Biol. Control, 20(2): 197-204, 2006

Seed-borne biocontrol agents for the management of rice sheath rot caused by *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw

C. GOPALAKRISHNAN and V. VALLUVAPARIDASAN
Department of Plant Pathology
Centre for Plant Protection Studies, Tamil Nadu Agricultural University
Coimbatore 641 003, Tamil Nadu, India
E-mail:pcgopal@yahoo.co.in

ABSTRACT: All the antagonist isolates obtained from rice seeds showed *in vitro* inhibitory effect on *Sarocladium oryzae* ranging from 10.90 to 82.18 per cent. *Bacillus subtilis* isolate 9 was found to be highly effective in inhibiting the mycelial growth of *S. oryzae* by 82.18 per cent. The biocontrol agents significantly reduced *S. oryzae* seed infection and increased seed germination and seedling vigour. Field trials conducted at four different locations indicated that the seed-borne *S. oryzae* in rice could be successfully managed by seed treatment followed by foliar application at boot leaf stage with *B. subtilis* (or) *P. fluorescens*.

KEY WORDS: Biocontrol agents, mycelial growth, rice seeds, *Sarocladium oryzae*, sheath rot

INTRODUCTION

Sheath rot of rice incited by *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw is seed-borne and present in all rice growing countries worldwide. The disease is highly destructive in Tamil Nadu and other rice growing states of India (Chakravarty and Biswas, 1978; Lakshmanan, 1993). Rice seeds are the natural habitat of many microorganisms particularly bacteria. Abundance of these bacteria varies, depending on sources of seed, status of seed health and whether germinated or whole seed before germination. Not all microorganisms associated with seed are pathogens. Some microorganisms possess biological control properties. Cottyn *et al.* (2001) and Xie *et al.* (2001) proved that seed-borne antagonistic bacteria are present in rice and promote seed germination and seedling vigour, and also suppress disease with an inoculum from the seed. About 4 per cent of the total bacterial population possesses biological control properties against most seed-borne pathogens of rice. Among the bacteria present on the seed, there were more diverse groups among those possessing antagonistic effects on selected rice fungal pathogens, such as *Rhizoctonia solani*, *Fusarium moniliforme*, *Pyricularia grisea*, *S. oryzae* and others, than those with a plant growth promotion effect. The present study was taken up to explore the possibility of using rice seed based biocontrol agents for managing rice sheath rot.

MATERIALS AND METHODS

Isolation and screening of biocontrol agents

Antagonistic fungi and bacteria were isolated from rice seeds by using direct plating technique (Ashura *et al.*, 1999). The seeds were surface sterilized for 10 minutes with sodium hypochlorite (2%). Then the seeds were rinsed five times with sterile water to remove the disinfectant, dried on sterile paper towels and plated on selective media. Ten isolates of *Pseudomonas fluorescens*, fifteen isolates of *Bacillus subtilis*, and three isolates of *Trichoderma viride*, were isolated, purified, identified and maintained in Kings B medium, Nutrient agar medium and *Trichoderma* selective medium, respectively. The biocontrol agents were screened against *S. oryzae* using dual culture technique.

Effect of selected biocontrol agents and fungicide on *S. oryzae* seed infection, seed germination and seedling vigour

Two rice cultivars, namely, ADT 39 and ADTRH 1 having higher seed infection (32.5 and 51 %, respectively) were used for this study. Seeds were incubated in blotter immediately after seed treatment with biocontrol agents *viz.*, *Bacillus subtilis* isolate 9, *Trichoderma viride* isolate 3 and *Pseudomonas fluorescens* isolate 10 at the rate of 10 g/ kg and carbendazim at the rate of 2 g/kg and the seed infection by *S. oryzae* (%) was assessed using standard blotter test. Talc based formulations of biocontrol agents were used for seed treatment and foliar application. The initial population of biocontrol formulations was maintained at 3×10^8 cfu/ g. Carbendazim was used at the rate of 2 g/ kg and 0.2 per cent for seed treatment and foliar application, respectively.

Seed germination

Seed germination test was conducted in between paper medium (roll towel) as per the method described in ISTA (1993).

Root and shoot length

On the final count day, ten normal seedlings were carefully removed at random from each replication. Length of root (start of the root to the tip of the primary root) and shoot (from base of the shoot to tip of the primary leaf) was measured separately in cm.

