Efficacy of acibenzolar-*S*-methyl and rhizobacteria for the management of foliar blight disease of amaranth

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Abstract

We evaluated the influence of Acibenzolar-*S*-Methyl (ASM; 0 to 100 ppm concentrations), a chemical activator, and four Plant Growth Promoting Rhizobacteria (PGPR; *Pseudomonas fluorescens* PN026R, *P. putida* 89B61, *Bacillus pumilus* SE34, and *B. subtilis* GB03) on amaranth (*Amaranthus tricolor* L.) foliar blight (*Rhizoctonia solani* Kühn) suppression. *In vitro* and *in vivo* experiments were conducted both under sterile and non-sterile soil conditions in which the PGPR and activator were tried both individually and in combination. Results indicate PGPR induced resistance against *R. solani* in a susceptible amaranth variety, 'Arun'. A native isolate, *P. fluorescens* PN026R was particularly effective in suppressing the disease and promoting plant growth. Plants treated with PN026R showed lower disease incidence and disease severity; 67 and 35 % respectively compared to 92 and 52 % for plants inoculated with pathogen alone. Combined application of PGPR and ASM was, however, more effective with disease incidence and disease severity of 42 and 21 % respectively.

Keywords: Biological control, Induced systemic resistance, Plant Growth Promoting Rhizobacteria, *Rhizoctonia solani,* Plant activator.

Introduction

Amaranth (*Amaranthus tricolor* L.) is considered to be the cheapest leafy vegetable in the market and it could rightly be described as the 'poor man's spinach'. It is a short duration crop that fits well with the crop rotations of Kerala. However, foliar blight caused by *Rhizoctonia solani* is a serious threat to amaranth growers (Kamala et al., 1996). Susceptibility of popular cultivars and the humid conditions in Kerala aggravate the problem. Although disease management using fungicides (e.g., foliar spray of mancozeb 0.4% in cow dung supernatant at fortnightly intervals; KAU, 2002) is effective, repeated use of chemicals pose health hazards. Biological control practices, which reduce the risk of pesticide residues and enable maintenance of ecological balance, therefore, assume significance.

Biocontrol using microbial agents, especially Plant Growth Promoting Rhizobacteria (PGPR), has been particularly effective under field conditions (Hass and Defago, 2005). PGPR stimulates plant growth, maintains soil health, and induces the plant defense system against diseases. Plant activators, a category of novel chemicals, also induce the defense capabilities of plants. For example, acibenzolar-*S*-methyl (ASM), a structural analog of salicylic acid, has been reported to be effective against plant diseases caused by fungal, bacterial, and viral agents (Métrauxs, 2001). ASM, although considered as a safe chemical, has not been tested previously in amaranth against any diseases. In view of this, a study was conducted to evaluate the efficacy of four PGPR (two *Pseudomonas* and *Bacillus* strains each) and a chemical activator (ASM), both individually as well as in combinations, for managing amaranth foliar blight disease.

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Materials and Methods

In vitro *experiments*

An *in vitro* experiment was conducted to test whether there is any direct antagonistic effect of rhizobacteria or the chemical activator on the pathogen. The rhizobacterial strains used were *Pseudomonas fluorescens* PN026R, *P. putida* 89B61, *Bacillus pumilus* SE34, and *B. subtilis* GB03. All these were reported to be efficient biocontrol agents against various phytopathogens (Anith et al., 2003; 2004). *In vitro* antagonism of the four rhizobacterial strains against *Rhizoctonia solani* was tested by dual culture plate assay on four culture media: Potato Dextrose Agar (PDA), King's medium B (KB), Carrot Agar (CA), and Nutrient Agar (NA). The *Pseudomonas* strains were streaked on KB medium and the *Bacillus* strains on NA medium to obtain single colonies. 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. A mycelial disc of 5 mm diameter from 7 day-old culture of *R. solani* was placed at the centre 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 observations on the mycelial growth of *R. solani* were recorded. Zone of inhibition by the rhizobacteria was measured after 5 days of incubation.

The effect of different concentrations of ASM was studied using the poison food technique. Two hundred milligram Actigard 50WG (Novartis Crop Protection Inc., Greensboro, NC, USA) was dissolved in 1 L of sterile water to get a concentration of 100 ppm ASM. To 100 ml of sterile molten PDA medium in 250 ml conical flasks, varying quantities of the stock solution were added aseptically to get concentrations 5, 12.5, 25, 37.5, 50, 75, and 100 ppm. For each combination, three replications were maintained. Plates were inoculated at the centre with a mycelial disc of *R. solani*. Plates without ASM inoculated with *R. solani* at the centre served as control. Inhibition of mycelial growth of the pathogen by the chemical activator was measured after a period of 7 days.

