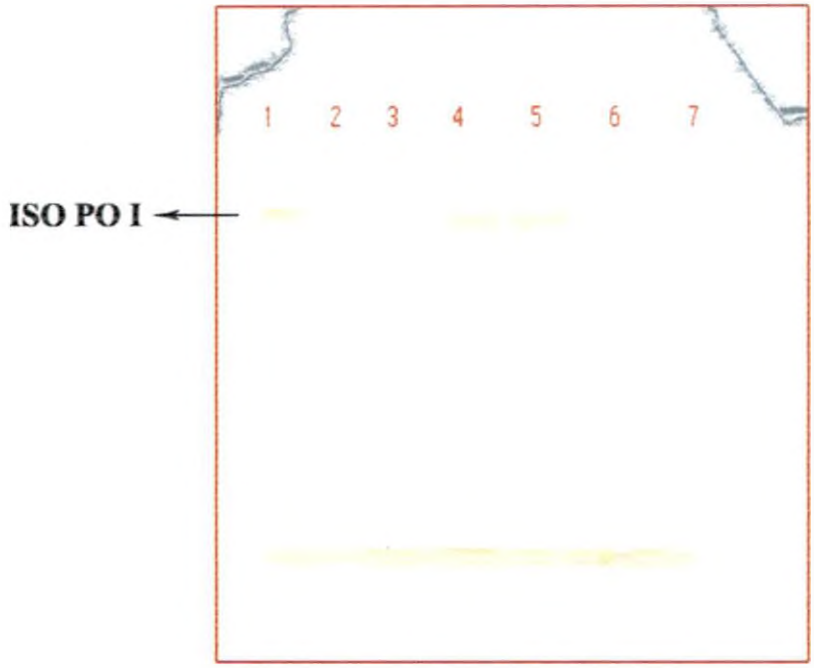


Plate 37.

case of root fresh weight. The maximum root fresh weight was recorded when 10 ppm ASM was provided (0.52 g). The lowest root fresh weight was recorded for the treatment with 5 ppm ASM (0.37 g).

4.3.4.4 Shoot and Root Dry Weight

Table 17 gives the shoot dry weights of the treated and control plants. The treatments showed no significant differences between each other. The maximum shoot dry weight was recorded for the control (0.306) and the lowest in the case of treatment with 5 ppm ASM (0.156 g).

None of the treatments differed significantly in case of root dry weight Table 18. A maximum root dry weight of 0.085 g and a minimum of 0.054 g were recorded for treatments with 5 ppm and 20 ppm ASM respectively.

Table 17 Effect of ASM on length, fresh and dry weight of shoots of amaranthus plants*

Treatments	Shoot length (cm)**	% variation over control	Shoot fresh weight (g)/plant**	% variation over control	Shoot dry weight(g)/ plant**	% variation over control
0 ppm	10.43 b		9.48 b		0.31 a	
5 ppm	11.75 ab	12.66	9.43 b	19.3	0.16 a	-40.2
10 ppm	14.50 a	39.02	12.50 ab	3.51	0.23 a	-76.88
20 ppm	13.30 ab	27.52	11.70 ab	23.68	0.17 a	-25.16
25 ppm	14.63 a	40.27	14.90 a	-7.89	0.18 a	-49.02

*Mean of four replications having one plant each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range test (P=0.05)

Table 18 Effect of ASM on length, fresh and dry weight of roots of amaranthus plants

Treatments	Root length (cm)**	% variation over control	Root fresh weight (g)/ plant**	% variation over control	Root dry weight (g)/ plant**	% variation over control
0 ppm	9.48 b		0.37 a		0.061 a	
5 ppm	9.43 b	-0.53	0.37 a	9.43	0.085 a	6.56
10 ppm	12.50 ab	31.86	0.52 a	2.16	0.067 a	-11.48
20 ppm	11.70 ab	23.42	0.38 a	39.35	0.054 a	9.83
25 ppm	14.90 a	57.17	0.41 a	-1.09	0.065 a	39.34

*Mean of four replications having one plant each

**Values followed by same letters in a column do not differ significantly according to Duncan's Multiple Range Test ($P=0.05$)

Discussion

5. DISCUSSION

Amaranthus is the most common leafy vegetable grown in Kerala. Leaves being the economic part of the crop foliar blight caused by *R. solani* poses a serious threat to amaranthus cultivation (Kamala *et al.*, 1996; Gokulapalan *et al.*, 2000). The recommended control measure for the disease at present is foliar spraying of mancozeb (0.4%) in cowdung supernatant at fortnightly intervals (Gokulapalan *et al.*, 1999). It is always recommended that use of chemical fungicides is to be reduced to the minimum in any leafy vegetable. Hence a better option would be resorting to biological control.

The present investigation involved the use of plant growth promoting rhizobacteria and a chemical activator for managing the foliar blight of amaranthus. Previous works on the management of foliar blight of amaranthus conducted in the Department of Plant pathology at College of Agriculture, Vellayani, include the use of microbial antagonists like *Trichoderma longibrachiatum* and a fluorescent *Pseudomonas* sp. (Smitha, 2000). Priyadarsini (2003) has made an attempt to manage the disease using *T. harzianum*, *P. fluorescens*, *Piriformospora indica* (a newly discovered endophyte) and indigenous materials like turmeric powder-baking soda and rice husk ash.

In both these experiments the antagonistic effect of the biocontrol agent was given focus as a mode of biocontrol. The bioagents were directly delivered to the site of infection in almost all cases. Plant growth promoting rhizobacteria can suppress pathogen attack by different modes of action like production of siderophores, production of antibiotics, competition for nutrients and space, production of HCN and induction of systemic resistance (ISR) (Kloepper, 1993). In the present investigation importance is given to ISR as a mode of biocontrol. Non- pathogenic biotic agents like PGPR have been used to induce ISR against many crop-

pathogen interaction like downy mildew of arabidopsis, anthracnose and halo blight of beans, anthracnose of cucumber and angular leaf spot of cucumber (van Loon *et al.*, 1998). Chemical activators like ASM is shown to induce multiple resistance to fungal and bacterial diseases (Ishii, 2003). Exploitation of ISR in amaranthus involved avoiding foliar application of the PGPR and the chemical activator. Thus attempts were made to induce defense responses against the foliar pathogen by drenching the rhizosphere soil with PGPR and ASM.