Vigour index

The vigour index was calculated using the following formula and expressed as whole number (Abdul – Baki and Anderson, 1973).

Vigour index = Germination (%) x Mean total length of seedlings (cm)

Field Experiments

The experiments were conducted in four agro-climatic zones of Tamil Nadu (western, southern, north east and Cauvery delta) during *Rabi* 2004 in a Randomized Block Design with three replications in a plot of 5×4 m^2. Sheath rot incidence and severity were recorded 10 days prior to harvest. The seeds were used for assessing seed infection and seed discolouration. The treatment combinations were as follows.

T_1 - Seed treatment with *Bacillus subtilis* isolate 9 @ 10g/ kg

T_2 - Seed treatment with *Pseudomonas fluorescens* isolate 10 @ 10g/ kg

T_3 - Seed treatment with carbendazim @ 2g/ kg

T_4 - Seed treatment with *Bacillus subtilis* isolate 9 @ 10g/ kg + foliar application (1%) at boot leaf stage

T_5 - Seed treatment with *Pseudomonas fluorescens* isolate 10 @ 10g/kg + foliar application (1%) at boot leaf stage

T_6 - Seed treatment with carbendazim @ 2g/ kg + foliar application (0.2%) at boot leaf stage

T_7 – Un-inoculated control

RESULTS AND DISCUSSION

Effect of biocontrol agents on mycelial growth of *S. oryzae*

All the isolates of biocontrol agents were found inhibitory to the mycelial growth of *S. oryzae* (Table 1). *Bacillus subtilis* isolate 9 recorded the minimum growth of 6 mm, which was 82.18 per cent

Table 1. Effect of biocontrol agents on the mycelial growth of *S. oryzae*

Bio control agent	Radial growth (mm) *	Reduction over control (%)
Pseudomonas fluorescens 1	25.67 ^{de}	23.76
P. fluorescens 2	23.00 ^{cd}	31.69
P. fluorescens 3	16.67 ^{b}	50.49
P. fluorescens 4	23.33 ^{cd}	30.71
P. fluorescens 5	19.33 ^{bc}	42.59
P. fluorescens 6	19.33 ^{bc}	42.59
P. fluorescens 7	30.00 ^{ef}	10.90
P. fluorescens 8	14.67 ^{b}	56.43
P. fluorescens 9	27.33 ^{de}	18.83
P. fluorescens 10	9.00 ^{a}	73.27
Bacillus subtilis 1	24.00 ^{e}	28.72
B. subtilis 2	20.67 ^{de}	38.61
B. subtilis 3	20.00 ^{de}	40.60
B. subtilis 4	11.00 ^{b}	67.33
B. subtilis 5	20.33 ^{de}	39.62
B. subtilis 6	22.00 ^{de}	34.66
B. subtilis 7	16.67 ^{cd}	50.49
B. subtilis 8	13.00 ^{bc}	61.39
B. subtilis 9	6.00 ^{a}	82.18
B. subtilis 10	23.00 ^{e}	31.69
B. subtilis 11	23.33 ^{e}	30.71
B. subtilis 12	25.33 ^{e}	24.77
B. subtilis 13	14.33 ^{bc}	57.44
B. subtilis 14	14.33 ^{bc}	57.44
B. subtilis 15	25.67 ^{e}	23.76
Trichoderma viride 1	22.67 ^{b}	32.67
T. viride 2	23.67 ^{b}	29.70
T. viride 3	7.67 ^{a}	77.22
Control	33.67 ^{c}	—

Means followed by a common letter are not significantly different at the 5% level by DMRT reduction of mycelial growth over control followed by 7.67 mm (77.22%) in *T. viride* isolate 3. *Pseudomonas fluorescens* isolate 7 was found to be least effective (10.9%).