In vivo *screening for disease suppression*

To test the effect of rhizobacteria and ASM on the management of foliar blight of amaranth, an *in vivo* test was done using 15 day old seedlings in plastic cups of diameter 7.5 cm and height of 9 cm filled with sterile potting mixture (soil and sand in 1:1 proportion). Each cup contained a single plant and four replications were maintained. The test variety was 'Arun', a susceptible amaranth cultivar. There were 13 treatments, which consisted of PGPR and ASM individually and in combination. The bacterial cells as well as the chemical activator were applied at the base of the plant only and any direct interaction of the biocontrol agent with the pathogen was completely avoided. Soil application with rhizobacteria was done by drenching the base of the plants with the bacterial suspension at the rate of 5 ml per plant 15 days after transplanting (Nair et al., 2007). The suspension contained bacterial cell density of approximately 10^8 cfu·ml^{-1}. ASM (25 ppm) solution (5 ml·plant^{-1}) was drenched twice, 1 and 12 days after transplanting. Challenge inoculation with the pathogen was done 7 days after rhizobacterial drenching, which corresponded to 10 days after ASM drenching. Small mycelial bits of the pathogen were cut from the agar plates and placed on the upper surface of the three lower 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 maintain high humidity levels. Inoculation was done on all plants except the control. Observations on disease incidence, disease severity, shoot length, root length, shoot and root fresh weight, and shoot and root dry weights were taken 21 days after transplanting.

Pot culture evaluation of disease suppression

Another *in vivo* experiment was performed in pots of size 22 x 21 cm containing soil, sand, and cowdung in 2:1:1 proportion to further evaluate eight treatments selected based on the initial screening. It involved the promising bacterial cultures (PN026R and GB03) and

their combinations with ASM. Three plants (15 days old) per pot were used. Rhizobacterial suspension was drenched at the base of the plants (5 ml·plant^{-1}) on15 and 22 days after transplanting. ASM solution was drenched thrice (1, 12, and 19 days after transplanting). Challenge inoculation with the pathogen was done 7 days after the last rhizobacterial drenching, which corresponded to 10 days after the last ASM drenching. Inoculation was done on 5th and 6th leaves from the top. The plants were uprooted 2 months after transplanting and growth observations recorded as in the first set of experiments.

Statistical analysis was done for all the parameters using one way analysis of variance and Duncan's Multiple Range Test (DMRT) for comparing the means, using the statistical package SAS version 8.1 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The data presented in Table 1 indicate the presence or absence of a zone of inhibition in the *in vitro* dual culture. *Pseudomonas* sp. PN026R showed no antagonism against the pathogen on PDA, KB, and CA. However, it showed antagonism on NA medium. Mycelial growth inhibition of the pathogen by different concentrations of ASM is shown in Table 2. Maximum mycelial growth inhibition was recorded with 37.5 ppm concentration of ASM. Direct inhibitory effect of ASM on growth of *R. solani* has been reported earlier too (Meyer et al., 2006).

Although PN026R did not show any direct antagonism *in vitro*, it substantially reduced the severity of the disease in the *in vivo* screening experiment (Fig. 1). This reduction in disease severity could be due to induction of systemic resistance as the pathogen and

Table 1. Antagonism of plant growth promoting rhizobacteria against *Rhizoctonia 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

Table 2. Effect of Acibenzolar-S- Methyl (ASM) on the growth of *Rhizoctonia solani.*

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

Mean of four replications having one plate each (not subjected to statistical analysis)

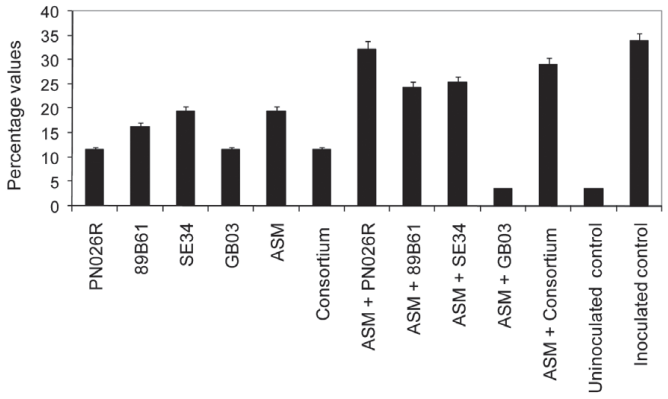

Figure 1. Disease severity during screening of plant growth promoting rhizobacteria and chemical activator against foliar blight disease in pot culture under sterile soil conditions. Mean of four replications having one plant each. Bars represent standard error.

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). The present study suggests that induction of systemic resistance is one of the probable mechanisms of disease suppression by PN026R.