Proven biocontrol agents procured from different sources and Acibenzolar-S-methyl were used for the ISR studies. Four PGPR strains, their consortium and their combination with ASM were tested *in vivo*. van Loon *et al.* (1998) suggested the absence of toxic effects of the inducing agent on the challenging pathogen as one of the criteria for involvement of ISR in biocontrol. This could be verified by *in vitro* dual culture assay conducted using the pathogen and the four antagonists. The studies were conducted using four different media as earlier reports suggests that different biological traits and / or production of varying metabolites are influenced by various media components and that these traits might be altered by simple changes in laboratory routines (Borowicz and Omar, 2000). It was interesting to note that some rhizobacterial strains which showed mycelial inhibition of the pathogen in one medium failed to show such trait in another media. PN026R showed antagonism against the pathogen only in NA where as in PDA, KB and CA it showed no inhibition of mycelial growth. 89B61 showed antagonism against the pathogen in PDA, NA and CA only. This could be attributed to lack of production of specific antimicrobial metabolites against the pathogen in these culture media. The antagonistic action would also depend on the species of pathogenic fungi. It was earlier reported that PN026R was able to show antagonism against *Phytophthora capsici* in PDA and CA (Anith *et al.*, 2004).

The effect of different concentrations of ASM on the growth of the pathogen was tested *in vitro*. Seven different concentrations of ASM ranging from 5ppm-100 ppm were used. It was observed that ASM had inhibitory effect on mycelial growth of pathogen. But earlier reports are contradictory to this as in most of the experiments ASM did not show direct inhibitory effect on the pathogen (Gullino *et al.*, 2000; Akinwunmi *et al.* 2001; Geetha and Shetty, 2002). Cytological observations on fungal progress revealed no differences in infection type between controls and ASM treated sunflower plants infected by rust pathogen (Prats *et al.* 2002). On plants activated with ASM the wheat powdery mildew infections are stopped by the faster formation of papillae at the sites of attempted penetration (Görlach *et al.*, 1996). In the present study the least percentage of mycelial inhibition was observed for 5 ppm concentration of ASM. The maximum percentage of mycelial inhibition was observed for 37.5ppm ASM. The concentration of ASM selected for further *in vivo* experiment based on previous reports was 25 ppm. A suspension of ASM (Actigard 50 WG) at 25 mg/litre when applied as foliar spray was found to be effective against bacterial spot in bell pepper (Romero *et al.*, 2001). ASM at concentrations upto 25 ppm in sterile deionized water was used for seed treatment of cowpea seeds by Akinwunmi *et al.* (2001).

To determine if ISR is a mechanism for biological control it is necessary to use a system that spatially separates the inducer and the challenging pathogen (Liu *et al.*, 1995). In the experiment to screen the PGPR strains for possible resistance inducing ability, the bacterial cells as well as the chemical activator were applied only to the base of the plant and direct interaction of the biocontrol agent with the pathogen was completely avoided. Though PN026R showed no direct antagonism *in vitro*, it could effectively reduce the severity of the disease in the *in vivo* screening experiment. The reduction in severity brought about by the bacterial strain PN026R could be due to induction of systemic resistance as the pathogen and the bacteria were spatially separated throughout the

experiment. PN026R has been reported as an efficient biocontrol agent against nursery wilt of black pepper (Anith *et al.*, 2003; 2004). The present study suggests that induction of systemic resistance also is one of the mechanisms of disease suppression by the strain PN026R. In the screening test treatment with GB03+ASM gave the lowest disease severity. The *Bacillus* strain GB03 showed antagonistic activity against *R. solani* in all the four test media. It could thus be inferred that the strain, if used as a foliar spray to prevent amaranthus blight may also become effective, owing to its high degree of antagonism. A single bacterial strain exhibiting multiple modes of disease suppression is not uncommon. Alström (1991) have reported that although ISR was inferred in the suppression of halo blight of bean by *P. fluorescens* S97, the rhizobacterium exhibited bacteriostatic activity against the pathogen. Thus it could be inferred that GB03 has multiple disease suppressive mechanisms.

It was noted that GB03 alone when used as a treatment in sterile soil recorded a disease severity of 8.33. But when GB03 was combined with ASM the disease severity recorded was zero. Chen *et al.* (1996) reported that combination of chemically induced SAR in tobacco seedlings with application of the biocontrol strain *Bacillus cereus* resulted in additive suppression of disease caused by *Pythium torulosum*, *Pythium aphanidermatum* or *Phytophthora parasitica*.

In all the *in vivo* experiments, the rhizobacteria and ASM were applied as soil drench and root dip of seedlings at the time of transplanting. This was to provide spatial separation between the pathogen and the antagonist. There was not much reduction in disease severity in combination treatments when compared to treatments with rhizobacteria alone except in case of GB03+ASM. Though *in vitro* experiments of interaction between ASM at 100 ppm concentration and the rhizobacterial strains showed no apparent effect on the growth of rhizobacteria on PDA, the combinations resulted in decreased suppression of disease severity in

the screening tests. Interestingly, ASM when used alone performed better than when it was combined with other rhizobacteria. The failure of the rhizobacterial combination with ASM could be due to the poor colonization of rhizobacteria in the presence of ASM. For effective induction of ISR response a minimum number of bacteria should colonize the roots of the plants (van Loon *et al.* 1998). Though in the present study no colonization patterns were studied on the roots, in sterile soil conditions, ASM some how prevented the rhizobacteria either from colonizing the roots or limiting some other way its ISR inducing capacity.

Present trend in biocontrol strategies is to use a consortium of rhizobacteria for disease management rather than using them singly. Weller and Cook (1983) reported that *P. fluorescens* 2-79 used in combination with *P. fluorescens* 13-79 was superior to either strain alone. Multiple strains of PGPR as formulated product are thought to have increased efficiency in biocontrol compared with application of a single strain (Pierson and Weller, 1994). For effective utilization of the additive effects if any, of the mixtures of biocontrol agents their interaction under *in vivo* conditions are to be studied. Combined application of *Trichoderma harzianum* and *Acaligenes* sp. strain AMB 8 had additive effect on suppression of nursery wilt of black pepper (Anith and Manmohandas, 2001). The *Acaligenes* sp. had inhibitory effect on the growth of *Trichoderma* under *in vitro* conditions. When the *in vivo* population dynamics was analysed, however, it was found that in the rhizosphere of black pepper both of the bioagents were performing well. In the present study such *in vivo* interactions were not performed. The disease severity observed for ASM + consortium of bacteria was more than that in consortium of bacteria as the treatment. From the results it is inferred that there was no additive effect due to the consortium.

Based on disease severity in the screening test, PN026R, GB03, their combination with ASM and ASM with controls were selected for

further studies. PN026R was selected as it was a native isolate belonging to *Pseudomonas* showing reduced severity than the other *Pseudomonas* strain 89B61. Anith *et al.* (2003) reported that suppression of the incidence of nursery wilt of black pepper was obtained when the cuttings were treated with PN026R. Plants treated with PN026R also showed improved root and shoot growth compared to the other treatments. Thus strain PN026R was considered to be belonging to PGPR *per se* with biocontrol potential.