Effect of selected biocontrol agents and fungicide on seed infection of *S. oryzae*, seed germination and seedling vigour of rice cv. ADTRH 1

All the treatments significantly reduced the *S. oryzae* seed infection (Table 2). The seeds treated with *P. fluorescens* isolate 10 reduced seed infection from 51.00 per cent (untreated) to 10.75 per cent, which was followed by carbendazim (12.75%), *B. subtilis* isolate 9 (14.25%) and *T. viride* isolate 3 (18.00%). Though all the treatments significantly improved the seed germination, maximum being (92.25%) in *P. fluorescens* isolate 10 treated seeds, which followed by *T. viride* isolate 3 (92%) compared to control (69%). The maximum root length (25.45 cm), shoot length (15.90 cm) and seedling vigour index (3756) were recorded in *P. fluorescens* isolate 10 treatment. The minimum seedling vigour of 2305 was observed in control. *Pseudomonas fluorescens* isolate 10 and *Bacillus subtilis* isolate 9 have significantly reduced *S. oryzae* seed infection and increased germination and seedling vigour of rice. Maximum seedling vigour index of 3756 in ADTRH 1 and 3592 in ADT 39 was recorded in *P. fluorescens* isolate 10 treatment. This may be due to growth promoting ability of fluorescent pseudomonads (Mew *et al.*, 2004).

Effect of selected biocontrol agents and fungicide on seed infection of *S. oryzae*, seed germination and seedling vigour of rice cv. ADT 39

Seeds treated with carbendazim reduced seed infection from 32.5 per cent (untreated) to 5.75 per cent followed by *T. viride* isolate 3 (12.25%), *P. fluorescens* isolate 10 (13.5%) and *B. subtilis* isolate 9 (15.5%) (Table 3). *Pseudomonas fluorescens* isolate 10 recorded maximum seed germination (90.0%), followed by *B. subtilis* isolate 9 (90.0%) and carbendazim (90.0%) compared to control (73.0%). The maximum root length (24.05 cm), shoot length (15.88 cm) and seedling vigour (3592) were recorded in *P. fluorescens* isolate 10. The minimum seedling vigour index of 2335 was observed in

Table 2. Effect of selected biocontrol agents and fungicide on *S. oryzae* seed infection, seed germination and seedling characters of ADTRH 1

Treatment	Seed infection* (%)	Germination* (%)	Root Length* (cm)	Shoot Length * (cm)	Vigour Index*
1. *B. subtilis* isolate 9 @ 10 g/kg	14.25 abc (22.00)	90.75 b (72.44)	24.90 b	13.98 ab	3531 cd
2. *P. fluorescens* isolate 10 @ 10 g/kg	10.75 a (18.91)	92.25 b (74.81)	25.45 b	15.90 b	3756 d
3. *T. viride* isolate 3 @10 g/kg	18.00 abc (25.02)	92.00 b (73.70)	21.08 a	13.25 ab	3163 bc
4. Carbendazim @ 2g/kg	12.75 ab (20.79)	89.25 b (71.08)	21.53 a	11.33 a	2934 b
5. Control	51.00 d (45.62)	69.00 a (56.47)	20.63 a	12.50 ab	2305 a

Figures in parentheses are arcsine- transformed values.

Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 3. Effect of selected biocontrol agents and fungicide on *S. oryzae* seed infection, seed germination and seedling characters of ADT 39

Treatment	C. delta zone (CO 43)		Southern zone (ASD 16)		N. E. zone (ADTRH 1)		Western zone (CO 43)	
	ShR incidence (%)*	ShR severity (%)*	ShR incidence (%)*	ShR severity (%)*	ShR incidence (%)*	ShR severity (%)*	ShR incidence (%)*	ShR severity (%)*
1. Seed treatment with *Bacillus subtilis* isolate 9 @ 10g/kg	33.00 ^{ab} (35.03)	7.0 ^{a}	14.30 ^{abc} (22.13)	4.33 ^{a}	14.70 ^{bc} (22.19)	3.33 ^{ab}	14.00 ^{a} (21.80)	4.33 ^{ab}
2. Seed treatment with *Pseudomonas fluorescens* isolate 10 @ 10g/kg	25.00 ^{abc} (29.67)	5.0 ^{a}	11.70 ^{bc} (19.75)	3.00 a	17.00 ^{abc} (24.15)	4.33 ^{ab}	15.30 ^{a} (22.91)	5.67 ^{a}
3. Seed treatment with carbendazim @ 2g/kg	26.00 ^{abc} (30.50)	5.7 ^{a}	13.00 ^{abc} (21.11)	5.00 ^{a}	16.7 ^{bc} (23.76)	4.33 ^{ab}	15.30 ^{a} (22.87)	5.67 ^{a}
4. Seed treatment with *B. subtilis* isolate 9 @ 10g/kg + foliar application (1%)	21.00 ^{bc} (27.25)	4.3 ^{a}	11.7 ^{bc} (19.76)	3.00 ^{a}	13.00 ^{bc} (21.06)	3.00 ^{b}	13.30 ^{a} (21.37)	3.00 ^{b}
5. Seed treatment with *P. fluorescens* isolate 10 @ 10g/kg + foliar application (1%)	22.00 ^{bc} (27.70)	5.0 ^{a}	13.00 ^{abc} (20.85)	3.00 ^{a}	12.30 ^{c} (20.14)	3.00 ^{b}	12.70 ^{a} (20.64)	3.00 ^{b}
6. Seed treatment with carbendazim @ 2g/kg + foliar application (0.2%)	18.70 ^{c} (25.51)	4.3 ^{a}	14.70 ^{abc} (22.47)	4.67 ^{a}	13.0 ^{bc} (20.98)	3.67 ^{ab}	12.30 ^{a} (20.47)	3.00 ^{ab}
7. Uninoculated Control	37.30 ^{a} (37.53)	7.0 ^{a}	19.7 0 ^{ab} (26.13)	5.00 ^{a}	29.00 ^{a} (32.46)	5.7 ^{a}	19.00 ^{a} (25.74)	6.33 ^{a}