In the screening test, the treatment with GB03+ASM gave the lowest disease severity. *Bacillus* strain GB03 showed antagonistic activity against *R. solani* in all the four test media. It could thus be inferred that this strain, if used as a foliar spray, may be effective to prevent amaranth blight. A single bacterial strain exhibiting multiple modes of disease suppression is not uncommon. For example, Alström (1991) reported the role of induced systemic resistance (ISR) in the suppression of halo blight of bean by *P. fluorescens* S97 and the rhizobacterium exhibited bacteriostatic activity. Thus GB03 may possess multiple disease suppressive mechanisms. When it was used alone as a treatment in sterile soil, the disease severity was 8.33% (Fig. 1). But when GB03 was combined with ASM, the disease severity dropped to zero, implying synergistic effects. This is consistent with the report of Chen et al. (1996) who found the combination of chemically induced systemic acquired resistance in tobacco seedlings and the biocontrol strain *Bacillus cereus* resulted in additive suppression of disease caused by *Pythium torulosum*, *Pythium aphanidermatum,* or *Phytophthora parasitica*. In the present study, however, ASM alone performed better than when combined with other rhizobacteria.

GB03+ASM recorded the highest disease incidence in the pot culture experiment (Fig. 2), which was contradictory to the results of the screening test. Such results are, however, not surprising as many a time, the results of the same experiment conducted under sterile and unsterile soil conditions differ profoundly. Thus under non-sterile conditions, when other native microflora were presumably present, the combination of GB03+ ASM may not work well. Under pot culture conditions, however, the combination of PN026R+ASM was effective in reducing disease severity and incidence. It was observed that PN026R+ASM treated plants had the highest shoot and root length, shoot fresh weight, shoot and root dry weight (Table 3). In all the *in vivo* experiments, treatments with ASM, however, had a stunting effect. Consistent with this, Prats et al. (2002) reported earlier that use of higher doses (0.25 and 2 $mg \cdot ml^{-1}$) of ASM led to reduction in shoot fresh weight of sunflower plants. However, application of some of the PGPR strains along with ASM could possibly mitigate stunting effects due to the plant activator (Nair et al., 2007).

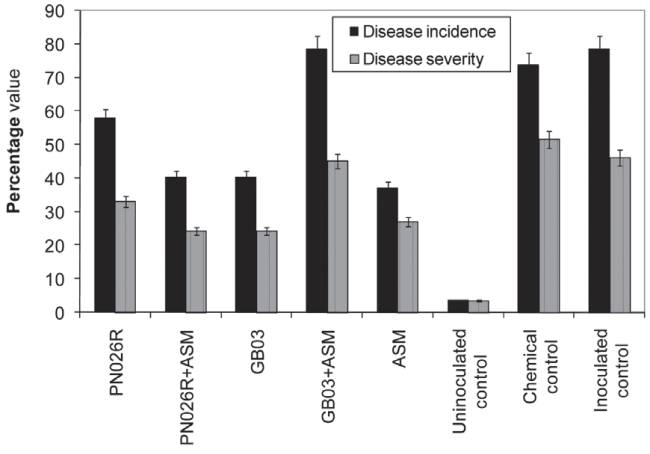

Figure 2. Effect of biocontrol gents and chemical activator on disease incidence and severity in pot culture under non-sterile soil conditions. Mean of four replications having three plants each. Bars represent standard error.

Table 3. Effect of biocontrol agents and chemical activator on length, fresh and dry weight of shoots and roots of amaranth plants.

Treatments	Shoot length (cm)	Shoot fresh weight per plant (g)	Shoot dry weight per plant (g)	Root length (cm)	Root fresh weight per plant (g)	Root dry weight per plant (g)
PN026R	33.2[a]	11.28[ab]	2.13[a]	19.5[ab]	4.23[a]	0.80[ab]
PN026R+ASM	36.4[a]	13.35[a]	2.14[a]	18.7[ab]	2.61[a]	1.10[a]
GB03	25.6[a]	11.30[ab]	1.46[b]	19.1[ab]	4.00[a]	0.85[ab]
GB03+ASM	32.2[a]	9.18[ab]	2.18[a]	18.9[ab]	4.19[a]	0.82[ab]
ASM	23.4[b]	11.18[ab]	1.20[b]	17.7[ab]	3.08[a]	0.70[ab]
Uninoculated control	20.6[b]	9.92[ab]	1.51[b]	21.2[ab]	2.98[a]	0.89[ab]
Chemical control	20.2[b]	8.98[a]	1.36[b]	16.7[b]	2.49[a]	0.56[b]
Inoculated control	22.6[b]	6.88[b]	1.56[b]	24.8[a]	2.65[a]	0.79[ab]

Means of four replications having three plants each; values followed by same superscripts within a column do not differ significantly according to Duncan's Multiple Range Test (p=0.05).

The present study highlights the use of microbe and chemically induced systemic resistance for managing *R. solani* induced foliar blight in amaranth. The results indicated that PGPR strains could induce resistance against *R. solani* in a susceptible variety of amaranth, 'Arun'. The native *Pseudomonas* isolate PN026R was effective in suppressing the disease and also improved growth of the plant. Thus treating the rhizosphere with PGPR strain would have dual benefits of plant growth promotion and disease suppression. The chemical activator ASM was compatible with the bacterial strain and combining the chemical activator, ISR inducing bacterial strains, and antagonists clearly is a better approach as it would not only promote biocontrol but also stimulate amaranth growth.

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