The combination of GB03 and ASM was selected as it showed the least disease severity. GB03 was taken as it gave the minimum disease severity among the *Bacillus* group. Treatment with ASM was taken as it has not been tested in amaranthus earlier. ASM has been reported to induce resistance in wheat against fungal pathogens (Görlach *et al.*, 1996; Morris *et al.*, 1998) and in bean against bacterial and fungal infections (Siegrist *et al.*, 1997).

Many of the rhizobacterial strains act as plant growth promoters. There are reports of plant growth promotion by PGPR in different crops (Mashooda *et al.*, 2003; Alka *et al.*, 2001; Deepak *et al.*, 2003; Abdul *et al.*, 2003). As certain strains of rhizobacteria improve plant growth in addition to biocontrol, they are collectively called PGPR (Kloepper *et al.*, 1980). The economic yield of amaranthus is determined by the vegetative growth. Observations on shoot length, root length, shoot fresh weight, root fresh weight, shoot and root dry weight were taken for all the *in vivo* experiments to determine the plant growth promoting ability of the biocontrol bacteria.

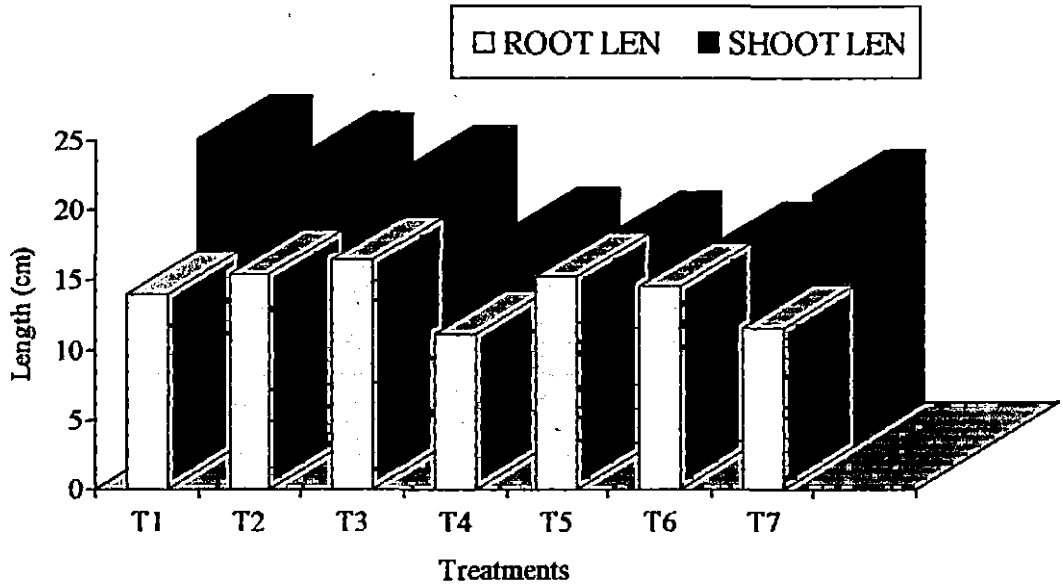
In the *in vivo* screening experiment the maximum shoot length, shoot and root dry weight was recorded for 89B61. The maximum root length was recorded for GB03. The increase in plant growth might be associated with secretion of auxins, giberllins and cytokinins (Ramamoorthy and Samiyappan, 2001). The use of fluorescent

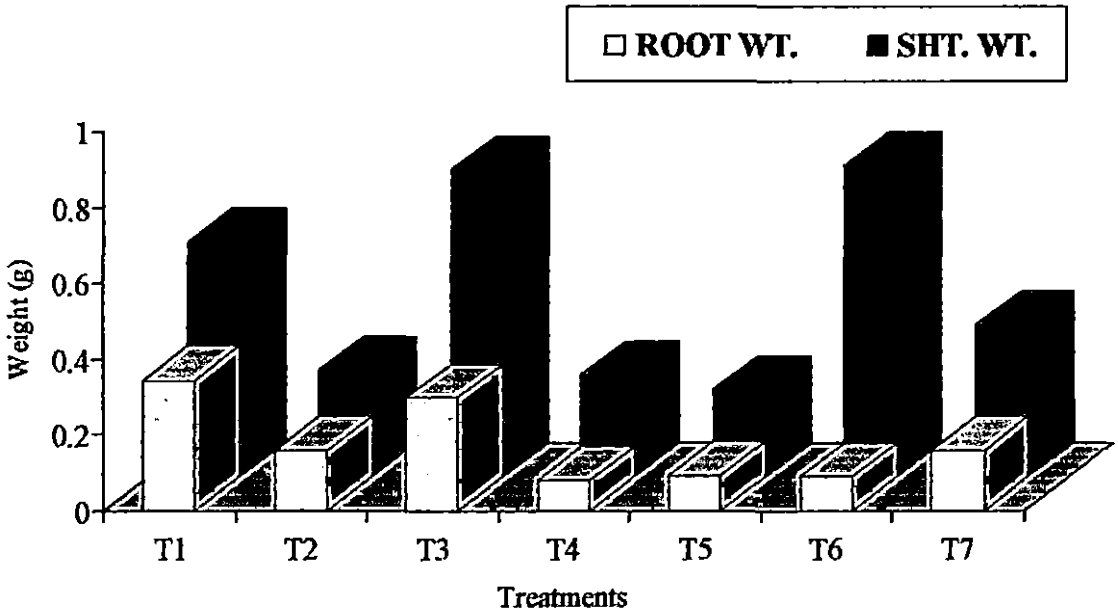
Pseudomonas having antagonistic activity and increasing the plant growth would certainly be promising in evaluating suitable isolates in biological control (Viswanathan and Samiyappan, 1999). Earlier reports also suggest that GB03 and SE34 strains increased the biomass of arabidopsis plants, total number of leaves, fruits, mean length, girth and biomass of the fruits (Mashooda *et al.*, 2003). Growth parameters of amaranthus plants treated with PGPR and chemical activator in the screening experiment is given in Fig. 1.

In all the *in vivo* experiments, treatments with ASM had a stunting effect on plants. It has been reported by Prats *et al.* (2002) that use of higher doses (0.25 and 2 mg/ml) of ASM lead to reduction in shoot fresh weight of sunflower plants. Higher concentration of ASM were phytotoxic resulting in plant stunting and blighted appearance of leaves of cucumber plants (Ishii, 2003). Activation of defense related genes is the mechanism behind induction of resistance. There is an optimum dose which would trigger defense mechanisms without compromising plant health.

An experiment was carried out to test the effect of ASM ranging from 5 ppm-25 ppm on the growth of amaranthus plants. It was noted that the maximum shoot length and root length was recorded for plants treated with 25 ppm ASM when compared with the control. There was no previous report of use of ASM in amaranthus or any similar crops. Based on the previous reports, the concentration for application was selected as 25 ppm (Romero *et al.*, 2001; Akinwunmi *et al.*, 2001).