Figures in parentheses are arcsine - transformed values.
Means followed by a common letter are not significantly different at the 5% level by DMRT.

control. Sakthivel and Gnanamanickam (1987) reported that seed treatment with *P. fluorescens* reduced *S. oryzae* seed infection by 8 –20 per cent and increased seedlings height (11%) and number of tillers (27%). Meena *et al.* (1999) reported that seed treatment with *P. fluorescens* significantly reduced the seed infection of *S. oryzae*. Seed bacterization of rice with *P. fluorescens* and *B. subtilis* promoted seed germination and seedling growth (Mew and Gonzales, 2002; Mew *et al.*, 2004).

Effect of selected biocontrol agents on sheath rot incidence and severity

All the treatments recorded minimum sheath rot incidence and severity when compared to control irrespective of locations and cultivars tested (Table 4). However, the effectiveness of treatments varied from location to location. In Aduthurai, minimum sheath rot incidence (18.67%) was recorded in carbendazim seed treatment + foliar

Table 4. Effect of selected biocontrol agents and fungicide on sheath rot incidence and severity

Treatment	Seed infection* (%)	Germination* (%)	Root Length* (cm)	Shoot Length * (cm)	Vigour Index*
1 *B. subtilis* isolate 9 @ 10 g /kg	15.50 ^{b} (22.99)	90.00 ^{b} (71.68)	23.63 ^{b}	14.90 ^{bc}	3465 ^{cd}
2 *P. fluorescens* isolate 10 @ 10 g/kg	13.50 ^{b} (21.44)	90.00 ^{b} (71.97)	24.05 ^{b}	15.88 ^{c}	3592 ^{d}
3 *T. viride* isolate 3 @ 10 g kg	12.25 ^{b} (20.40)	88.50 ^{b} (70.80)	21.20 ^{ab}	14.15 ^{bc}	3130 ^{bc}
4 Carbendazim @ 2g kg	5.75 ^{a} (13.21)	90.00 ^{b} (71.98)	19.80 ^{a}	13.43 ^{ab}	2988 ^{b}
5 Control	32.50 ^{c} (34.69)	73.00 ^{a} (58.83)	20.00 ^{a}	11.95 ^{a}	2335 ^{a}

Figures in parentheses are aresine- transformed values.
Means followed by a common letter are not significantly different at the 5% level by DMRT.

application compared to control (37.33%). The minimum sheath rot incidence was recorded in *P. fluorescens* isolate 10 seed treatment + foliar application and *B.subtilis* isolate 9 seed treatment + foliar application (19.67%). Seed treatment with *P.fluorescens* isolate 10 followed by foliar application and carbendazim seed treatment + foliar application has recorded minimum sheath rot incidence (12.33%) and severity (3.0) in Arakkonam and N. D. palayam, respectively. In general, seed treatment followed by foliar application was found more effective in reducing both sheath rot incidence and severity than seed treatment alone. Among four locations, the maximum sheath rot incidence in untreated control (37.33%) was recorded in Aduthurai in the cv. CO 43, followed by Arakkonam (29.00%) in ADTRH 1. The maximum sheath rot severity (7.0) was recorded in cv. CO 43 at Aduthurai while minimum (5.0) was recorded in cv. ASD 16 at Ambasamudram.