The *in vivo* screening experiment was mainly focused on the management aspect. An experiment was exclusively conducted for plant growth promotion which was performed in the absence of the pathogen. The results after 21 days of transplanting showed that there was significant difference in shoot length of the plants when compared to the control. At harvest it was noted that all the treatments which received the PGPR had produced higher number of leaves compared to the control as well as ASM. The growth parameters of the amaranthus plants treated with





T_1 –*P. fluorescens* strain PN026R
T_2 –*P. fluorescens* strain PN026R + ASM
T_3 –*B. pumilus* strain GB03
T_4 –*B. pumilus* strain GB03 + ASM
T_5 –ASM
T_6 – Uninoculated control
T_7 – Pathogen Inoculated control

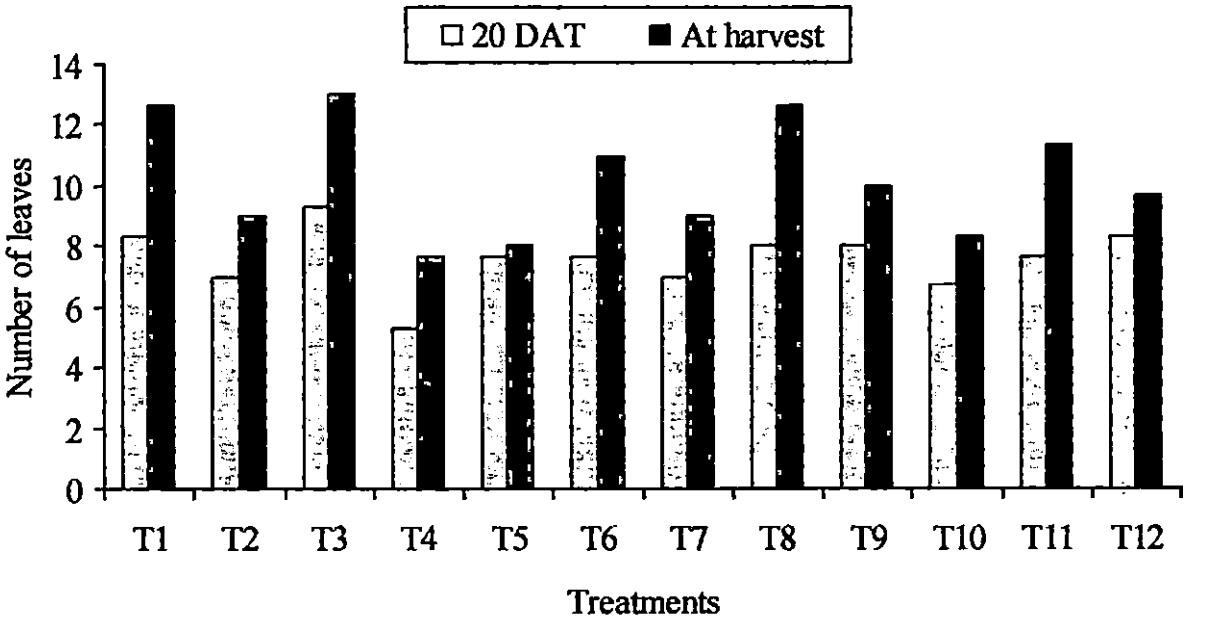
Fig. 1 Growth parameters of amaranthus plants treated with PGPR isolates and chemical activator in screening experiment

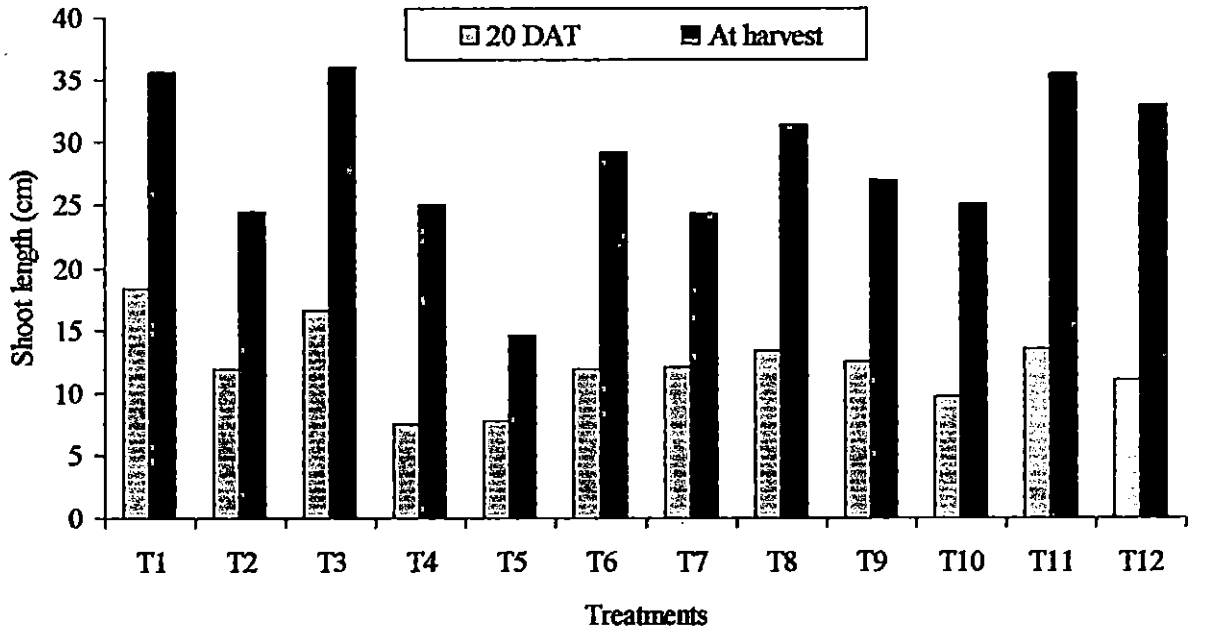
PGPR and chemical activator in the plant growth promotion experiment is depicted in Fig. 2. Though plant growth promotion by the application of PGPR is a continuous process once the bacteria get colonized on the roots, its continued application at the root zone has been found to improve the efficiency. In this study as compared to 20 DAT, at harvest the results of plant growth promotion are not that conspicuous. It could be speculated that continued application of the rhizobacteria at regular intervals could have improved the plant stand on harvest.

The screening of the rhizobacteria was performed under sterile soil condition in which only the treatment alone was allowed. Therefore no external factors, which normally present in natural soil conditions were able to influence the rhizobacteria-pathogen-plant interaction. This usually doesn't happen in normal field conditions. Therefore a pot culture experiment with non sterile soil as planting medium was conducted using the treatments selected after screening. Disease incidence, disease severity and plant growth promotion parameters were noted. Some biochemical studies were also performed to make sure that there was induction of defense responses.

The maximum disease incidence in pot culture was recorded for GB03+ASM. This was contradictory to the results in the screening test. Such results are not surprising as many a time same experiments conducted under sterile and unsterile soil conditions differ substantially. Here it could be deduced that under non sterile conditions, when other native microflora were present, the combination of GB03+ ASM could not work well. Under pot culture conditions, however, the combination of PN026R+ASM was found to be effective in reducing disease severity and incidence.

It was examined that PN026R+ASM treated plants had the maximum shoot and root length, shoot fresh weight, shoot and root dry weight. Growth parameters of the amaranthus plants treated with PGPR



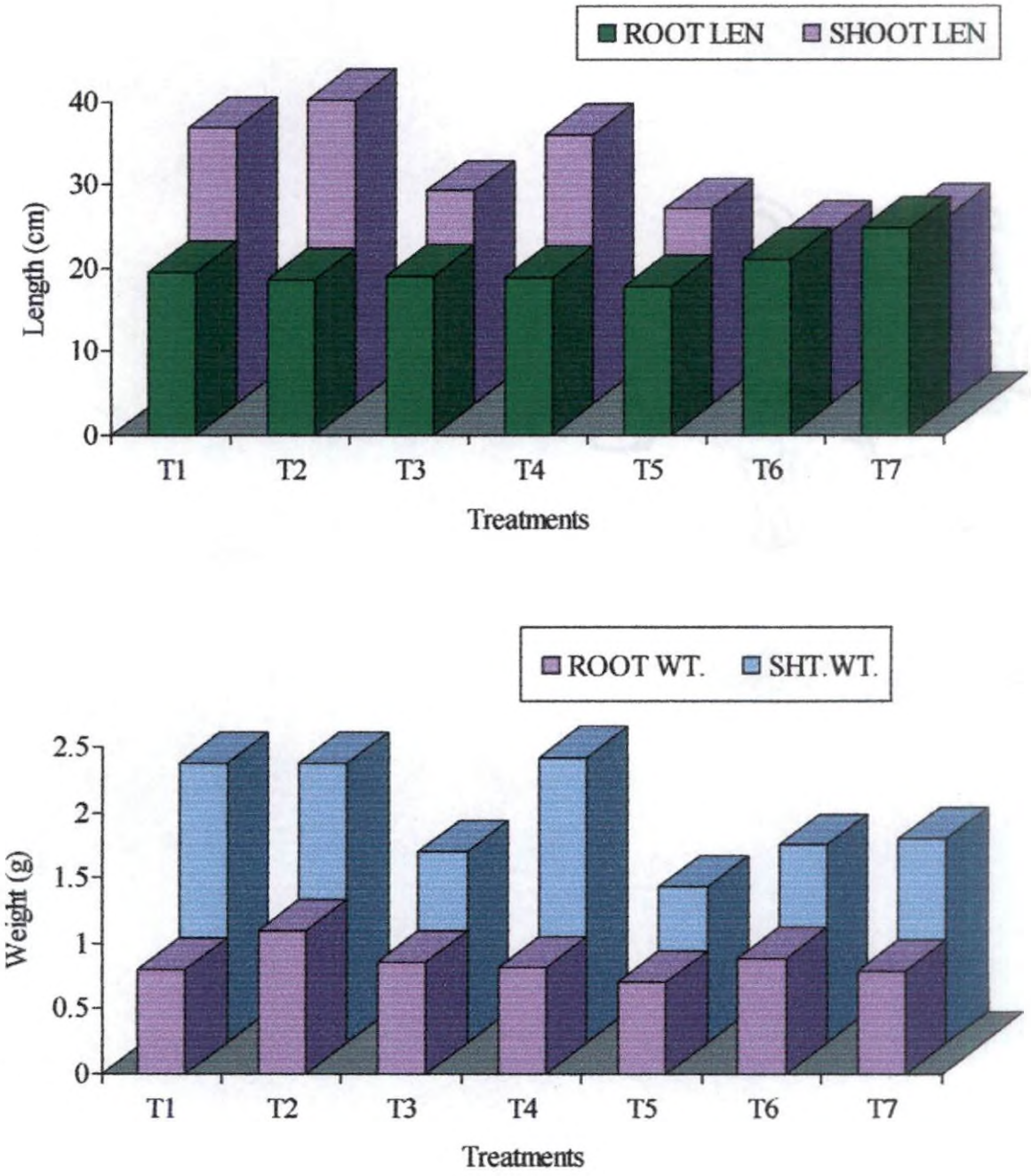


T_1 – *P. fluorescens* strain PN026R
T_2 – *P. fluorescens* strain 89B61
T_3 – *B. pumilus* strain SE34
T_4 – *B. subtilis* strain GB03
T_5 – Acibenzolar-*S*-methyl (25 ppm)
T_6 – Consortium of bacteria
T_7 – *P. fluorescens* strain PN026R + ASM
T_8 – *P. fluorescens* strain 89B61 + ASM
T_9 – *Bacillus pumilus* strain SE34 + ASM
T_{10} – *Bacillus subtilis* strain GB03 + ASM
T_{11} – Consortium of bacteria + ASM
T_{12} – Control

Fig. 2 Growth parameters of amaranthus plants treated with PGPR isolates and chemical activator in plant growth promotion experiment

and chemical activator in the pot culture experiment is given in Fig. 3. It was reported that many plant activators have a negative influence on plant-growth. Several reports suggested that observable stunting effect was evidenced when plant activators were used at higher concentrations (Prats *et al.*, 2002; Ishii, 2003). In the studies conducted under pot culture conditions and experiment on plant growth promotion, it was observed that 25 ppm of ASM reduced the plant growth substantially when compared to other treatments. However when ASM was combined with other rhizobacterial strains especially with PN026R the reduction in growth was compensated. This could be attributed to the plant growth promoting activity of the rhizobacteria even in the presence of ASM. The combination of GB03 and ASM though showed comparable plant growth promoting activity it had less disease suppressive ability. This may be because the threshold population on roots required for disease suppression is more than that for plant growth promotion.