Sakthivel and Gnanamanickam (1987) reported that *P. fluorescens* treated rice plants cv. IR 20 showed 54 per cent reduction in the length of sheath rot lesions and reduced the sheath rot severity by 20 – 42 per cent in five rice cultivars and enhanced plant height, number of tillers and grain yields by 3 – 160 per cent. The simultaneous inoculation of rice with *P. oryzae* and antagonistic *Trichoderma* spp. resulted in 71 to 88 per cent reduction in foliar symptoms of blast disease, compared to plants inoculated with *P. oryzae* alone (Ouazzani Touhami *et al.*, 1998). A combination of antagonists *T. harzianum* and *B. subtilis* proved to be highly effective against sheath blight of rice (Sarmah, 1999). Seeds treated with *T. viride* and *T. harzianum* showed significant reduction in sheath blight infection and increased grain yield, where efficacy of the former was found more than the latter (Das and Hazarika, 2000).

Effect of selected biocontrol agents on grain discolouration and seed infection

Irrespective of locations and cultivars tested, all the treatments recorded minimum grain discolouration and seed infection when compared to control (Table 5). Carbendazim seed treatment followed by foliar application recorded minimum per cent grain discolouration and per cent resultant seed infection in Aduthurai (23.33 and 18.67%) and Ambasamudram (11.67 and 10.33%), respectively. Whereas at N. D. palayam, minimum grain discolouration was recorded in carbendazim seed treatment followed by foliar application but resultant seed infection (8.33%) was minimum in *B. subtilis* isolate 9 seed treatment followed by foliar application. The results at Arakkonam showed

Table 5. Effect of selected biocontrol agents and fungicide on grain discolouration and resultant seed infection

Treatment	C. delta zone (CO 43)		Southern zone (ASD 16)		N. E. zone (ADTRH 1)		Western zone (CO 43)	
	Grain dis. (%)*	Seed infn. (%)*	Grain dis. (%)*	Seed infn. (%)*	Grain infn. (%)*	Seed infn. (%)*	Grain dis (%)*	Seed infn. (%)*
1. Seed treatment with *Bacillus subtilis* isolate 9 @ 10g/kg	26.0 ^{ab} (30.32)	24.7 ^{a} (29.46)	13.3 ^{abc} (21.31)	13.3 ^{a} (21.36)	24.0 ^{bc} (29.07)	12.3 ^{ab} *(20.38)*	10.07 ^{a} (19.01)	17.7 ^{ab} (20.81)
2. Seed treatment with *Pseudomonas fluorescens* isolate 10 @ 10g/kg	32.7 ^{abc} (34.73)	24.7 ^{a} (29.46)	13.0 ^{bc} (21.00)	12.3 a (20.50)	21.3 ^{abc} (26.89)	12.3 ^{ab} (20.30)	12.0 ^{a} (20.23)	12.7 ^{a} (18.01)
3. Seed treatment with carbendazim @ 2g/kg	33.3 ^{abc} (35.21)	22.0 ^{a} (27.84)	14.7 ^{abc} (22.09)	13.7 ^{a} (21.65)	15.0 ^{bc} (22.68)	10.0 ^{ab} (18.09)	10.3 ^{a} (18.40)	10.3 ^{ab} (20.77)
4. Seed treatment with *B. subtilis* isolate 9 @ 10g/kg + foliar application (1%)	27.7 ^{bc} (31.08)	23.3 ^{a} (28.65)	15.0 ^{bc} (22.60)	11.7 ^{a} (19.73)	12.7 ^{c} (20.76)	8.7 ^{a} (16.78)	11.0 ^{a} (19.08)	8.3 ^{b} (18.72)
5. Seed treatment with *P.fluorescens* isolate 10 @ 10g/kg + foliar application (1%)	28.0 ^{bc} (31.44)	18.7 ^{a} (25.38)	14.3 ^{abc} (22.17)	10.7 ^{a} (18.89)	16.3 ^{abc} (23.81)	8.7 ^{a} (16.96)	11.0 ^{a} (19.32)	8.7 ^{b} (16.47)
6. Seed treatment with carbendazim @ 2g/kg + foliar application (0.2%)	23.3 ^{b} (28.87)	18.7 ^{a} (25.18)	11.7 ^{a} (19.87)	10.3 ^{a} (18.36)	19.7 ^{abc} (26.24)	11.7 ^{a} (19.8)	9.3 ^{a} (17.64)	9.7 ^{ab} (19.85)
7. Uninoculated Control	44.7 ^{a} (41.87)	34.7 ^{a} (35.97)	17.7 ^{ab} (24.74)	15.7 ^{a} (23.12)	27.7 ^{a} (31.71)	16.0 ^{a} (23.53)	12.3 ^{a} (20.17)	23.3 ^{a} (23.94)