Several reports indicate that phenolic content and activities of defense related enzymes are increased after treatment with PGPR (Matta, 1969; van Peer *et al.*, 1991; Wei *et al.*, 1991). Vidhyasekaran *et al.* (1997) reported that phenolic compounds are formed through phenylpropanoid metabolism, which has been associated with disease resistance. In the present investigation the maximum phenol content was recorded in plants treated with PN026R and ASM five days after inoculation. Sivakumar and Sharma (2003) reported that maize leaf sheaths challenged with *R. solani* showed an increase in phenolic content and PO, PPO and PAL activities. Increase in phenolic content, PO, PPO and PAL activities were noticed in tomato plants treated with *P. fluorescens*. PAL plays an important role in the biosynthesis of phytoalexins (Daayf *et al.*, 1997). De Meyer *et al.* (1999) reported that rhizosphere colonization by *P. aeruginosa* 7NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. In the present experiment the maximum PAL activity was noted for plants treated with ASM after one day of



T_{-1} –*P. fluorescens* strain PN026R
T_2 –*P. fluorescens* strain PN026R + ASM
T_3 –*B. pumilus* strain GB03
T_4 –*B. pumilus* strain GB03 + ASM
T_5 –ASM
T_6 – Uninoculated control
T_7 – Pathogen Inoculated control

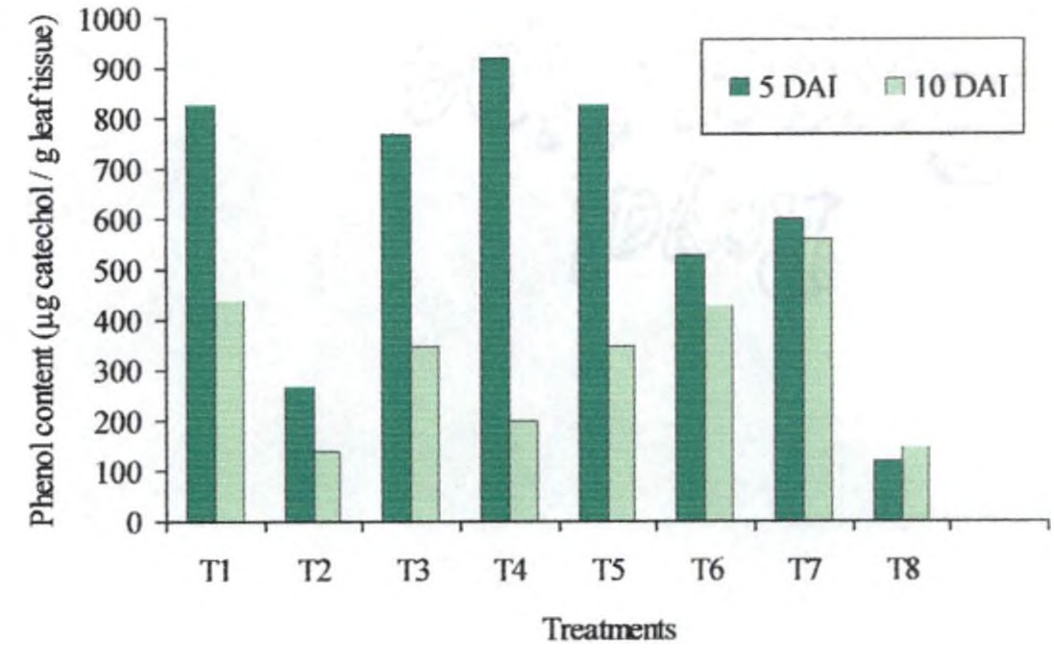
Fig.3 Growth parameters of amaranthus plants treated with PGPR isolates and chemical activator in pot culture experiment

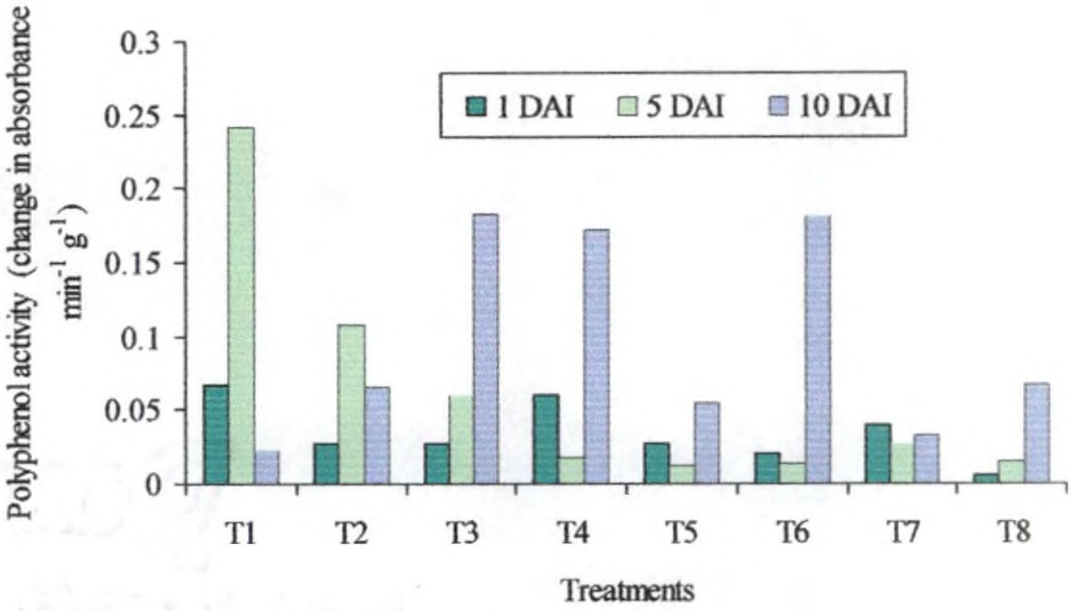
challenge inoculation with the pathogen. The maximum PPO activity was noted for GB03 and the maximum PO activity was recorded for ASM, which was in confirmatory with the earlier reports. The phenol content, PPO activity, PO activity and PAL activity of the challenged amaranthus plants is given in Fig. 4 and Fig. 5.

Molecular level studies were conducted for the better understanding of the nature of induction of the defense related enzymes due to the chemical activator, PGPR and their combinations. Rice plants treated with *P. fluorescens* isolate Pf1 and FP7 and challenge inoculated with *R. solani* showed an increase in the activity of PO, PPO, PAL and phenol (Radjacommare *et al.*, 2003). Isoform analysis revealed that a unique PPO2, PPO3, PPO4 isoform was induced in bacterised rice sheath tissues challenged with the pathogen. In the present investigation the isoform analysis of peroxidase enzyme revealed only one ISO PO was induced in treatments *viz.*, PN026R, GB03+ASM and ASM. The failure of the other treatments to express the isoform of peroxidase may be due to the late induction of PO or due to lesser concentration of the same. Chen *et al.* (2000) reported that the isoperoxidase in native PAGE analysis indicated that the peroxidase isoforms in cucumber roots induced by rhizobacteria were different from that in roots infected with *Pythium aphanidermatum*..

The present study is first of its kind in amaranthus where microbe and chemically induced systemic resistance was exploited for managing *R. solani* induced foliar blight. The results indicated that PGPR strains could induce resistance against *R. solani* in a susceptible variety of amaranthus, Arun. The native isolate PN026R was effective in suppressing the disease and also improved growth of the plant. Thus treating the rhizosphere soil with the PGPR strain would have dual benefit of plant growth promotion as well as disease suppression. The chemical activator ASM was also found to be compatible with the bacterial strain.

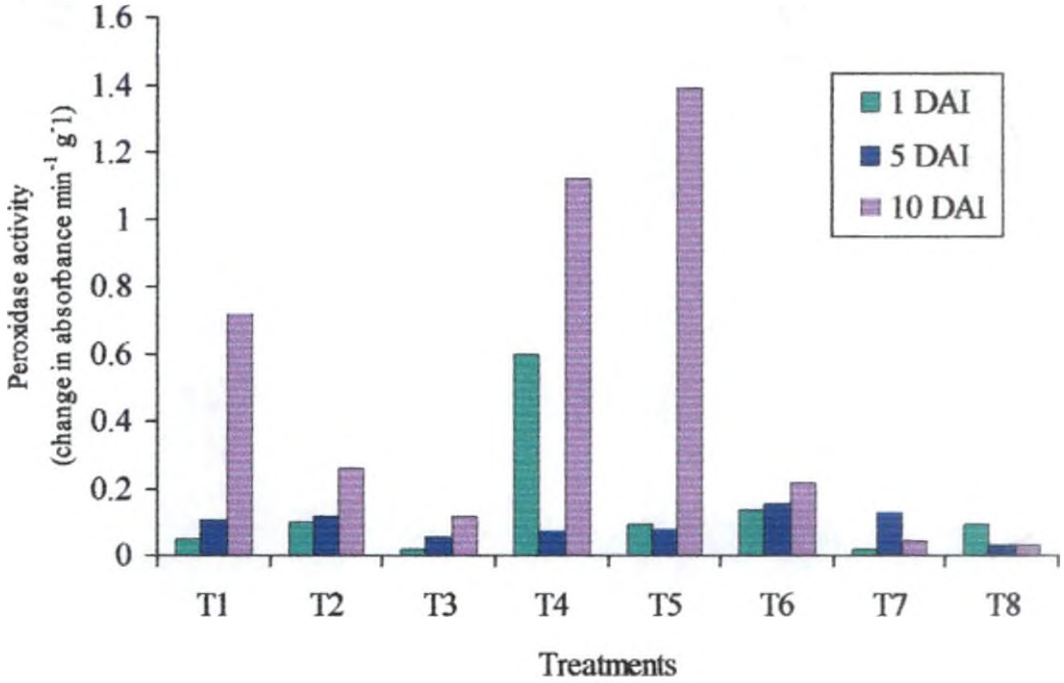
Combining the chemical activator, the ISR inducing bacterial strains and antagonists with direct suppression of the growth of the

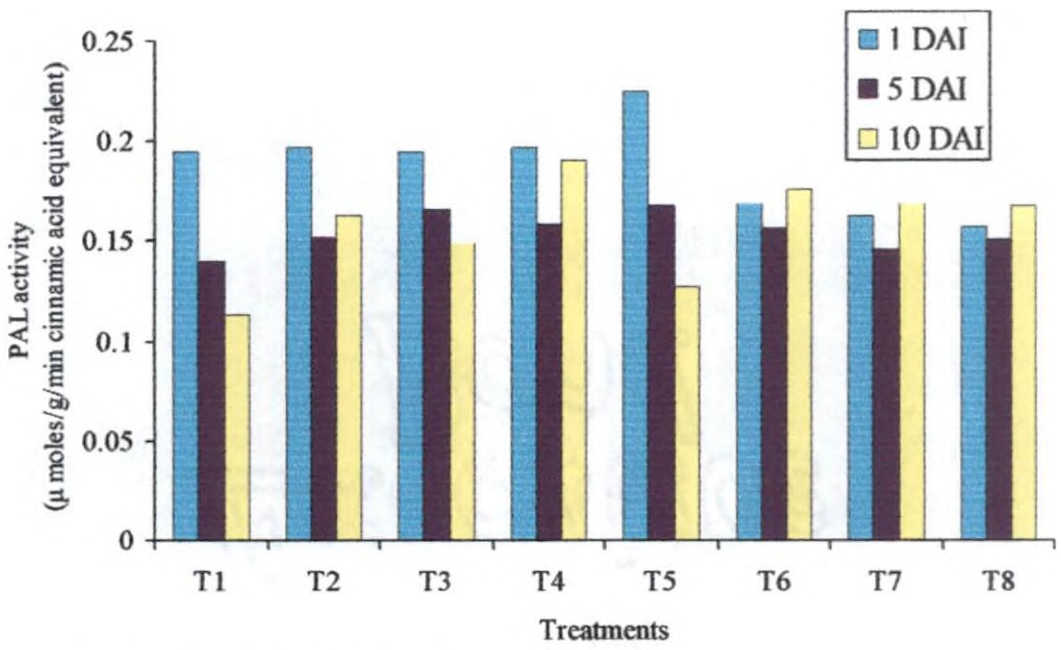




T_{-1} –*P. fluorescens* strain PN026R
T_2 –*P. fluorescens* strain PN026R + ASM
T_3 –*B. pumilus* strain GB03
T_4 –*B. pumilus* strain GB03 + ASM
T_5 –ASM
T_6 – Uninoculated control
T_7 – Pathogen Inoculated control
T_8 – Chemical control

Fig. 4 Biochemical analysis of challenged amaranthus plants





T_1 –*P. fluorescens* strain PN026R
T_2 –*P. fluorescens* strain PN026R + ASM
T_3 –*B. pumilus* strain GB03
T_4 –*B. pumilus* strain GB03 + ASM
T_5 –ASM
T_6 – Uninoculated control
T_7 – Pathogen Inoculated control
T_8 – Chemical control

Fig. 5 Biochemical analysis of challenged amaranthus plants

pathogen is expected to perform in a better way through multiple action of biocontrol as well as plant growth promotion in amaranthus.

Summary

6. SUMMARY

Foliar blight caused by *Rhizoctonia solani* has emerged as a serious disease affecting the leafy vegetable amaranthus (*Amaranthus tricolor* L.) in Kerala. The present investigation was undertaken to manage the disease using PGPR and chemical activator. Four proven PGPR isolates *viz.*, *Pseudomonas fluorescens* strain PN026R, *Pseudomonas putida* strain 89B61, *Bacillus pumilus* strain SE34 and *Bacillus subtilis* strain GB03 and a chemical activator Actigard™ 50WG containing the active ingredient Acibenzolar-S-methyl (ASM) were used for the study.

Disease suppression and plant growth promotion studies were conducted using the PGPR strains and ASM. An *in vitro* study was conducted to assess the direct effect of the PGPR on the pathogen. Dual culture plate assay was performed in four different media. It was found that PN026R showed antagonism against the pathogen only in NA where as in PDA, KB and CA it showed no inhibition of mycelial growth. 89B61 showed antagonism against the pathogen in PDA, NA and CA only. The effect of different concentrations of ASM on the growth of the pathogen was also tested *in vitro*. Seven different concentrations of ASM ranging from 5ppm-100 ppm were used. It was observed that ASM had inhibitory effect on mycelial growth of pathogen.