Figures in parentheses are arcsine-transformed values. Means followed by a common letter are not significantly different at the 5% level by DMRT.

minimum grain discolouration and seed infection (12.67 and 8.67%, respectively) in *B. subtilis* isolate 9 seed treatment followed by foliar application. Among the four locations, Aduthurai recorded maximum grain discolouration and seed infection (44.67 and 34.67%, respectively) in untreated control. In the present study, seed treatment + foliar application of *B. subtilis* isolate 9 recorded minimum per cent grain discolouration at Arakkonam and N. D. palayam and recorded significantly minimum seed infection.

REFERENCES

Abdul Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soybean by multiple criteria. *Crop Science*. **13**: 630-632.

Ashura, L. K., Mabagala, R. B. and Mortensen, C. N. 1999. Isolation and characterization of seed-borne pathogenic bacteria from rice in Tanzania. *Tanzania Journal of Agricultural Science*, **2**(1): 71-80.

Chakravarty, D. K. and Biswas, S. 1978. Estimation of yield loss in rice affected by sheath rot. *Plant Disease Reporter*, **62**: 226-227.

Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T. W. and Swings, J. 2001. Bacterial populations associated with rice seed in the tropical environment. *Phytopathology*, **91**(3): 282-292.

Das, B. C. and Hazarika, D. K. 2000. Biological management of sheath blight of rice. *Indian Phytopathology*, **23**(4): 433-435.

ISTA. 1993. International rules for seed testing. *Seed Science and Technology*, **21**: 141-186.

Lakshmanan, P. 1993. Studies on sheath rot disease of rice due to the interactions of *Sarocladium oryzae* and rice insect pests. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Meena, B., Radhajeyalakshmi, R., Vidyasekaran, P. and Velazhahan, R. 1999. Effect of foliar application of *Pseudomonas fluorescens* on activities of phenylalanine ammonia-lyase, chitinase and â-1,3-glucanase and accumulation of phenolics in rice. *Acta Phytopathologica et Entomologica Hungarica*, **34**(4): 307-318.

Mew, T. W., Cottyn, B., Pamplona, R., Barrios, H., Xiangmin, L., Zhiyi, C., Fan, L., Nilpaint, N., Arunyanart, P., Kim, P. V. and Du, R. V. 2004. Applying rice seed-associated antagonistic bacteria to manage rice sheath blight in developing countries. *Plant Disease*, **88**: 557-564.

Mew, T. W. and Gonzales, 2002. *A hand book of rice seed-borne fungi*. T.W. Mew and B. Cottyn (Eds.). IRRI Science Publishers, 83 pp.

Quazzani touhami, A., Mouria, A., Douira, A., Hmouni, A., Mlaiki, A. and El- Yachioui, M. 1998. The ability of *Trichoderma* species to reduce leaf infection in rice. *Al Awamia*, **96**: 25-31.

Sakthivel, N. and Gnanamanickam, S. S. 1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yield in rice. *Applied and Environmental Microbiology*, **53** (4): 2056-2059.

Sarmah, D. K. 1999. Combined effect of *Gliocladium virens* and *Bacillus subtilis* on sheath blight of rice. *Journal of the Agricultural Science Society of North East India*, **12** (2): 271-273.

Xie, G. L., Pamplona, R. S., Cottyn, B., Swings, J. and Mew, T. W. 2001. Rice seed: Source of bacteria antagonistic against rice pathogens, pp. 9-17. In Mew, T. W. and Cottyn, B. (Eds.), *Seed health and seed associated microorganisms for rice disease management*. Limited Proceedings No. 6. Los Banos (Philippines): International Rice Research Institute.