An *in vivo* screening experiment was conducted to assess the role of ISR by the PGPR isolates and ASM in reducing the severity of the disease. Though PN026R showed no direct antagonism *in vitro*, it could effectively reduce the severity of the disease in the *in vivo* screening experiment. The reduction in severity brought about by the bacterial strain PN026R could be due to induction of systemic resistance as the pathogen and the bacteria were spatially separated throughout the experiment. The treatment GB03+ASM recorded the least disease

severity. The disease severity observed for ASM + consortium of bacteria was more than that in the consortium of bacteria. From the results it was inferred that there is no additive effect due to the consortium.

Based on disease severity PN026R, GB03, their combination with ASM and ASM with controls were selected for further studies. The maximum disease incidence was recorded in combined treatment with GB03 + ASM (91.67%). The minimum disease incidence was recorded for uninoculated control. It was followed by PN026R + ASM, GB03 and ASM. The maximum disease severity was recorded in the chemical control (61.11%) followed by inoculated control. GB03 and PN026R + ASM recorded same disease severity (20.84). The total phenol content and the different enzyme activities were also assessed in this experiment after challenge inoculation. Changes in the levels of PAL, PO and PPO were recorded at one, five and ten days after inoculation with the pathogen. After five days of inoculation the maximum phenol content was recorded for PN026R. Treatment with ASM alone showed maximum PAL activity five days after inoculation. There was a progressive increase in peroxidase activity from one day after inoculation to ten days after inoculation in the plants treated with PN026R, PN026R + ASM and GB03.

An experiment was carried out to test the effect of ASM on the growth of amaranthus plants. It was noted that the maximum shoot length and root length was recorded for plants treated with 25 ppm ASM when compared with the control.

An experiment was exclusively conducted for plant growth promotion, which was performed in the absence of the pathogen. Observation on 21 days after transplanting showed that there was significant difference in shoot length of the plants when compared to the control. At harvest it was noted that all the treatments, which received

the PGPR, had higher number of leaves compared to the control as well as ASM.

It has been observed that PN026R has shown better ISR and growth promotion activities. PN026R was able to compensate the adverse effects of ASM. The combination of ASM + PN026R performed well in pot culture experiments.

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* Original not seen

Appendices

APPENDIX – 1

Composition of different media

A. Potato dextrose agar (1 litre)

Potato	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1 litre

B. King's B medium (1 litre)

Peptone	:	20.00 g
Dipotassium hydrogen phosphate	:	1.50 g
Magnesium sulphate	:	1.50 g
Glycerol	:	10 ml
Distilled water	:	1 litre

Adjust pH to 7.2

C. Carrot Agar (1 litre)

Carrot	:	200 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1 litre

D. Nutrient Agar medium(1 litre)

Beef extract	:	3 g
Peptone	:	5 g
Sodium chloride	:	5 g
Agar	:	20 g
Distilled water	:	1 litre

APPENDIX – II

Buffers for enzyme analysis

A) 0.1 M sodium borate buffer (pH 8.8)

A: 0.2 M solution boric acid (12.4 g in 1000 ml)

B: 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml

B) 0.1 M sodium phosphate buffer (pH 6.5)

A: 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 ml)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of Na_2HPO_4. 7 H_2O in 1000 ml)

68.5 ml of A is mixed with 31.5 ml of B, diluted to a total of 200 ml

C) 0.6 M acetate buffer (pH 5.4)

A: 0.6 M solution of acetic acid (34.5 ml in 1000 ml)

B: 0.6 M solution of sodium acetate (49.2 g in 1000 ml)

8.8 ml of A is mixed with 41.2 ml of B, diluted to a total of 100 ml

MANAGEMENT OF FOLIAR BLIGHT OF AMARANTHUS USING RHIZOBACTERIA AND CHEMICAL ACTIVATOR-ACIBENZOLAR-*S*-METHYL

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Abstract of the
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ABSTRACT

The study, "Management of foliar blight of amaranthus using plant growth promoting rhizobacteria (PGPR) and a chemical activator Acibenzolar-S-Methyl" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani. Four proven PGPR isolates *viz.*, *Pseudomonas fluorescens* strain PN026R, *Pseudomonas putida* strain 89B61, *Bacillus pumilus* strain SE34 and *Bacillus subtilis* strain GB03 and a chemical activator Actigard^{TM} 50WG containing the active ingredient Acibenzolar-S-Methyl (ASM) were used for the study. Disease suppression and plant growth promotion studies were performed using these PGPR isolates and ASM. *In vitro* studies were conducted to check whether the bioagents are having a direct antagonistic effect on the pathogen. Dual culture plate assay was performed in four different media. It was noted that the antagonism showed by these rhizobacteria ranged from slight antagonism to a zone of more than 5 mm. The range of antagonism even by same antagonist varied in different media. The mycelial growth inhibition of the pathogen by different concentration of ASM in the medium was also noticed.

A screening experiment was conducted to assess the involvement of ISR by different PGPR and the chemical activator individually and in combination against the disease caused by *R. solani* in amaranthus var. Arun. Sterile potting mixture was used in the study. The minimum disease severity was observed for combined treatment with GB03 and ASM. Plants treated with PN026R, GB03 and the consortium of bacteria showed same disease severity of 8.33%. Observations on plant growth promotion were also taken. Based on the effect on disease suppression and plant growth promotion two PGPR strains were selected for further pot culture studies *viz.*, GB03 and PN026R. PN026R+ASM, GB03 and ASM treatments recorded the lowest disease incidence. The minimum disease

severity was recorded for uninoculated control. GB03 and PN026R+ASM recorded same disease severity. Maximum shoot length and shoot fresh weight was observed for the treatment with PN026R+ASM.

The total phenol content and the different enzyme activities were also recorded in this experiment. Changes in the levels of PAL, PO and PPO were recorded at one, five and ten days after inoculation with the pathogen. After five days of inoculation the maximum phenol content was recorded for PN026R. Treatment with ASM alone showed maximum PAL activity five days after inoculation. There was a progressive increase in peroxidase activity from one day after inoculation to ten days after inoculation in the plants treated with PN026R, PN026R+ASM and GB03.

Plant Growth promotion experiments were also carried out using the four PGPR strains and ASM. The maximum shoot length was recorded for the treatment with SE34 followed by PN026R, consortium of bacteria+ASM and the control. The maximum root length was recorded for GB03+ASM.

The results of the study indicate that PN026R showed better growth promotion and ISR activities. PN026R can also be used in combination with ASM which helps to compensate the adverse effects of ASM.